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Original Article

RUNX3 polymorphisms and the susceptibility to cervical cancer and cervical intraepithelial neoplasia in Western China

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Abstract: Runt-related transcription factor 3 (*RUNX3*) is recognized to play essential roles in various tumors. But the effect between genetic variations and the susceptibility to tumor remains unclear, so does it in cervical cancer (CC) and cervical intraepithelial neoplasia (CIN). Three single nucleotide polymorphisms (SNP) of the *RUNX3* gene were selected to evaluate whether they were associated with CC and CIN. The three polymorphisms were genotyped in 260 CC patients, 212 CIN patients and 286 healthy controls in a hospital based case-control study in Western China. The three SNPs of *RUNX3* were analyzed between every two groups. We found TT genotype of rs760805 (P=0.01, OR=0.55, 95% CI=0.35-0.88) compared with AA/AT genotype and AG genotype of rs2236852 (P=0.0024, OR=1.72, 95% CI=1.25-2.5) compared with AA/GG genotype were significantly associated with susceptibility to CC. The former was also associated with the clinical stage of CC. We also found SNPs rs760805 (P=0.0042, OR=8.33, 95% CI=1.12-50) and rs7528484 (P=0.027, OR=0.14, 95% CI=0.027-0.72) were closely connected with HPV infection in CIN patients. Moreover, we found rs2236852 AG genotype of *RUNX3* was more related with the progression from CIN to CC (P<0.0001, OR=2.27, 95% CI=1.54-3.45). *RUNX3* mRNA expression was significantly decreased in CC (P<0.0001) while no significant relationship between *RUNX3* mRNA expression and the three SNPs. Those results probably mean that the SNPs of *RUNX 3* influence not only the genetic susceptibility to CC, but also the pathogenesis of CC and CIN in Western China. The decreased expression of *RUNX3* mRNA might indicate the inhibition of CC.

Keywords: *RUNX3*, cervical cancer, cervical intraepithelial neoplasia, polymorphism

Introduction

In less developed countries, cervical cancer (CC) is the second most common cancer and the third leading cause of cancer death among female until year 2012 [1]. It was estimated that there were 527,600 new CC cases and 265,700 deaths worldwide in 2012 [1]. Cervical intraepithelial neoplasia (CIN) is a precancerous lesion of CC [2]. Both CC and CIN are closely related to human papillomavirus (HPV), which is regarded to be the leading etiology [3]. However, not all HPV infections will induce cervical lesions-only with persistent HPV infection, some will develop to CIN or even progress to CC [3, 4]. In previous studies, researchers showed that the polymorphisms in genes was associated with the susceptibility to HPV [5, 6] and also with the susceptibility to CIN and CC [5, 7-10]. In addition, in our previous studies, we also found interleukin 1 (*IL-1*) and interleukin 6 *(IL-6*) genes were associated with the susceptibility to CC [11, 12]. These findings strongly implied that genetic polymorphisms may have potential associations with CC.

Runt-related transcription factor 3 (*RUNX3*) is a member of Runt domain family [13] and is a key downstream effector of transforming growth factor beta (*TGFβ*) signaling pathway [14]. In

many previous studies, such as gastric, hepatocellular, and breast cancers, *RUNX3* was recognized to play an essential role in tumor suppressing [15-18]. The primary inhibiting inactivation of *RUNX3,* which might induce oncogenesis, included promoter hypermethylation, inactivating mutations, gene deletions, protein mislocalization and so on [15, 18-22]. While some researchers thought RUNX3 was an oncogene [23, 24], and Lotem et al. speculated the gene played important functions in immunity and inflammation and might indirectly influence epithelial tumor development [25]. For figure out the relationship between *RUNX3* and cancer, some studies found the polymorphisms of *RUNX3* (rs760805 and rs2236852) were connected with bladder cancer and gastric cancer, and concluded the gene polymorphisms may affect tumor occurrence [26-28]. Byungho et al. found a risk-associated allele of rs7528484 increased the distal promoter activity and possibly stimulated nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity which may be associated with susceptibility to intestinal-type gastric cancer [26]. But so far, it is still not fully understand how *RUNX3* regulate the signal pathway and affect tumorigenesis. Further, we knew little about the association of *RUNX3* with CC and CIN. Therefore, in this study, we focus on exploring the association of two tag SNPs (rs760805, rs226852 at proximal promoter) and one dbSNP (rs7528484 at distal promoter) in the *RUNX3* gene with the individual susceptibility to CC and CIN. And a hospital based case-control study was performed in Western China.

Materials and methods

Subjects

The study was approved by the ethics committee of the West China Second University Hospital of Sichuan University and all women signed informed consents before the research. This hospital based case-control study enrolled 260 women with CC (mean ± SD, 45.89±8.78) and 212 with CIN (mean ± SD, 39.15±9.15) (**Table 1**) from 22 to 72 years old in the West China Second University Hospital from January 2012 to December 2014. All patients met the study criteria as follows *(i)* had not been previously diagnosed with CC or other cancer; *(ii)* had a cervix; *(iii)* had no history of cervical surgery; *(iv)* were not pregnant; and *(v)* were physically able to undergo routine pelvioscopy. Women were excluded if they never had sexual intercourse or had history of serious disease or cancer. 286 healthy women aged from 18 to 72 years old were recruited as control group when they had a regular gynecological examination (mean ± SD, 40.07±10.34). Diagnoses of all cases were confirmed by pathological diagnosis. All subjects were living in western China. We reviewed medical records for patients' characteristics, including age at diagnosis, menstrual status, pathological type, clinical stage, tumor differentiation, lymph node status, and parametrial invasion, vessel invasion and HPV infection status.

Genotyping

DNA was extracted from patients' cervical smear using a DNA isolation kit from Bioteke (Peking, China) according to the instruction of manufacturer. Genotyping of *RUNX3* polymorphisms was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The SNPs information of *RUNX3*, such as primers [27, 28], annealing temperature and restriction enzymes, were all summarized in **Table 2**. The PCR reactions were performed in a total volume of 10 μl, including 5 μl 2×Taq PCR Mastermix (Tiangen, Peking, China) and 50 ng of genomic DNA. PCR products for SNPs were digested for 3 hours and the digested PCR products were separated by a 6% polyacrylamide gel and stained with 1.0 mg/ml argent nitrate. We randomly selected about 20% samples to perform the repeated assays and the results were 100% concordant. The genotypes were confirmed by DNA sequencing analysis (TsingKe, Peking, China).

RNA isolation and qRT-PCR

RUNX3 mRNA expression was analyzed in 114 CC and 66 controls. Total RNA was extracted and purified from blood samples using TRIzol® Reagent (Life Technologies, USA) according to the manufacturer's protocol. Reverse transcription-PCR (RT-PCR) were performed by one step RT-PCR kit (BIONEER, South Korea) followed the manufacturer instructions. Quantitative real-time PCR was carried out using SYBR green PCR Master Mix (Roche, Switzerland) and samples were amplified in a thermocycler

Table 1. The socio-demographic characteristics of the study subjects

Characteristics	Controls	Cases		P		
		Cervical cancer (CC)	CIN	Controls vs CC	Controls vs CIN	CIN vs CC
Sample size	286	260 (squamous=234, adeno-carcinoma=17, other=9)	212 (CIN1=55, CIN2=40, CIN3=117)	-	-	
Age (years), mean (SD)	40.07 (10.34)	45.89 (8.78)	39.15 (9.15)	<0.001***	0.318	<0.001***
Menstrual status						
Premenopausae	275	182	209	<0.001***	0.17	<0.001***
Postmenopause	11	78	3			

***P<0.001.

Table 2. The SNPs informations of RUNX3

SNP ID	Primer (5'-3')	Annealing temperature (°C)	Restriction enzymes	Cuttable allele	Uncuttable allele
rs760805	F: TCTCCCACTCAGCAGTTCACAC	58.7°C	BstZ17I	A (152 and 22 bp)	T (174 bp)
	R: TACAGCTCTCAATATGCGCCAG				
rs2236852	F: TGGAGTGGCTCCCCTCTTTCTG	63.6°C	NdeI	A (100 and 20 bp)	G (120 bp)
	R: TATGGCAGGGCTGCCACCTC				
rs7528484	F: TGCGAGGCCCAGGGTGTTGA	60°C	HincII	C (107 and 18 bp)	T (125 bp)
	R: CATGGAAGGGCACTCTGGTG				

as follows: 95°C for 10 min (1 cycle), 95°C for 15 s, 60°C for 1 min (48 cycles). The primer information of *RUNX3* was as follows: sense GGGCGAGGGAAGAGTTTCAC and antisense GTCTGGTCCTCCAGCTTCTG (product=140 bp). Data were normalized for beta-actin (β-actin) expression with comparative threshold cycle method. Triplicate Ct values were averaged and the relative expression levels were determined as $2^{-\Delta\Delta Ct}$.

Statistical analysis

All data analyses were calculated by SPSS 13.0 statistical software (SPSS Inc, Chicago, IL, USA). The baseline characteristics of participants were assessed by Student's t test and Single-factor Pearson chi-square. The genotype and allele frequencies of SNPs rs760805, rs2236852 and rs7528484 were obtained by direct counting. Hardy-Weinberg equilibrium was evaluated by the chi-square test. Genotypic association tests including codominant, dominant, recessive and overdominant genetic models were completed using SNPstats [29] in a case-control pattern. The linkage disequilibrium (LD) between the polymorphisms was implemented by using SHEsis software http://analysis.bio-x.cn/myAnalysis.php. Odds ratio (OR) and respective 95% confidence intervals (95% CI) were reported to assess the different effects between alleles and genotypes. *RUNX3* mRNA expression levels were compared between CC and controls using Mann-Whitney nonparametric test. Statistical comparisons of the relative expression of mRNA between the different genotypes of the three SNPs were performed with the Kruskal-Wallis test. It was regarded as statistically significant if *P* value < 0.05.

Results

The socio-demographic characteristics of the study subjects are shown in **Table 1**. There were significant differences in the distribution of age between CC and controls (P<0.001), CC and CIN (P<0.001). The menstrual status was also significantly different. We analyzed the data after adjustments for age and menstrual states, but there were no significant differences between crude and adjusted values (the crude values are not shown in **Tables 3**-**5**).

Analysis of allele frequencies

The three polymorphisms of rs760805, rs2236852 and rs7528484 were all successfully genotyped in 260 CC patients, 212 CIN patients and 286 healthy control subjects. The genotype distribution of the three variants in con-

Table 3. Genotype distributions of rs760805 in cases/controls and the association with CC/CIN risk estimates

rs760805	Genotype	CC cases N (%)	CIN cases N (%)	Controls N (%)	CC vs Control		CIN vs Control		CC vs CIN	
					Logistic regression OR (95% CI)[a]	P	Logistic regression OR (95% CI)	P	Logistic regression OR (95% CI)[a]	P
Genetic model										
Codominant	A/A	101 (38.9%)	74 (34.9%)	85 (29.7%)	1.00	0.01*	1.00	0.47	1.00	0.2
	A/T	122 (46.9%)	92 (43.4%)	133 (46.5%)	1.38 (0.93-2.06)		0.79 (0.53-1.19)		1.04 (0.68-1.61)	
	T/T	37 (14.2)	46 (21.7%)	68 (23.8%)	0.46 (0.27-0.76)		0.78 (0.48-1.27)		1.63 (0.92-2.86)	
Dominant	A/A	101 (38.9%)	74 (34.9%)	85 (29.75%)	1.00	0.016*	1.00	0.22	1.00	0.42
	A/T-T/T	159 (61.1%)	138 (65.1%)	201 (70.3%)	0.63 (0.44-0.92)		0.79 (0.54-1.15)		0.85 (0.56-1.27)	
Recessive	A/A-A/T	223 (85.8%)	166 (78.3%)	218 (76.2%)	1.00	0.01*	1.00	0.58	1.00	0.076
	T/T	37 (14.2%)	46 (21.7%)	68 (23.8%)	0.55 (0.35-0.88)		0.88 (0.58-1.35)		0.63 (0.38-1.05)	
Overdominant	A/A-T/T	138 (53.1%)	120 (56.6%)	153 (53.5%)	1.00	0.79	1.00	0.49	1.00	0.57
	A/T	122 (46.9%)	92 (43.4%)	133 (46.5%)	1.05 (0.74-1.49)		1.13 (0.79-1.46)		1.12 (0.76-1.64)	
Allele										
	A	324 (62.3%)	240 (56.6%)	303 (53.0%)	1.00	0.002**	1.00	0.27	1.00	0.08
	T	196 (37.7%)	184 (43.4%)	269 (47.0%)	0.68 (0.53-0.87)		0.86 (0.67-1.11)		0.79 (0.61-1.02)	

*P<0.05, **P<0.01, [a]Adjusted by age and menstrual status.

Table 4. Genotype distributions of rs2236852 in cases/controls and the association with CC/CIN risk estimates

rs2236852	Genotype	CC cases N (%)	CIN cases N (%)	Controls N (%)	CC vs Control		CIN vs Control		CC vs CIN	
					Logistic regression OR (95% CI)[a]	P	Logistic regression OR (95% CI)	P	Logistic regression OR (95% CI)[a]	P
Genetic model										
Codominant	G/G	60 (23.1%)	76 (35.9%)	90 (31.5%)	1.00	0.0098**	1.00	0.28	1.00	<0.0001**
	A/G	165 (63.5%)	90 (42.5%)	142 (49.6%)	1.69 (1.12-2.56)		0.75 (0.50-1.12)		2.27 (1.45-3.57)	
	A/A	35 (13.5%)	46 (21.7%)	54 (18.9%)	0.93 (0.53-1.64)		1.01 (0.60-1.67)		0.98 (0.54-1.79)	
Dominant	G/G	60 (23.1)	76 (35.9%)	90 (31.5%)	1.00	0.05*	1.00	0.31	1.00	0.0049**
	A/G-A/A	200 (76.9%)	136 (64.2%)	196 (68.5%)	1.49 (1.00-2.22)		0.82 (0.56-1.19)		1.85 (1.20-2.86)	
Recessive	G/G-A/G	225 (86.5%)	166 (78.3%)	232 (81.1%)	1.00	0.086	1.00	0.44	1.00	0.04*
	A/A	35 (13.5%)	46 (21.7%)	54 (18.9%)	0.65 (0.40-1.06)		1.19 (0.76-1.85)		0.58 (0.34-0.98)	
Overdominant	G/G-A/A	95 (36.5%)	122 (57.5%)	144 (50.4%)	1.00	0.0024**	1.00	0.11	1.00	<0.0001**
	A/G	165 (63.5%)	90 (42.5%)	142 (49.6%)	1.72 (1.25-2.5)		0.75 (0.52-1.08)		2.27 (1.54-3.45)	
Allele										
	A	235 (45.2%)	182 (42.9%)	250 (43.7%)	1.00	0.63	1.00	0.85	1.00	0.51
	G	285 (54.8%)	242 (57.1%)	322 (56.3%)	0.94 (0.74-1.19)		1.03 (0.80-1.33)		0.91 (0.70-1.18)	

*P<0.05, **P<0.01, [a]Adjusted by age and menstrual status.

trols were all in accordance with the Hardy-Weinberg equilibrium, the *P* value were 0.249, 0.904 and 0.657 respectively. The genotype and allele frequencies of the three polymorphisms were analyzed in **Tables 3-5**. It was indicated the allele T of rs760805 (P=0.002, OR=0.68, 95% CI=0.53-0.87) was observed to be associated with the risk of CC. But there was no significant association between CC risk and alleles of rs2236852 (P=0.63, OR=0.94, 95% CI=0.74-1.19) or rs7528484 (P=0.49, OR=1.11, 95% CI=0.85-1.47). In addition, there was no statistically significant association between CIN risk and alleles of rs760805 (0.27, OR= 0.86, 95% CI=0.67-1.11), rs2236852 (P=0.85, OR=1.03, 95% CI=0.80-1.33) and rs7528484 (P=0.61, OR=1.09, 95% CI=0.81-1.45). And we neither found strong LD in both patients and controls about the three SNPs.

Comparison between CC and controls

We compared the SNPs between CC and controls and found that a significantly reduced risk

Table 5. Genotype distributions of rs7528484 in cases/controls and the association with CC/CIN risk estimates

rs7528484	Genotype	CC cases N (%)	CIN cases N (%)	Controls N (%)	CC vs Control		CIN vs Control		CC vs CIN	
					Logistic regression OR (95% CI)[a]	P	Logistic regression OR (95% CI)	P	Logistic regression OR (95% CI)[a]	P
Genetic model										
Codominant	T/T	151 (58.1%)	113 (53.3%)	153 (53.5%)	1.00	0.34	1.00	0.16	1.00	0.06
	C/T	91 (35%)	93 (43.9%)	115 (40.2%)	0.76 (0.52-1.10)		1.10 (0.76-1.59)		0.74 (0.49-1.10)	
	C/C	18 (6.9%)	6 (2.8%)	18 (6.3%)	1.06 (0.51-2.21)		0.45 (0.17-1.18)		2.13 (0.79-5.89)	
Dominant	T/T	151 (58.1%)	113 (53.3%)	153 (53.5%)	1.00	0.18	1.00	0.97	1.00	0.33
	C/T-C/C	109 (41.9%)	99 (46.7%)	133 (46.5%)	0.78 (0.55-1.11)		1.01 (0.70-1.45)		0.83 (0.56-1.22)	
Recessive	T/T-C/T	242 (93.1%)	206 (97.2%)	268 (93.7%)	1.00	0.89	1.00	0.066	1.00	0.066
	C/C	18 (6.9%)	6 (2.8%)	18 (6.3%)	1.05 (0.52-2.13)		0.43 (0.17-1.11)		2.44 (0.90-6.67)	
Overdominant	T/T-C/C	169 (65%)	119 (56.1%)	171 (59.8%)	1.00	0.15	1.00	0.41	1.00	0.072
	C/T	91 (35%)	93 (43.9%)	115 (40.2%)	0.76 (0.53-1.10)		1.16 (0.81-1.67)		0.69 (0.47-1.03)	
Allele										
	C	127 (24.4%)	105 (24.8%)	151 (26.4%)	1.00	0.49	1.00	0.61	1.00	0.94
	T	393 (75.6%)	319 (75.2%)	421 (73.6%)	1.11 (0.85-1.47)		1.09 (0.81-1.45)		1.02 (0.76-1.37)	

[a]Adjusted by age and menstrual status.

of CC was associated with the TT homozygous carriers of rs760805 in a codominant model, compared with AA genotype (P=0.01, OR=0.46, 95% CI=0.27-0.76). Then, compared with AA and AT homozygous carriers, TT genotype carriers also have a reduced CC risk in a recessive model (P=0.01, OR=0.55, 95% CI=0.35-0.88). In addition, there was significantly reduced CC susceptibility associated with allele T carriers (P=0.016, OR=0.63, 95% CI=0.44-0.92) in a dominant model (**Table 3**). For rs2236852, we found that, compared with GG homozygous, AG genotype may significantly increase the risk of CC in a codominant model (P=0.0098, OR=1.69, 95% CI=1.12-2.56). And compared with homozygous of AA and GG in an overdominant model, AG genotype carriers also increased the risk of CC (P=0.0024, OR=1.72, 95% CI=1.25-2.5) (**Table 4**). But for rs7528484, there was no statistically significant difference between CC and controls (**Table 5**). Further, we performed stratification analysis of genotype distribution with CC patients for different age, clinical stage, pathological type, tumor differentiation, lymph node status, parametrial invasion, vessel invasion and HPV type. As shown in **Table 6**, only AT genotype of rs760805 in an overdominant model was identified significant association between clinical stage I and stage II-IV of CC, compared with the homozygote AA/TT genotypes (P=0.038, OR=0.6, 95% CI=0.36-0.97). While no significant association was detected between other clinical features of rs760805, rs2236852 and rs7528484 (data were not shown completely).

Comparison between CIN and controls

We also compared the three SNPs between CIN and controls, but there was no significant difference of the risk of CIN (**Tables 3-5**). And we conducted stratified analyses with different age, HPV type and pathological type. Part of analysis results were summarized in **Table 6**. We found TT genotype carriers of rs760805 had a significant association between high risk type (HR) HPV infection and negative ones in codominant model (0.015, OR=9.09, 95% CI=1.18-100) and recessive model (0.0042, OR=8.33, 95% CI=1.12-50.0). Moreover, CC genotype carrier for rs7528484 of CIN patients were significantly different between HR-HPV and negative ones in a codominant model (0.031, OR=0.18, 95% CI=0.03-0.96) and recessive model (0.027, OR=0.14, 95% CI=0.027-0.72). However, no significant association was detected between other clinical features and the three polymorphisms.

Comparison between CC and CIN

Moreover, we compared CIN and CC for the three variants (**Tables 3-5**) and found that AG genotype carriers of rs2236852 also increased the risk of CC in the codominant model (P<0.0001, OR=2.27, 95% CI=1.45-3.57), overdominant model (P<0.0001, OR=2.27, 95%

Table 6. Analysis of CC/CIN patients characteristics and polymorphisms of RUNX3

	Clinical features	rs760805									rs7528484								
		Genotype			Genetic model						Genotype			Genetic model					
		AA	AT	TT	Dominant (AA vs AT/TT)		Recessive (AA/AT vs TT)		Overdominant (AA/TT vs AT)		CC	CT	TT	Dominant (TT vs CC/CT)		Recessive (TT/CT vs CC)		Overdominant (TT/CC vs CT)	
					OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P				OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
CC	Age																		
	<45 (n=133)	41%	45%	14%	1.24 (0.75-2.05)	0.4	1.12 (0.56-2.25)	0.74	1.16 (0.71-1.89)	0.55	5%	36%	59%	1.05 (0.64-1.72)	0.85	1.71 (0.64-4.55)	0.28	0.91 (0.54-1.51)	0.71
	≥45 (n=127)	36%	49%	15%							9%	34%	57%						
	Clinical stage																		
	I (n=125)	34%	54%	12%	0.70 (0.42-1.15)	0.16	1.43 (0.70-2.89)	0.32	0.60 (0.36-0.97)	0.038*	6%	34%	59%	1.09 (0.67-1.79)	0.72	1.17 (0.45-3.07)	0.75	1.05 (0.63-1.75)	0.85
	II-IV (n=135)	43%	41%	16%							7%	36%	57%						
CIN	Age																		
	<40	32%	43%	25%	0.79 (0.0.45-1.41)	0.42	0.69 (0.56-1.35)	0.27	1.07 (0.60-1.79)	0.9	3%	48%	49%	0.73 (0.42-1.25)	0.25	1.00 (0.19-4.76)	0.93	0.73 (0.42-1.25)	0.26
	≥40	38%	44%	18%							3%	40%	57%						
	HPV type																		
	HRHPV	33%	43%	24%	1.72 (0.78-3.85)	0.18	8.33 (1.12-50.0)	0.0042**	0.74 (0.33-1.64)	0.46	2%	46%	52%	1.43 (0.64-3.23)	0.38	0.14 (0.027-0.72)	0.027*	2.17 (0.91-5.26)	0.071
	Negative	46%	50%	4%							11%	29%	61%						

*P<0.05, **P<0.01.

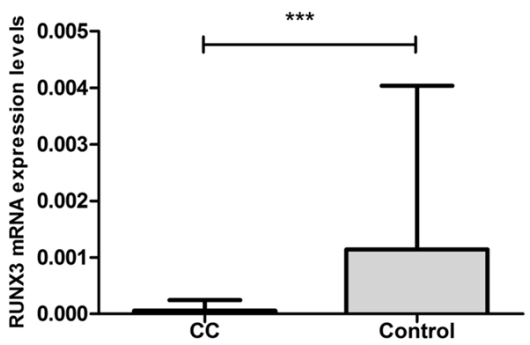

Figure 1. RUNX3 mRNA expression level was significantly decreased in CC (P<0.0001).

CI=1.54-3.45) and dominant model (P=0.0049, OR=1.85, 95% CI=1.2-2.86), but AA homozygous carriers might reduce the risk of CC in a recessive model (P=0.04, OR=0.58, 95% CI=0.34-0.98).

mRNA expression level of RUNX3

At last, we analyzed the expression levels of *RUNX3* mRNA in CC with controls. *RUNX3* mRNA expression was significantly decreased in CC (P<0.0001) (**Figure 1**). However, no significant relationship was found between RUNX3 mRNA expression and polymorphisms of rs760805, rs2236852 and rs7528484 in CC (P=0.4005, 0.67450 and 0.1437 respectively) or controls (P=0.8798, 0.7500 and 0.2990 respectively).

Discussion

It is well known that CC and CIN are closely related to the HR-HPV infection [3]. But most infected women will eliminate virus and only a small part of infected women will progress to CIN or even CC [2-4]. In the previous studies, the immunological mechanism and genetic factors of hosts may play an important role in cervical lesions [30, 31]. In this study, we continued to explore the potential effect of gene for CIN and CC and selected three SNPs (2 tag SNPs from proximal promoter and 1 dbSNP from distal promoter) in the *RUNX3* gene. According to the results, we conclude that *RUNX3* is probably a potential gene for CC susceptibility. The polymorphisms of *RUNX3* may be associated with HPV infection and CIN progression in Western China.

RUNX3 gene is a member of *RUNX* family and located in chromosome *1p36*, which is deemed to be a tumor suppress gene for gastric cancer and bladder cancer etc [13, 20, 21]. In previous study, Qing et al. found that gastric epithelial cells from *RUNX3*$^{-/-}$ nude mice with *p53*$^{-/-}$ background were tumorigenic, while those from *RUNX3*$^{+/+}$*p53*$^{-/-}$ mice were not [16]. But controversially, some studies have demonstrated it over-expressed in carcinomas of head and neck, BCC and ovarian [23, 32, 33]. Nevadunsky et al. found *RUNX3* had a role in cell proliferation and viability in ovarian cancer because of the overexpression of immunohistochemistry and qRT-PCR. In addition, the overexpressed *RUNX3* in SKOV3 ovarian cancer cells resulted in increased cell viability while silencing *RUNX3* expression by siRNA transfection resulted in a decrease in proliferation demonstrate the potential oncogenic role [32]. To date, scientists cannot fully elucidate the mechanism of *RUNX3* with cancer and the genetic variations in *RUNX3* that may affect signaling pathway for the development of cancer [17]. Researchers recognized *RUNX3* is a downstream target gene of *TGFβ* signaling which is a tumor suppress pathway. It regulates the expression of *Bim* and *p21*, negatively regulates *VEGF*, and thereby affects apoptosis, cell growth arrest and angiogenesis, respectively [14, 17]. In previous studies, only a few studies investigated on *RUNX3* polymorphisms with cancer. The studies of bladder cancer and gastric cancer of an eastern Chinese population found that the genetic variants of proximal promoter (rs11249206 at intron 1, rs760805 at intron 3 and rs2236852 at intron4) in *RUNX3* may modulate the risk of both cancer which may be identified with function in gene transcription and protein expression [27, 28]. In addition, in a Korean population study, the change of SNP rs7528484 located in the *RUNX3* distal promoter may contribute differentially to intestinal-type gastric cancer and the proximal and distal promoters may have opposite regulatory actions [26]. But the specific affection of RUNX3 polymorphisms to cancer is still not clear and need further functional studies.

In this study, we found that TT genotype of rs760805 polymorphism was associated with a significantly reduced risk of CC in Western China, which was contrary to the previous studies of bladder and gastric cancer [27, 28]. Further, we found that rs2236852 AG genotype was related to a significantly increased risk of CC, the same as the discovery of Wu et al. [27]

but not as Suárez-Villanueva et al. [34]. In addition, we also investigated that rs2236852 was associated with CIN and CC and might predict CIN progression. After stratification analysis, we found rs760805 of RUNX3 was associated with not only the susceptibility to CC but also the clinical stage of CC and HR-HPV infection of CIN. And rs7528484 was also associated with the risk of HR-HPV infected in CIN patients. All these evidences suggested that the rs760805 polymorphism may be a contributor to pathogenesis of CC and CIN while the specific functions should be demonstrated. Further, we found the expression of *RUNX3* mRNA decreased significantly in CC group. It might suggest *RUNX3* inhibited the tumor growth [35, 36]. However, no relationship was found between the three SNPs and the low expression. It may be some other factors to regulate *RUNX3* transcription rather than the polymorphisms. And it might also be the limitation of sample selections in our study. These findings also indicate that *RUNX3* may play complex roles for different tumors, such as functions of immunity and inflammation, and indirectly influence tumor development [25]. In the future, the polymorphisms of *RUNX3* might become useful diagnostic biomarkers for CC and CIN. We also look forward novel *RUNX3* related genetic therapeutic could be considered for advanced CC. The results also encourage us to explore how does *RUNX3* gene affect tumor and whether it could be used for therapy.

In conclusion, we demonstrated that *RUNX3* SNPs may play important roles with the susceptibility to CC and CIN. However, due to the restriction of samples, further studies are needed to explore the association between *RUNX3* and CC/CIN. Most importantly, further researches about functional evaluations of *RUNX3* are also needed to confirm our findings.

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Disclosure of conflict of interest

None.

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