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Association of Foxp3 promoter polymorphisms with susceptibility to endometrial cancer in the Chinese Han women

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Abstract

To evaluate the association between *Foxp3* gene polymorphisms (rs3761548 and rs5902434) and susceptibility to endometrial cancer (EC), we report a hospital case–control study involving 602 women, consisting of 269 patients with EC and 333 healthy controls. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism. Our results suggest that the frequency of the A allele in rs3761548 in patients with EC was significantly lower than that in healthy controls (20.3% vs 26.4%, odds ratio [OR] 0.71, 95% confidence interval [CI]: 0.54–0.93, $P = .012$), while the heterozygous AC genotype showed a significant protective effect on EC in codominant, dominant, and overdominant models (adjusted OR 0.64, 95% CI: 0.45–0.91, $P = .039$; OR 0.65, 95% CI: 0.47–0.91, $P = .011$; OR 0.67, 95% CI: 0.47–0.94, $P = .02$, respectively), and AA genotype was more frequent in patients with cervical invasion (recessive model: OR 3.55, 95% CI: 1.10–11.44, $P = .046$). Moreover, ATT/ATT genotype (rs5902434) was conferred a lower risk of EC in the recessive model (adjusted OR 0.58, 95% CI: 0.35–0.96, $P = .031$). From the data generated, we conclude that Foxp3 promoter polymorphisms are associated with susceptibility to EC in Chinese Han women.

Abbreviations: ACS = acute coronary syndrome, BMI = body mass index, CI = confidence interval, CRC = colorectal cancer, DTC = differentiated thyroid carcinoma, EC = endometrial cancer, FIGO = International Federation of Gynecology and Obstetrics, Foxp3 = transcription factor forkhead box protein 3, NSCLC = non-small cell lung cancer, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, RRP = recurrent respiratory papillomatosis, Treg cell/Treg = regulatory T cell.

Keywords: endometrial cancer, Foxp3, polymorphisms, Treg cells

1. Introduction

Endometrial cancer (EC) is one of the 3 major malignant female genital tumors, ranking on top of the list of gynecologic malignant tumors in developed countries and some developed cities in China.[1] In recent years, with the changes of lifestyle and increase in metabolic diseases, the incidence of EC has increased, with a younger age of onset.[2] According to the data from Beijing Tumor Registry Office, EC overtook cervical cancer to become the highest incidence of female reproductive system cancers in Beijing since 2008.[2]

Regulatory T cells (Treg cells or Tregs) act as indispensable inhibitors to immune responses and participate in maintaining peripheral immune tolerance while suppressing anti-tumor immunity.[3] As early as 2001, Woo et al[4] reported for the first time that there was an increase in the number of Treg cells found in non-small cell lung cancer (NSCLC) and patients with ovarian cancer compared to healthy controls. Recent studies also revealed that a significant increase of Treg cells was observed in peripheral blood of patients with EC [5] and high Treg counts in EC tissue implied a worse prognosis.[6]

The Foxp3 (transcription factor forkhead box protein 3), located on the short arm of the X-chromosome (Xp11.23), belongs to the forkhead/winged-helix transcription factor family and is an indispensable regulatory gene that influences the development and function of Treg cells.[7] Many studies have examined the frequency of Foxp3 polymorphisms and its association with cancers and autoimmune diseases,[8] particular-

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ly the putative functional ones located in the promoter region of Foxp3, which may influence expression of Foxp3.[9,10] rs3761548 (C/A) and rs5902434 (del/ATT)[10] are both located in the promoter region of Foxp3.

We hypothesize that the promoter gene polymorphisms of Foxp3 would be associated with the risk of developing EC. To test our hypothesis, we performed the following study to evaluate the role of rs3761548 (C/A) and rs5902434 (del/ATT). As far as we are aware, this study would be the first to evaluate the association between Foxp3 promoter gene polymorphisms and susceptibility to EC.

2. Materials and methods

2.1. Study subjects

A total of 602 women (269 patients with EC and 333 matched healthy women) were recruited from the West China Second University Hospital of Sichuan University between June 2008 and June 2014. Diagnosis of EC was based on histopathologic biopsies. To prevent compounding factors, patients with autoimmune diseases or other cancers were excluded from this study. Clinical staging was performed using the guidelines from the International Federation of Obstetrics and Gynecology (FIGO) standards. Control group consisted of cancer-free women from routine gynecologic examination recruited from the outpatient department. Clinical and demographical characteristics (age, body mass index [BMI], menopausal status, family history of cancer, history of pregnancy, etc) were collected from medical records, and the details are depicted in Table 1. Our Hospital Ethics Committee approved the study and all patients signed informed consent forms.

2.2. DNA extraction and genotyping

DNA, stored at −20°C until analysis, was extracted using a whole-blood DNA isolation kit from BioTeke (Beijing, China) according to the manufacturer's instructions. Genotyping of rs3761548 (C/A) and rs5902434 (del/ATT) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers used were: F: 5'-GAAGGGCAAATTGAAGACCA-3' and R: 5'-GGTGCTGAGGGGTAAACTGA-3' for rs3761548 (C/A); F: 5'-CCCTGCCCATGCATTAAGTA-3' and R: 5'-TACCCAGCTACCGTGATTCC-3' for rs5902434 (del/ATT). PCR-RFLP analysis was performed using the following conditions; 100 ng of DNA was amplified in a total volume of 10 μL reaction mixture using 2 × Power Taq PCR MasterMix (BioTeke). PCR conditions for amplification were 4°C for 4 minutes, 32 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and a final elongation step at 72°C for 10 minutes. For rs3761548, 0.5 μL of PCR products was digested with 0.5 μL of PstI (New England Biolabs, Beijing, China) in a 10 μL reaction mixture for 2 hours at 37°C, then separated by 6% polyacrylamide gel and stained with 1.5 g/L argent nitrate: a 147 bp band for the undigested A allele, while 123 and 24 bp bands for the C allele was used to detect the different genotypes. To detect rs5902434 genotype, PCR product was separated by 6% polyacrylamide gel and stained with 1.5 g/L argent nitrate directly without digestion. The genotype was determined as follows: a 99-bp band for del-type and a 102-bp band for ATT-type. Furthermore, 10% of the samples were randomly selected for retesting with the results being 100% concordant.

Table 1

Descriptive characteristic of patients with endometrial cancer and health controls.

Characteristics	Number of cases (%)	Number of controls (%)	*P* value
Sample size	269	333	
Age mean ± SD (y)	51.77 ± 9.86	50.46 ± 11.87	.149
BMI mean ± SD (kg/m^2)	24.23 ± 3.48	23.96 ± 3.46	.346
Family history of cancer			.174
Yes	22 (8.2)	18 (5.4)	
No	247 (91.8)	315 (94.6)	
Memopausal status			.731
Premenopausal	129 (48.0)	155 (46.5)	
Postmenopausal	140 (52.0)	178 (53.5)	
History of pregnancy			.823
Yes	255 (94.8)	317 (95.2)	
No	14 (5.2)	16 (4.8)	
Uterine bleeding		–	
Yes	256 (95.2)		
No	13 (4.8)		
FIGO stage		–	
I	202 (75.1)		
II	23 (8.6)		
III	29 (10.8)		
IV	13 (4.8)		
Unknown*	2 (0.7)		
FIGO grade		–	
G1	95 (35.3)		
G2	98 (36.4)		
G3	76 (28.3)		
Histology		–	
Endometrioid adenocarcinoma	228 (84.8)		
Non-endometrioid adenocarcinoma†	41 (15.2)		

SD = standard deviation, BMI = body mass index, FIGO = International Federation of Gynecology and Obstetrics.

* No surgery.

† Serous adenocarcinoma: 9; clear cell adenocarcinoma: 5; carcinosarcoma: 4; neuroendocrine carcinoma: 2; mixed: 21.

2.3. Statistical analysis

Statistical analysis was performed using SPSS 22.0 (SPSS, Inc, Chicago, IL) and SNPstats online software (www.snpstats.net/start.htm). Student *t* test or chi-square test was used to compare the clinical and demographic characteristics of the 2 groups. The genotype frequencies were counted directly and the Hardy–Weinberg equilibrium was tested by a chi-square test. SNPstats analyzed genotype-related associations by constructing codominant, dominant, recessive, or overdominant genetic models.[11] Logistic regression was used to detect the association of Foxp3 polymorphisms and EC by the odds ratio (OR) for risk at 95% confidence interval (95% CI). $P < .05$ was considered statistically significant.

3. Results

3.1. Clinical and demographical characteristics of patients with EC and healthy controls

Table 1 depicts the characteristics of 269 patients and 333 healthy controls with age matched between the 2 groups. In addition, the 2 groups were similar in terms of BMI, menopausal status, family history of cancer, and pregnancy history ($P > .05$).

Table 2

Genotype and allele distribution of two Foxp3 promoter gene polymorphisms in patients with EC and health controls.

		Patients	Control	Logistic regression (crude)		Logistic regression (adjusted)*	
Genotype or allele	Genotype	N = 269 (%)	N = 333 (%)	OR (95% CI)	*P* value	OR (95% CI)	*P* value
rs3761548							
Genetic model							
Codominant	C/C	173 (64.3)	178 (53.5)	1.00	.027†	1.00	.039†
	A/C	83 (30.9)	134 (40.2)	0.64 (0.45–0.90)		0.64 (0.45–0.91)	
	A/A	13 (4.8)	21 (6.3)	0.64 (0.31–1.31)		0.68 (0.33–1.42)	
Dominant	C/C	173 (64.3)	178 (53.5)	1.00	.0071†	1.00	.011†
	A/C-A/A	96 (35.7)	155 (46.5)	0.64 (0.46–0.89)		0.65 (0.47–0.91)	
Recessive	C/C-A/C	256 (95.2)	312 (93.7)	1.00	.43	1.00	.56
	A/A	13 (4.8)	21 (6.3)	0.75 (0.37–1.54)		0.81 (0.39–1.66)	
Overdominant	C/C-A/A	186 (69.1)	199 (59.8)	1.00	.017†	1.00	.02†
	A/C	83 (30.9)	134 (40.2)	0.66 (0.47–0.93)		0.67 (0.47–0.94)	
Log-additive	–	–	–	0.71 (0.54–0.93)	.012†	0.72 (0.55–0.95)	.02†
Allele							
	C	429 (79.7)	490 (73.6)				
	A	109 (20.3)	176 (26.4)	0.71 (0.54–0.93)	.012†		
rs5902434							
Genetic model							
Codominant	D/D	113 (42.0)	139 (41.7)	1.00	.082	1.00	.077
	I/D	129 (48.0)	141 (42.3)	1.13 (0.80–1.59)		1.13 (0.80–1.60)	
	I/I	27 (10.0)	53 (15.9)	0.63 (0.37–1.06)		0.62 (0.36–1.05)	
Dominant	D/D	113 (42.0)	139 (41.7)	1.00	.95	1.00	.94
	I/D-I/I	156 (58.0)	194 (58.3)	0.99 (0.71–1.37)		0.99 (0.71–1.37)	
Recessive	D/D-I/D	242 (90.0)	280 (84.1)	1.00	.033†	1.00	.031†
	I/I	27 (10.0)	53 (15.9)	0.59 (0.36–0.97)		0.58 (0.35–0.96)	
Overdominant	D/D-I/I	140 (52.0)	192 (57.7)	1.00	.17	1.00	.17
	I/D	129 (48.0)	141 (42.3)	1.25 (0.91–1.73)		1.26 (0.91–1.75)	
Log-additive	–	–	–	0.88 (0.69–1.11)	.27	0.87 (0.69–1.11)	.27
Allele							
	D	355 (66.0)	419 (0.63)				
	I	183 (34.0)	247 (37.1)	0.87 (0.69–1.11)	.27		

CI = confidence interval, EC = endometrial cancer, OR = odds ratio.

* Adjusted for age, body mass index, family history of cancer, memopausal status, and history of pregnancy using the logistic regression model.

† $P < .05$.

3.2. Association of Foxp3 polymorphisms with genetic susceptibility to EC

Table 2 lists the genotype and allele composition of Foxp3 polymorphisms in 269 EC patients and 333 healthy women. The frequencies of the allele distribution of rs3761548 and rs5902434 in both groups were in keeping with the Hardy Weinberg equilibrium ($P > .05$), which means random distribution. The *P* values (case group, control group) of rs3761548 and rs5902434 are ($P = .45$, $P = .58$) and ($P = .34$, $P = .10$), respectively.

For rs3761548, the frequency of A allele in patients with EC was significantly lower than that in healthy controls (20.3% vs 26.4%, OR 0.71, 95% CI: 0.54–0.93, $P = .012$). A larger percentage of AC, the heterozygous genotype, was found in controls compared with that in patients with EC (40.2% vs 30.9%). By genetic model analysis, AC showed a statistically significant protective effect from EC in codominant, dominant, and overdominant models (adjusted OR 0.64, 95% CI: 0.45–0.91, $P = .039$; OR 0.65, 95% CI: 0.47–0.91, $P = .011$; OR 0.67, 95% CI: 0.47–0.94, $P = .02$, respectively). For rs5902434, no significant difference was observed in allele frequency in any genetic models except for the ATT/ATT homozygote genotype frequency in the recessive model (adjusted OR 0.58, 95% CI: 0.35–0.96, $P = .031$). Thus, the A allele, the AC heterozygous genotype of rs3761548, and the ATT/ATT homozygous genotype of rs5902434 indicate a relatively reduced risk of EC.

3.3. Association of Foxp3 polymorphisms with clinical characteristics of patients with EC

We conducted a stratified analysis to explore the relationship between the Foxp3 polymorphisms and certain clinical features (FIGO stage, FIGO grade, pathologic type, myometrial invasion, cervical invasion, parametrial invasion, lymph node status, peritumor intravascular cancer emboli). For rs3761548 (Table 3), the AA homozygous genotype was considered to be a high-risk factor for cervical invasion in EC (recessive model: OR 3.55, 95% CI: 1.10–11.44, $P = .046$). For rs5902434 (Table 4), no genotype or allele was significantly associated with clinical features.

4. Discussion

In this study, we examined the association of Foxp3 promoter polymorphisms with susceptibility to EC and its clinical characteristics in Chinese Han women. To the best of our knowledge, this is the first study to report the association between Foxp3 polymorphisms and risk of EC. Our results suggested that both Foxp3 polymorphisms (rs3761548 and rs5902434) were associated with the risk of EC, whereas the AA genotype

Table 3

Association between the genotype distribution of rs3761548 polymorphism of *Foxp3* gene and clinical features.

	rs3761548										
	Genotype			Genetic model							
				Codominant (C/C vs A/C vs A/A)		Dominant (C/C vs A/C-A/A)		Recessive (C/C-A/C vs A/A)		Overdominant (C/C-A/A vs A/C)	
Clinical features	C/C	A/C	A/A	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
FIGO stage											
I	133	61	8	A/C: 1.26 (0.69–2.31)	.39	1.37 (0.77–2.43)	.28	2.02 (0.64–6.41)	.25	1.18 (0.65–2.14)	.58
II–IV	38	22	5	A/A: 2.19 (0.68–7.08)							
FIGO grade											
G1	65	27	3	A/C: 1.25 (0.72–2.17)	.45	1.32 (0.78–2.25)	.30	1.87 (0.50–6.97)	.33	1.20 (0.69–2.07)	.52
G2–G3	108	56	10	A/A: 2.01 (0.53–7.56)							
Histology											
Endometrioid adenocarcinoma	142	74	12	A/C: 0.56 (0.25–1.23)	.23	0.53 (0.25–1.14)	.093	0.45 (0.06–3.56)	.40	0.59 (0.27–1.29)	.17
Non-endometrioid adenocarcinoma	31	9	1	A/A: 0.38 (0.05–3.05)							
Myometrial invasion											
<1/2	130	68	10	A/C: 0.70 (0.36–1.35)	.56	0.73 (0.39–1.36)	.32	1.06 (0.28–3.99)	.93	0.70 (0.37–1.35)	.28
≥1/2	41	15	3	A/A: 0.95 (0.25–3.62)							
Cervical invasion											
Negative	146	70	8	A/C: 1.08 (0.52–2.25)	.13	1.35 (0.69–2.62)	.38	3.55 (1.10–11.44)†	.046†	0.95 (0.47–1.94)	.89
Positive	25	13	5	A/A: 3.65 (1.10–12.06)							
Parametrial invasion											
Negative	154	79	12	A/C: 0.46 (0.15–1.41)	.35	0.50 (0.18–1.39)	.16	0.92 (0.11–7.49)	.94	0.47 (0.15–1.43)	.15
Positive	17	4	1	A/A: 0.75 (0.09–6.17)							
Lymph node status											
Negative	140	62	10	A/C: 1.29 (0.51–3.23)	.86	1.25 (0.52–3.03)	.62	0.92 (0.11–7.51)	.94	1.29 (0.52–3.20)	.59
Positive	14	8	1	A/A: 1.00 (0.12–8.40)							
Peritumor intravascular cancer emboli											
Negative	145	72	11	A/C: 0.85 (0.40–1.82)	.91	0.87 (0.43–1.79)	.71	1.07 (0.23–5.01)	.94	0.85 (0.40–1.80)	.67
Positive	26	11	2	A/A: 1.01 (0.21–4.84)							

CI = confidence interval, FIGO = International Federation of Gynecology and Obstetrics, OR = odds ratio.
† $P < .05$.

(rs3761548) was considered to be a risk factor for cervical invasion in stratified analysis.

For rs3761548, the A allele and AC heterozygous genotype showed a statistically significant protective effect on EC, indicating a lower risk. Although there were no other EC-related data reported, similar results have been observed in other disease studies. In a study of triple negative breast cancer in Brazil, interestingly, it showed AC genotype was a protective factor while AA was a risk one.[12] Genotype analysis of acute coronary syndrome (ACS) among Chinese population[13] implied the C allele was a risk factor for ACS, but detailed data analysis by us revealed a larger percentage of AC genotype and A allele in

Table 4

Association between the genotype distribution of rs5902434 polymorphism of *Foxp3* gene and clinical features.

	rs5902434										
	Genotype			Genetic model							
				Codominant (C/C vs A/C vs A/A)		Dominant (C/C vs A/C-A/A)		Recessive (C/C-A/C vs A/A)		Overdominant (C/C-A/A vs A/C)	
Clinical features	D/D	I/D	I/I	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
FIGO stage											
I	87	93	22	I/D: 1.40 (0.78–2.54)	.40	1.29 (0.73–2.30)	.38	0.68 (0.25–1.88)	.44	1.45 (0.83–2.55)	.19
II–IV	24	36	5	I/I: 0.82 (0.28–2.40)							
FIGO grade											
G1	39	44	12	I/D: 1.02 (0.6–1.73)	.59	0.94 (0.57–1.56)	.81	0.65 (0.29–1.46)	.30	1.11 (0.67–1.83)	.69
G2–G3	74	85	15	I/I: 0.66 (0.28–1.55)							
Histology											
Endometrioid adenocarcinoma	90	112	26	I/D: 0.59 (0.30–1.18)	.042	0.51 (0.26–1.00)	.049	0.19 (0.03– 1.47)	.043	0.73 (0.37–1.44)	.36
Non-endometrioid adenocarcinoma	23	17	1	I/I: 0.15 (0.02–1.17)							
Myometrial invasion											
<1/2	87	96	25	I/D: 1.25 (0.68–2.27)	.076	1.05 (0.58–1.89)	.87	0.26 (0.06–1.12)	.031	1.48 (0.83–2.65)	.18
≥1/2	24	33	2	I/I: 0.29 (0.06–1.31)							
Cervical invasion											
Negative	95	106	23	I/D: 1.29 (0.64–2.58)	.76	1.24 (0.63–2.44)	.52	0.90 (0.29–2.74)	.85	1.28 (0.67–2.46)	.46
Positive	16	23	4	I/I: 1.03 (0.32–2.44)							
Parametrial invasion											
Negative	104	115	26	I/D: 1.81 (0.70–4.65)	.28	1.58 (0.62–4.01)	.33	0.40 (0.05–3.10)	.32	1.98 (0.80–4.89)	.13
Positive	7	14	1	I/I: 0.57 (0.07–4.85)							
Lymph node status											
Negative	96	94	22	I/D: 2.55 (0.95–6.86)	.15	2.34 (0.89–6.18)	.07	0.82 (0.18–3.75)	.80	2.35 (0.96–5.79)	.056
Positive	6	15	2	I/I: 1.45 (0.27–7.70)							
Peritumor intravascular cancer emboli											
Negative	96	106	26	I/D: 1.39 (0.69–2.82)	.099	1.16 (0.58–2.34)	.67	0.20 (0.03–1.55)	.052	1.65 (0.83–3.30)	.15
Positive	15	23	1	I/I: 0.25 (0.03–1.95)							

CI = confidence interval, FIGO = International Federation of Gynecology and Obstetrics, OR = odds ratio.

healthy controls compared to patients, suggesting a concordance with our findings. Shen et al study of patients with psoriasis[14] suggested that the AA genotype may abolished E47/c-Myb binding, leading to transcriptional defects in *Foxp3* gene. Wildin et al[15] demonstrated that *Foxp3* gene polymorphisms might modulate $CD4^+CD25^+$Treg cell function, resulting in the manifestation of some autoimmune diseases. These findings may provide possible explanations to our results, that is, decreasing functional Tregs might enhance immune surveillance, making EC risk relatively reduced.

However, majority of the literature describes the A allele of rs3761548 as a risk factor for several cancers and autoimmune diseases. A allele was more frequent in patients with differentiated thyroid carcinoma (DTC) than in healthy controls, and individuals with the AC genotype had a higher risk of DTC.[16] Similarly, the AA/AC genotypes and A allele represented a significantly greater risk or a higher susceptibility to colorectal cancer (CRC)[17] and NSCLC.[18] Marson et al[19] and Zheng et al[20] observed many *Foxp3* genes regulated in $FOXP3^+$ T cells, indicating that Foxp3 functions as a transcriptional activator and repressor. The dual-acting nature of Foxp3 may explain the different results of *Foxp3* gene polymorphisms and susceptibility to diverse diseases.

Similar to rs3761548, the ATT/ATT homozygous genotype of rs5902434 was also associated with a relatively low risk of EC. Although rs5902434 has scarcely been associated with cancer, its association with other diseases has been established. Examples of which include reported frequencies of ATT/ATT genotypes being significantly decreased in severe recurrent respiratory papillomatosis (RRP) compared to controls, indicating it may be a protective factor in the risk of severe RRP in a cohort of Korean women.[21] Furthermore, Chu et al[22] suggested that the del/del genotype led to a higher expression of Foxp3 mRNA and may be associated with a reduced risk of chronic obstructive pulmonary disease and lung dysfunction in a cohort of male Chinese. These associations of rs5902434 with other diseases were also evaluated and significant associations were observed in unexplained recurrent pregnancy loss in Indian[23] and Chinese[24] women, pre-eclampsia in Chinese[25] and ACS in Iranian,[26] suggesting that rs5902434 (del/ATT) may confer a significant susceptibility to a number of diseases among the Asian population.

Furthermore, in a stratified analysis, the AA genotype of rs3761548 was considered as a high-risk factor for cervical invasion in EC. Previous studies has demonstrated that there was a positive correlation between the presence of $Foxp3^+$ Treg cells and vascular density in endometrial adenocarcinoma, indicating a correlation between immune function and intratumoral angiogenesis.[27] Moreover, the frequency of AA/AC genotype was higher in patients with DTC with tumor diameter >1 cm compared with patients with tumor diameter <1 cm[16] and was observed at higher frequency in patients with stage II NSCLC.[18] AA genotype was found highly significantly associated with advanced breast cancer.[28] However, no significant association between Foxp3 polymorphisms (rs3761548) and CRC[17] was established.

5. Conclusion

From our analysis, we conclude that the Foxp3 promoter polymorphisms (rs3761548, rs5902434) were associated with the susceptibility for EC. The A allele, AC genotype (rs3761548), and ATT/ATT genotype (rs5902434) were associated with a lower risk of EC in Chinese Han women, while the AA genotype of rs3761548 was more frequent in patients with EC with cervical invasion. Larger sample size of patients and other ethnic groups should be considered to confirm our findings. Additionally, the molecular mechanism of Foxp3 in endometrial tumorigenesis is a crucial and need to be investigated further.

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