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Original Article

Correlation between 1082G/A gene polymorphism of interleukin 10 promoter and cervical carcinoma

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Abstract: Cervical carcinoma has now become the second popular malignant tumors in females. In studies of interleukin-10 (IL-10) gene polymorphism, its incidence was significantly elevated in cervical cancer women. This study thus recruited cervical cancer patients, whose gene polymorphism of 1082G/A of IL-10 promoter was analyzed to elucidate its correlation with occurrence and progression of cervical cancer. Cervical cancer patients along with cervical intraepithelial neoplasia and chronic cervicitis patients were recruited. PCR primers based on loci 1082 of IL-10 gene promoter were introduced to genotype patients and allele frequency, to elucidate their correlation with HPV infection and severity of cervical cancer. Three genotypes (GG, AA and GA) were detected at loci 1082 of IL-10 gene promoter. In cervical cancer group, genotype AA and allele A had significantly higher frequency than cervical intraepithelial neoplasia and chronic cervicitis groups ($p<0.05$). HPV16 (+) cervical cancer patients had significantly higher frequency of AA genotype and allele A than HPV16 (-) patients ($P<0.05$). Those patients with late TNM stage, lower differentiation grade, vascular infiltration or lymph node metastasis had higher AA genotype ($P<0.05$). Multifactorial analysis revealed the correlation between -1082 loci and TNM stage, differentiation grade, vascular infiltration and lymph node metastasis. -1082G/A gene polymorphism of IL-10 gene promoter was closely correlated with occurrence of cervical cancer, and may work as one novel marker for diagnosis and prognostic prediction of cervical cancer.

Keywords: Interleukin-10, 1082G/A gene polymorphism, cervical cancer

Introduction

The incidence of cervical carcinoma is increasing rapidly, and has become the second popular malignant tumor in women. A survey showed that about 500,000 people were newly diagnosed with cervical cancer each year all over the world. More than 250,000 people died from cervical cancer, consisting about 83% in developing countries, thus severely affecting women's health and life quality. Various risk factors including smoking, birth pills, multiple sex partners, multi-birth and human papilloma virus (HPV) infection are involved in the occurrence of cervical cancer. In addition, the body's immune function has drawn lots of research interests as the immune dysfunction is closely related with tumor progression, development and prognosis [1-3]. Interleukin-10 (IL-10) is one important anti-inflammatory factor, and is one critical member of cytokine superfamily. It participates in various aspects including anti-infection, auto-immune and tumor immune therapy, as it can facilitate tumor growth and is one critical factor for anti-tumor therapy [4]. Certain polymorphism exists in the promoter of IL-10 gene promoter, and is closely related with IL-10 production. There are three single nucleotide polymorphism (SNP) loci within the promoter region, namely, -1082G/A, -819C/T and -592C/A, whose distribution and genotype determine serum IL-10 level [5]. Previous study found the correlation between allele G at loci 1082 of IL-10 gene promoter and the lower susceptibility of malignant tumors, possibly via down-regulating IL-1β, TNF-α and VEGF to inhibit tumor activity [6, 7]. This study recruited cervical cancer patients in our hospital for testing gene polymorphism of 1082G/A loci of IL-10 promoter, in order to analyze its correlation with occurrence and progression of cervical cancer.

Materials and methods

General information

A total of 100 infiltrated cervical carcinoma patients (aging between 30 and 70 years old,

Table 1. Primers in PCR

Target gene	Primer sequence (5'-3')	Fragment length
-1082G/A	G: CTACTAAGGCTTCTTTGGGAG	258 bp
	A: ACTACTAAGGCTTCTTTGGGAA	
	R: CAGTGCCAACTGAGAATTTGG-	
β-globin	F: GTGCTCGGTGCCTTTAGT	319 bp
	R: ACACCAGCCACCACTTTC	

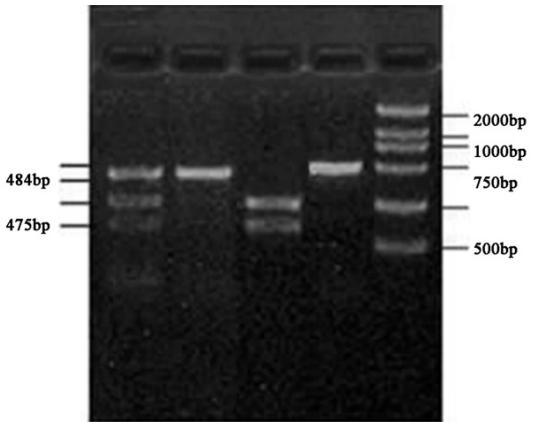

Figure 1. Agarose gel electrophoresis of Rsa I digested PCR products at loci 1082. From left to right, lane 1, GA; lane 2, GG; lane 3, AA; lane 4, PCR products; lane 5, 2000 bp DNA marker.

average age = 52.5±4.8 years) from January 2014 to January 2015 were recruited in Qingdao HaiCi hospital, including 21 stage I, 27 stage II, 28 stage III and 24 stage IV cases. In pathological grading, there were 30 G1 cases, 32 G2 patients and 38 G3 patients. Based on histology examination, there were 61 squamous cell carcinoma and 39 patients with adenoma. There were 36 patients with vascular infiltration, 40 patients with pelvic lymph node metastasis, 77 cases with HPV16 (+) and 20 cases with HPV18 (+). Another 100 patients with cervical intraepithelial neoplasia (aging between 35 and 65 years old, average age = 51.8±4.0 years) and 100 cases with chronic cervicitis (aging between 35 and 70 years old, average age = 52.6±4.2 years) were recruited in this study as two control groups. No significant difference regarding sex, age or body weight has been discovered among all groups, which were thus comparable. This study has been pre-approved by the ethical committee of our hospital and has obtained written consents from all participants.

Inclusive criteria

All patients have been confirmed by pathological examination, and have no connective tissue disease or immune disorders. No chemo-/radio-/bio-therapy has been performed before the surgery.

Sample collection

4 mL venous blood samples were collected from all research objects and aliquoted for EDTA-treatment and serum extraction by 3,000 rpm centrifugation for 10 min. Serum was kept at -20°C for further use.

Genomic DNA extraction

Total blood genomic DNA extraction kit was used to extract DNA from anti-coagulant blood. DNA concentration was detected and its purity was examined by agarose gel electrophoresis.

PCR amplification

PCR amplification was carried out in a 50 μL system containing 1 μL forward and reverse primers, 2 μL DNA template, 21 μL distilled water, and 25 μL 2X Taq PCR Master Mix. PCR conditions were: 94°C pre-denature for 3 min, followed by 30 sec and 30 cycles each containing 48°C for 90 sec and 72°C for 45 sec, and ended with 72°C elongation for 10 min. Agarose gel electrophoresis was used to detect amplified gene fragments. Primer sequences were shown in **Table 1**.

Endonuclease digestion

Rsa I enzyme reaction system contained 10 μL PCR products, 16 μL DD water, 2 μL 10Xbuffer, 1 μL Rsa I enzyme, and was carried out at 37°C for 2 h. Tsa I enzyme reaction system contained 10 μL PCR products, 16 μL DD water, 2 μL 10Xbuffer, 1 μL Tas I enzyme, and was carried out at 65°C for 3 h.

Genotype and allele frequency determination

We compared the genotype and allele frequency of all patients among cervical cancer, cervical intraepithelial neoplasia and chronic cervicitis groups.

Statistical analysis

SPSS17.0 software package was used to analyze all collected data, which were presented as

Table 2. Genotype and allele frequency at -1082 of IL-10 gene

Group	N	IL-10 -1082G/A				
		Genotype frequency			Allele frequency	
		GG	GA	AA	G	A
Cervical cancer	100	1 (1)*,#	11 (11)*,#	88 (88)*,#	14 (7)*,#	186 (93)*,#
Cervical intraepithelial neoplasia	100	3 (3)#	17 (17)#	80 (80)#	23 (11.5)#	177 (88.5)#
Control (cervicitis)	100	10 (10)	25 (25)	65 (65)	45 (22.5)	155 (77.5)

Note: *, p<0.05 compared to cervical intraepithelial neoplasia group; #, p<0.05 compared to control group.

Table 3. Genotype/allele frequency of IL-10 gene and HPV infection of cervical cancer patients

Group	N	Genotype (%)			Allele (%)	
		GG	GA	AA	G	A
HPV16 (+)	77	0	6 (7.8)	72 (93.5)	6 (3.9)	150 (97.4)
HPV16 (-)	23	1 (4.3)	5 (21.7)	16 (69.6)	7 (15.2)	13 (28.3)
HPV18 (+)	20	0	4 (20)	16 (80)	4 (10)	36 (90)
HPV18 (-)	80	1 (1.25)	7 (8.8)	72 (90)	9 (5.6)	151 (94.4)

Note: *, P<0.05 compared to HPV (-) patients.

mean ± standard deviation (SD). Measurement data were analyzed by student t-test, while enumeration data were analyzed by chi-square test. Logistic regression model was employed in multi-factorial analysis. A statistical significance was defined when P<0.05.

Results

Hardy-Weinberg equilibrium

Using chi-square test, we analyzed the -1082G/A allele frequency of IL-10 promoter and found that all groups fitted Hardy-Weinberg equilibrium (P>0.05), suggesting the representativeness all samples.

Genotyping loci 1082 of IL-10 gene promoter

After Rsa I enzyme digestion, three different genotypes, namely, GG, AA and GA, were found at loci 1082 of IL-10 gene promoter, as shown in **Figure 1**.

Genotype and allele frequency at -1082 loci of Il-10 gene

We further compared both genotype and allele frequencies at -1082 loci of IL-10 gene. The frequency of GG in cervical cancer, cervical intraepithelial neoplasia and chronic cervicitis groups were 1%, 3% and 10%, respectively. Whilst such frequencies were 11%, 17% and 25% for GA, and were 88%, 80% and 65% for AA. The frequency of AA was significantly higher in cervical cancer group compared to the other two groups (P<0.05). Regarding allele frequency, we found the frequency of G in cervical cancer; cervical intraepithelial neoplasia and chronic cervicitis groups were 7%, 11.5% and 22.5%, respectively. While A frequencies were 93%, 88.5% and 77.5%, respectively. The allele frequency of A in cervical cancer groups was significantly higher than the other two groups (P<0.05), as shown in **Table 2**.

Correlation between genotype/allele frequency and HPV

The analysis of genotype and allele frequency at -1082 loci of IL-10 gene in cervical cancer patients, along with HPV16 and HPV18 infection conditions found that 93.5% of HPV16 (+) cervical cancer patients had AA genotype, with allele A frequency at 97.4%, which was significantly higher than HPV16 (-) patients (P<0.05). In HPV18 (+) patients, AA genotype and A allele had frequencies at 80% and 90%, respectively, both of which were, however, no significantly different from HPV18 (-) ones. See **Table 3** for details.

Genotype at -1082 loci of IL-10 gene and severity of cervical cancer

We further described genotype at loci 1082 of IL-10 gene in all categories of cervical cancer patients based on TNM stage, differentiation grade, vascular infiltration and lymph node metastasis. Results showed the correlation between AA/GA/AA genotypes and all those parameters of cervical cancer. AA genotype frequency was found to be significantly elevated in

Table 4. One-factorial analysis of -1082 genotype of IL-10 gen and severity of cervical cancer

Item	N	GG	GA	AA
TNM stage				
I	21	0	0	17 (80.9)
II	27	0	1 (3.7)	23 (85.2)
III	28	0	3 (10.7)	25 (85.7)
IV	24	1 (4.2)	8 (33.3)	23 (95.8)
P value		P<0.05	P<0.05	P<0.05
Differentiation grade				
Moderate to high	43	0	1 (2.3)	37 (86.0)
Low	57	1 (1.8)	10 (17.5)	51 (89.5)
P value		P<0.05	P<0.05	P<0.05
Vascular infiltration				
Yes	36	1 (2.8)	9 (25)	34 (94.4)
No	64	0	2 (3.1)	54 (84.4)
P value		P<0.05	P<0.05	P<0.05
Lymph node metastasis				
Yes	40	1 (2.5)	8 (20)	38 (95)
No	60	0	3 (5)	50 (83.3)
P value		P<0.05	P<0.05	P<0.05

Table 5. Multi-factorial analysis of -1082 loci genotype of IL-10 gene and severity of cervical cancer

Observing index	Regression coefficient	*P* value	Relative risk
TNM stage	1.275	0.001	2.852
Differentiation grade	1.288	0.018	3.627
Vascular infiltration	1.797	0.001	6.031
Lymph node metastasis	1.004	0.001	2.730

those tumors with late TNM stage, lower differentiation grade, with vascular infiltration or lymph node metastasis (**Table 4**).

Multi-factorial analysis of -1082 genotype and severity of cervical cancer

We further performed Logstic multiple factorial analysis including TNM stage, differentiation grade, vascular infiltration and lymph node metastasis of cervical cancer. Results showed the correlation of -1082 loci genotype of IL-10 gene and all those abovementioned factors as shown in **Table 5**.

Discussion

Cervical cancer is one major malignant tumor that affects women health, and is mainly caused by the continuous infection of HPV. Current treatment strategies focus on the modulation of tumor cell growth and immune modulation on related cytokines [8]. The polymorphism of cytokine gene is closely related with its expression level and reactive strength, thus affecting tumor occurrence, progression and prognosis [9]. IL-10 is one cytokine that can be produced by various cells and is cleared by kidneys. Under physiological conditions, it can be secreted by mononuclear cells, macrophage, T cells, B cells, hyperplasia cells, eosinophilic cells and keratinocytes [10, 11]. Under normal condition, body level of IL-10 maintains at minimal. Only under certain circumstances such as malignant tumors can cells secrete IL-10 at high levels [12]. IL-10 exerts it biological functions mainly via inhibiting Th1 cell-induced immune response or antagonize cytokines such as IFN-γ, thus inhibiting body's antiviral immune reaction [13]. Current findings have shown three important SNP sites, namely, 1082G/A, 819T/C and 592C/A, all of which have important effects on IL-10 secretion [14].

In this study, we selected cervical cancer patients in our hospital as the experimental group, in parallel with cervical intraepithelial neoplasia and cervicitis patients as controls, and found three genotypes (GG, AA and GA) at loci 1082 of IL-10 gene promoter. Further comparison of genotype and allele frequency at such loci found significantly elevated AA genotype and A allele in cervical cancer patients compared to the other groups, suggesting higher risk of cervical cancer in individuals carrying allele A.

High-risk carcinogenic HPV is one important risk factor of cervical cancer after persistent infection, as it can induce potent innate and cellular immune response of the host body [15]. This study performed an analysis between genotype/allele frequency at -1082 loci of IL-10 gene and infection of HPV16 or HPV18 in cervical cancer patients, and found higher AA genotype and A allele in HPV16 (+) cervical cancer patients compared to HPV16 (-) patients, but not in HPV18 cases. Such results suggested that AA genotype and A allele could increase risk of HPV16 infection. Study has shown the critical action of HPV infection in occurrence

and progression of cervical carcinoma. IL-10 has potent inhibitory function against cell immunity. With increased secretion of IL-10, body immune system may be compromised, making it susceptible for HPV and further cervical cancer [16]. Persistent HPV infection could eventually interfere with body's normal immune system to cause cervical cancer. Thus IL-10 could inhibit cervical cancer via eliminating HPV [17].

This study further analyzed the correlation between genotype at -1082 loci of Il-10 gene and severity of cervical cancer and found the correlation between genotypes and TNM stage, differentiation grade, vascular infiltration and lymph node metastasis. In those tumors with advanced TNM stage, lower differentiation grade, vascular infiltration and lymph node metastasis the frequency of AA genotype was relatively higher than GA and GG. Further Logistic multi-factorial analysis also indicated the correlation between IL-10 gene SNP and all those abovementioned indexes governing cancer severity, further indicating the correlation between AA genotype and cervical cancer. Stanczuk et al reported that GA genotype at loci 1082 of IL-10 gene in cervical cancer patients led to large amounts of IL-10 production compared to healthy individuals [18]. Victor et al further pointed the close correlation between IL-10 expression level and malignancy of cervical cancer and HPV infection, probably due to the IL-10-induced immune suppression at the micro-environment of tumors during the advancement of cervical cancer [19]. Farzaneh et al further described both genotype frequency and distribution at loci 1082 of IL-10 gene promoter in cervical cancer patients and found significantly negative correlation between IL-10 G allele with continuous HPV infection [20].

In summary, -1082G/A gene polymorphism of IL-10 gene promoter is closely correlated with the occurrence and progression of cervical cancer. With elevation of AA genotype and A allele frequency, the risk of HPV16 infection is further elevated, thus increasing the susceptibility of cervical cancer in populations. With advancement of TNM stage, lower differentiation grade, occurrence of vascular infiltration or lymph node metastasis, AA genotype and A allele frequencies were elevated. This 1082G/A gene polymorphism of IL-10 gene promoter thus may work as one novel marker for diagnosis and prognostic prediction of cervical cancer, and is worth further study.

Disclosure of conflict of interest

None.

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References

[1] Herszényi L, Hritz I, Lakatos G, Varga MZ, Tulassay Z. The behavior of matrix metalloproteinases and their inhibitors in colorectal cancer. Int J Mol Sci 2012; 13: 13240-63.

[2] Nogueira-Rodrigues A, Melo AC, Werutsky G, Garces AH, Ferreira CG. Exploiting HPV-induced carcinogenesis for a rational drug development in cervical cancer. Curr Cancer Drug Targets 2016; 16: 249-60.

[3] Ma C, Zeng C, Jin L, Yang Y, Li P, Chen L, Wang J. GSK3beta mediates the carcinogenic effect of HPV16 in cervical cancer. Sci Rep 2015; 5: 16555.

[4] Zhang Z, Doel C and Bashiruddin JB. Interleukin-10 production at the early stage of infection with foot-and-mouth disease virus related to the likelihood of persistent infection in cattle. Vet Res 2015; 46: 132.

[5] Singh S, Sharma A and Arora SK. Combination of low producer AA-genotypes in IFN-gamma and IL-10 genes makes a high risk genetic variant for HIV disease progression. Cytokine 2016; 77: 135-44.

[6] Seifart C, Plagens A, Dempfle A, Clostermann U, Vogelmeier C, von Wichert P, Seifart U. TNF-alpha, TNF-beta, IL-6, and IL-10 polymorphisms in patients with lung cancer. Dis Markers 2005; 21: 157-65.

[7] Luo Y, Han R, Evanoff DP, Chen X. Interleukin-10 inhibits Mycobacterium bovis bacillus Calmette-Guerin (BCG)-induced macrophage cytotoxicity against bladder cancer cells. Clin Exp Immunol 2010; 160: 359-68.

[8] Lee JH, Piao MS, Choi JY, Yun SJ, Lee JB, Lee SC. Up-regulation of cyclooxygenase 2 and matrix metalloproteinases-2 and -9 in cutaneous squamous cell carcinoma: active role of inflammation and tissue remodeling in carcinogenesis. Ann Dermatol 2013; 25: 145-51.

[9] Zhou Y, Li N, Zhuang W, Liu GJ, Wu TX, Yao X, Du L, Wei ML, Wu XT. Interleukin-10 -1082 pro-

moter polymorphism associated with gastric cancer among Asians. Eur J Cancer 2008; 44: 2648-54.
[10] Zhou Y, Hu W, Zhuang W, Wu X. Interleukin-10 -1082 promoter polymorphism and gastric cancer risk in a Chinese Han population. Mol Cell Biochem 2011; 347: 89-93.
[11] Liu J, Song B, Wang JL, Li ZJ, Li WH, Wang ZH. Polymorphisms of interleukin-10 promoter are not associated with prognosis of advanced gastric cancer. World J Gastroenterol 2011; 17: 1362-7.
[12] Zeng X, Li Y, Liu T, Zhang J. Diverse H. pylori strains, IL-10 promoter polymorphisms with high morbidity of gastric cancer in Hexi area of Gansu Province, China. Mol Cell Biochem 2012; 362: 241-8.
[13] He B, Pan Y, Xu Y, Nie Z, Chen L, Gu L, Wang S. Increased risk for gastric cancer in carriers of the lymphotoxin-alpha+252G variant infected by Helicobacter pylori. Genet Test Mol Biomarkers 2012; 16: 9-14.
[14] Ni P, Xu H, Xue H, Lin B, Lu Y. A meta-analysis of interleukin-10-1082 promoter polymorphism associated with gastric cancer risk. DNA Cell Biol 2012; 31: 582-91.
[15] Bhairavabhotla RK, Verm V, Tongaonkar H, Shastri S, Dinshaw K, Chiplunkar S. Role of IL-10 in immune suppression in cervical cancer. Indian J Biochem Biophys 2007; 44: 350-6.
[16] Opdal SH. IL-10 gene polymorphisms in infectious disease and SIDS. FEMS Immunol Med Microbiol 2004; 42: 48-52.
[17] da Silva GN, Bacchi MM, Rainho CA, de Oliveira DE. Epstein-Barr virus infection and single nucleotide polymorphisms in the promoter region of interleukin 10 gene in patients with Hodgkin lymphoma. Arch Pathol Lab Med 2007; 131: 1691-6.
[18] Stanczuk GA, Sibanda EN, Perrey C, Chirara M, Pravica V, Hutchinson IV, Tswana SA. Cancer of the uterine cervix may be significantly associated with a gene polymorphism coding for increased IL-10 production. Int J Cancer 2001; 94: 792-4.
[19] Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol 2001; 166: 3915-22.
[20] Farzaneh F, Roberts S, Mandal D, Ollier B, Winters U, Kitchener HC, Brabin L. The IL-10 -1082G polymorphism is associated with clearance of HPV infection. BJOG 2006; 113: 961-4.