Original Paper

Polymorphisms of Receptor for Advanced Glycation end Products and Risk of Epithelial Ovarian Cancer in Chinese Patients

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Key Words

Receptor for advanced glycation end products • Epithelial ovarian carcinoma • Polymorphisms • Risk

Abstract

Background: Given the roles of receptor for advanced glycation end products (RAGE) in the pathogenesis of carcinogenesis, we propose that RAGE polymorphisms may be associated with risk of epithelial ovarian carcinoma (EOC). **Method:** This case-control study included 190 women over 40 years of age who were diagnosed with primary EOC and 210 healthy control subjects. RAGE gene polymorphisms, including 82G>S, -374T>A, -429C>T, and 1704G>T were determined. **Results:** We found that only the frequencies of the 82G>S polymorphisms were significantly different between the EOC cases and controls. The 82SS genotype was significantly higher in EOC patients than in controls (37.89% vs. 23.33%, $P < 0.001$). With the 82 GG genotype as reference, the OR for 82SS homozygous carriers reached to 2.65 (95% CI: 1.54-4.58; $P = 0.0004$) after adjustment for age, smoking status, body mass index, family history, usage of contraceptives, tubal ligation history, use of menopausal hormones and menopausal status. The 82S allele carriage presented a higher risk for EOC (OR=1.71; 95% CI, 1.29-2.26; $P = 0.0002$). The polymorphisms of 1704G>T, -374T>A and -429C>T did not affect the EOC risk. **Conclusion:** This result suggests that the 82G>S polymorphism of RAGE gene may be associated with the susceptibility of EOC.

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Introduction

Epithelial ovarian cancer (EOC) is the most common type of ovarian cancer in human [1]. To date, the pathogenesis of EOC is still not fully understood. The role of genetic background in the etiology and pathogenesis of EOC has been documented [2, 3]. Given that most cases present with advanced, incurable disease, high-risk women should be targeted for primary prevention and early detection. Several genetic polymorphisms have been reported to be involved in the development of EOC [4-6]. However, the documented genetic variants associated with EOC are limited, and more studies need be performed to elucidate the genetic mechanisms underlying EOC.

The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily of cell surface molecules and a receptor for advanced glycation end products (AGEs) [7, 8]. AGE-RAGE interaction alters several cell functions through modulation of multiple intracellular signaling pathways [9]. Accumulating evidence revealed that RAGE plays an important role in the pathogenesis and progression of cancer by promoting cancer cell migration, differentiation and invasion [10-12]. Clinical studies suggests that RAGE level may be used as a new biomarker for some type of cancers including lung cancer [13], breast cancer [14], prostate cancer [15], colorectal cancer [16].

The RAGE gene is found on chromosome 6p21.3 in the major histocompatibility locus (MHC) locus class II/III junction and is composed of a 1.7-kb 5' flanking region and 11 exons. To date, several genetic variants have been identified in RAGE gene, including 82 G>S (rs2070600), -429C>T (rs1800625), -374 T>A (rs1800624) and 1704G>T (rs184003). Previous studies documented positive associations between the genetic variants of RAGE and a variety of cancers, including gastric cancer [17], pancreas cancer [18] and breast cancer [14], however, no study regarding the role of RAGE genetic variants in EOC was reported. Given the roles of RAGE in the pathogenesis of carcinogenesis, we proposed that the RAGE genetic polymorphisms might be related to the risk of EOC. In present study, we performed a case-control study in a Chinese cohort to test the hypothesis.

Materials and Methods

Study population

This case-control study included 190 women over 40 years old who were diagnosed with primary histologically-confirmed EOC between Aug 2002 and Aug 2008 in our hospital. A total of 210 age-matched healthy control subjects were recruited as control. Socio-demographic, lifestyle, and health-related information, e.g. age, sex, body mass index, smoking status, cancer family history, use of oral contraceptives, menopausal status, had or had not tubal ligation, use of menopausal hormones were collected by using a structured questionnaire. The Institutional Review Board of the Shandong University approved the study protocol. All the participants provided written informed consent.

RAGE genotyping

Venous blood was collected from each patient into tubes containing 50 mmol of EDTA per liter, and genomic DNA was isolated with DNA blood Mini kit, according to manufacturer's instructions. A Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assay was used to determine the RAGE polymorphisms. The detection of RAGE genetic polymorphisms of 82G>S, -374 T>A, -429 C>T, and 1704G>T were performed as described previously [19, 20].

Statistical analysis

χ^2 tests were used to compare genotype frequency and demographic distributions between cases and controls. Multiple logistic regression analyses were used to evaluate if each polymorphism was independently associated with EOC risk with adjustment of the potential confounding effects of clinical variables. The odds ratios (OR) and 95% confidence intervals (CIs) were calculated. All analyses were performed by using SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc,

	EOC	Control	
Age(y)	53.55±3.8	53.45±4.6	0.582
body mass index (kg/m ²)			
Underweight(BMI<18.5)	25	31	0.719
Normal(BMI:18.5-25)	44	54	
Overweight(BMI:25-30)	67	63	
Obesity(BMI>30)	54	62	
Smoking			
Never Smoker	34	57	0.081
Past smoker	78	80	
Current smoker	78	73	
Cancer family history			
Yes	56	30	0.001
No	134	180	
Use of oral contraceptives			
Yes	88	77	0.032
No	102	133	
Had tubal ligation			
Yes	61	67	0.525
No	129	143	
Menopausal status			
Premenopausal	50	57	0.471
Postmenopausal	140	153	
Use of menopausal hormones			
Never used	101	121	0.302
Estrogen only	45	35	
Progesterone only	34	45	
Estrogen+Progesterone	10	9	

Table 1. The clinico-pathologic characteristics of the cohort

Chicago, IL, USA). The D' value for the genetic polymorphisms of 82G>S, -374 >A, -429C>T, and 1704G>T were calculated with the SHEsis software [21].

Results

The clinical characteristics of all participants are summarized in Table 1. The EOC and healthy control groups were similar in age, body mass index, smoking status, tubal ligation, menopausal status and use of menopausal hormones. The EOC patients had a higher rate of cancer family history and use of oral contraceptives (both $P<0.05$).

The genotype distributions of the genetic polymorphisms of 82G>S, -374 >A, -429C>T, and 1704G>T were in agreement with Hardy-Weinberg equilibrium for both cases and controls (both $P>0.05$). The 82SS genotype was significantly higher in EOC patients than in controls (37.89% vs. 23.33%, $P<0.001$). Furthermore, the 82S allele frequency was significantly higher in the case group than in the control group (59.47% vs. 46.19%, $P<0.001$). In contrast, no significant differences in the genotype frequencies of 1704G>T, -374 T>A and -429C>T were noted between EOC and control groups (all $P>0.05$). We further performed the multivariate logistic regression to determine the independent risk factors for EOC. With the 82 GG genotype as reference, the adjusted OR for 82SS homozygous carriers

Genotype	N	%	N	%	P trend	adjusted OR	95%CI	X ²	adjusted P	Power
82GG	36	18.95%	65	30.95%	<0.001	1				
82GS	82	43.16%	96	45.71%		1.54	0.93 2.55	2.87	0.0903	0.39
82SS	72	37.89%	49	23.33%		2.65	1.54 4.58	12.55	0.0004	0.94
82G	154	40.53%	226	53.81%	<0.001	1				
82S	226	59.47%	194	46.19%		1.71	1.29 2.26	14.12	0.0002	0.96

Table 2. Data for genotype distribution and allele frequencies of 82G>S between EOC subjects and controls

1704G>T	-374T>A	82G>S	-429C>T	Case(freq)	Control(freq)	P	Odds Ratio [95%CI]
G	A	S	C	54	15	0.00126	2.569 [1.399~4.286]
G	T	S	C	33	37	0.543	0.943 [0.707~1.698]
T	A	S	T	44	31	0.0214	1.569 [1.0131~2.986]
G	T	G	T	35	38	0.117	0.893 [0.717~1.924]
T	T	G	T	32	55	0.0017	0.523 [0.217~0.924]

Table 3. The estimated haplotype frequencies of the RAGE genetic polymorphisms in EOC patients and controls

reached to 2.65 (95% CI: 1.54-4.58; P =0.0004, power value 0.94) after adjustment for age, smoking status, BMI, family history, usage of contraceptives, tubal ligation history, use of menopausal hormones and menopausal status, while the adjusted OR for the 82GS genotype was 1.54 (P=0.0903). The 82S allele carriage also presented a higher risk for EOC (adjusted OR=1.71; 95% CI, 1.29-2.26; adjusted P=0.0002, power value 0.96). The polymorphisms of 1704G>T, -374T>A and -429C>T did not affect the EOC risk in logistic regression (data not shown).

The associations between the RAGE haplotypes and the risk for EOC were analyzed in this study. The D' values for the studied genetic polymorphisms were calculated with the SHEsis software. All the studied RAGE genetic polymorphisms were in strong LD (all D'>0.8). The estimated haplotype frequencies of the RAGE SNPs in the patients with EOC and controls are shown in Table 3. The haplotype of G₁₇₀₄-A₋₃₇₄-S₈₂-C₋₄₂₉ showed a significantly higher risk for EOC (OR=2.569, 95% CI: 1.399~4.286, P=0.00126). The haplotype of T₁₇₀₄-A₋₃₇₄-S₈₂-T₋₄₂₉ represented a higher risk for EOC as well (OR=1.569, 95% CI: 1.0131~2.986, P=0.0214). Meanwhile, the T₁₇₀₄-T₋₃₇₄-G₈₂-T₋₄₂₉ had a lower risk for EOC (OR=0.523, 95% CI: 0.217~0.924, p =0.0117).

Discussion

In the present study, we found that 82G>S variants were significantly associated with the risk of EOC in a Chinese cohort. The 82SS genotype carriers presented a 2.65 times higher risk for EOC than 82GG genotype carriers. These results suggest that the 82G>S polymorphisms of RAGE gene may be used as genetic marker for EOC occurrence. In addition, we also found that the haplotype G1704-A-374-S82-C-429 and T1704-A-374-S82-T-429 represented higher risk for EOC.

RAGE is encoded by chromosome 6 at the major MHC class II/III junction. To date, more than 20 several single nucleotide polymorphisms in the RAGE gene have been identified [22]. Some genetic variants in the RAGE gene could alter the expression and function of RAGE, thus affect disease development [22]. One of the most frequently studied and relatively high prevalence variants is the 82G>S r (Gly82Se) polymorphism. It is at codon 82 (GGC→AGC) in exon 3 of RAGE and leads to a change from glycine to serine within the putative ligand-

binding domain of the protein and it has been proposed as a functional polymorphism and associated with enhanced RAGE signaling [23]. Recent observations indicated that the 82G>S polymorphism was associated with various diseases, including skin complications in type 2 diabetes, diabetic advanced nephropathy and coronary artery disease [23-26].

Several previous studies explored the role of 82G>S polymorphism of *RAGE* gene in carcinogenesis in Chinese population. The polymorphism of G>S was reported to be associated with the increased risk of gastric cancer and a significant correlation of the *RAGE* variant genotypes with adjacent organ invasion [17]. In that study, the genotype frequency was 50.18% for GG, 44.52% for GS and 5.3% for SS. In another study in patients with non-small cell lung cancer, the 82SS genotype not only increased the lung cancer risk, but also related to a lower chemotherapy response rate and poor prognosis. The GG frequency in lung cancer patients were 14.95% for GG, 64.06% for GS and 21.00 for SS [27]. A recent published study showed that 82SS is associated with the cervical cancer in Chinese patients. The cervical cancer patients had a markedly higher percentage of 82SS carriage than controls. The 82SS genotype was associated with elevated risk for cervical cancer. In addition, the 82SS carriers had significantly lower serum soluble RAGE levels than 82GS and 82GG [28]. In the present study, we observed the 82G>S genotype frequencies were: GG: 18.95%, GS: 43.16%, SS: 37.89%. The 82G>S genotype distribution in our study was consistent with the genotype frequencies from cervical cancer [28]. Consistent with the results from Chinese cervical cancer patients, our study also suggests that carriage of 82SS genotype of 82G>S polymorphism predicts a significantly higher risk for EOC incidence.

It was reported that soluble RAGE concentration was significantly higher in subjects with the 82GG genotype ($1,038 \pm 33$ pg/mL) than in those with the 82GS (809 ± 19 pg/mL) or the 82SS (428 ± 43 pg/mL) genotype [29]. The 82G>S variant also was significantly influence the serum inflammatory marker, such as C reaction protein level in the Chinese Han population with coronary heart disease [30]. Factors contributing to chronic inflammation appear to be associated with increased risk of ovarian cancer [31]. A recent study assessed the association between circulating levels of inflammation mediators and subsequent risk of ovarian cancer. The authors found that inflammation markers, specifically interleukins, were associated with higher risk for EOC [32]. This study adds new evidence that inflammation is involved in the development of EOC. Based on the above-mentioned study, we postulate that the 82G>S genetic polymorphism may determine the EOC risk by influencing the inflammation status of carriers. Our future study will focus on the expression of inflammation mediators at serum and tissue level to better elucidate the association between the RAGE 82G>S polymorphism and EOC risk.

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