Biomedical Research 2017; 28 (16): 7131-7136 *ISSN 0970-938X*
www.biomedres.info

Association between IL-8 genetic polymorphisms and the risk of ovarian cancer in a Chinese population.

Yuchong Hu[1,2], Jingkun Lu[3], Aiming Wang[1,4*]

[1]Southern Medical University, Guangzhou, PR China

[2]Department of Obstetrics and Gynaecology, Inner Mongolia Autonomous Region People's Hospital, Hohhot, PR China

[3]School of Basic Medical Sciences, Inner Mongolia Medical College, Hohhot, PR China

[4]The PLA Navy General Hospital, Beijing, PR China

Abstract

Objective: To perform a 1:1 matched case-control study to evaluate the role of IL-8 rs4073, rs1126647 and rs2227306 polymorphisms in the development of ovarian cancer in a population of China.
Methods: A total of 280 patients with ovarian cancer and 280 healthy controls were recruited. Genotyping of IL-8 rs4073, rs1126647 and rs2227306 were run in a 384-well plate format on the sequenom MassARRAY platform.
Results: Patients with ovarian cancer were more likely to have higher BMI (OR=1.12, 95% CI=1.06-1.17), a long term use of hormone replacement therapy (OR=3.58, 95% CI=1.28-10.01) and a habit of alcohol drinking (OR=1.47, 95% CI=1.01-2.14). Moreover, those carrying the AT (OR=1.50, 95% CI=1.05-2.16) and TT (OR=2.26, 95% CI=1.18-4.35) genotypes were associated with a higher risk of ovarian cancer when compared with those with the AA genotype. The AT+TT genotype was correlated with higher risk of ovarian cancer in comparison to the AA genotype (OR=1.58, 95% CI=1.12-2.24). A linkage disequilibrium was found between rs4073 and rs1126647 (D'=0.572, r2=0.12). A total of four common haplotypes (frequency>0.03) were selected into analysis, and the A-C-T haplotype showed a reduced risk of ovarian cancer, with the OR (95% CI) of 0.74 (0.56-0.99).
Conclusions: Our results support direct association of IL-8 rs4073 polymorphism and A-C-T haplotype with the risk of ovarian cancer, suggesting that the IL-8 may be a new biomarker for the susceptibility to ovarian cancer.

Keywords: Ovarian cancer, IL-8, Polymorphism, Haplotypes.

Accepted on July 20, 2017

Introduction

Ovarian cancer is common malignant tumor with high mortality in females worldwide, and it is a serious threat to women's health [1,2]. The high mortality of this cancer is mainly due to the majority of the patients present with advanced stages at the time of diagnosis [3,4]. The etiology of ovarian cancer has been widely investigated and reported, but its pathogenesis is not fully clear. Many environment, dietary and lifestyle factors play an important role in the development of ovarian cancer, such as the choice not to or inability to bear children, higher body mass index and receiving long-term of estrogen replacement therapy and ovulation inducing drugs [3].

However, the incidence of ovarian cancer varies greatly across different populations even when they are exposure to the same environment, dietary and lifestyle factors, indicating that genetic factors play an important role in the induction of ovarian cancer.

Interleukin (IL-8) is a pro-inflammatory chemokine known for its angiogenetic activity, which is involved in regulating both inflammatory and immune processes [5-7]. IL-8 is also responsible for tumor-associated angiogenesis in several malignant tumors [5,8].

Previous studies have shown that IL-8-251A/T (rs4073), +1633C/T (rs1126647) and +781C/T (rs2227306) and genetic polymorphisms were correlated with the susceptibility of malignant tumors. Few study reported the association of IL-8 polymorphism with risk of ovarian cancer in a population of Germany [9].

Therefore, we performed a 1:1 matched case-control study to evaluate the role of IL-8 rs4073, rs1126647 and rs2227306 polymorphisms in the development of ovarian cancer in a Chinese population.

Materials and Methods

Subjects

A total of 280 patients with ovarian cancer were recruited in our study between May 2014 and May 2016, and all the patients were recruited from the Department of Obstetrics and Gynaecology at the Inner Mongolia Autonomous Region People's Hospital and Southern Medical University. All the patients with ovarian cancer were newly diagnosed within one month and confirmed by histopathology. Patients with a history of other malignant tumors, recurrent or metastasis ovarian cancer were excluded from this study.

Simultaneously, a total of 280 healthy controls which were frequency-matched to the cases by age (within 5 y old) were recruited into our study. All the healthy controls were selected from the outpatient clinics and physical examination center of the Inner Mongolia Autonomous Region People's Hospital and Southern Medical University.

The demographic and clinical characteristics were collected from medical records and questionnaires. All the patients and controls were interviewed face-to-face by trained graduate students. Ever smoking included current and former smoking, and it was defined as those who smoked at least 20 cigarettes within one week and lasted for 6 months. The others were defined as never smoking. Ever drinking included current and former drinking and it was defined as those who drank at least 50 ml white wine, 250 ml beer or 250 ml wine within one month and lasted for 6 months. The others were grouped into never drinking. Study subjects agreed to participate into our study and signed a consent form prior to enrolment. Our research was approved by the Research Ethics Committee of the Inner Mongolia Autonomous Region People's Hospital and Southern Medical University.

SNP genotyping

Each patient was asked to provide a 3 mL peripheral venous blood sample. DNA samples were isolated from peripheral venous blood of patients and controls by a TIANamp Blood DNA Kit (Tiangen, Beijing, China) using standard procedures. Primers for Polymerase Chain Reaction (PCR) amplification were designed by Sequenom Assay Design 3.1 software. Genotyping of IL-8 rs4073, rs1126647 and rs2227306 were run in a 384-well plate format on the sequenom MassARRAY platform (Sequenom, San Diego, USA). PCR amplification was carried out with an initial denaturation at 94°C for 2 min; 45 cycles of 95°C for 30 s; 56°C for 30 s and 72°C for 60 s; then a final extension of 72°C for 5 min. The PCR products were desalted, dispensed to a SpectroCHIP and analyzed with MALDI-TOF MS.

Statistics

The differences in the demographic and clinical characteristics and genotype frequencies were compared by Chi-square (χ^2) test and student t-test. The Hardy-Weinberg Equilibrium (HWE) of IL-8 rs4073, rs1126647 and rs2227306 was evaluated by Chi-square with one degree of freedom.

The association of IL-8 rs4073, rs1126647 and rs2227306 with the risk of ovarian cancer was evaluated by binary multivariate logistic regression, and the results were displayed by Odds Ratio (OR) and 95% confident intervals (95% CI). The linkage disequilibrium and haplotype analyses of IL-8 rs4073, rs1126647 and rs2227306 were analyzed using SHEsis software.

The analyses were performed by IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp, Armonk, NY, USA), and all the statistical tests were two-sided and $P<0.05$ was set as a statistical significance.

Results

By χ^2 or t-tests, we observed significant differences between patients with ovarian cancer and controls in terms of age at diagnosis (t=0.46, P=0.02), use of hormone replacement therapy (χ^2=7.66, P=0.01) and BMI (t=0.46, P=0.02) (Table 1).

Table 1. *Demographic and clinical data of included patients with ovarian cancer and controls.*

Category	Patients N=280	%	Controls N=280	%	χ^2 or t tests	P value
Age at diagnosis, years	51.60 ± 7.09		51.34 ± 6.24		0.46	0.02
Education						
Less than middle school	55	19.64	58	20.71		
Middle school or above	225	80.36	222	79.29	0.1	0.75
Smoking						
Never	241	86.07	240	85.71		
Ever	39	13.93	39	13.93	0.02	0.9
Drinking						
Never	187	66.79	208	74.29		

Ever	93	33.21	72	25.71	3.79	0.05
Use of hormone replacement therapy						
No	262	93.57	275	98.21		
Yes	18	6.43	5	1.79	7.66	0.01
BMI, kg/m^2	23.87 ± 3.49		22.53 ± 3.54		0.46	0.02
Family history of cancer				0		
No	249	88.93	260	92.86		
Yes	31	11.07	20	7.14	2.61	0.11
BMI: Body Mass Index.						

The AA, AT and TT genotype distributions of IL-8 rs4073 revealed significant difference between patients with ovarian cancer and controls (χ^2=8.35, P=0.02) (Table 2). However, the genotype distribution of IL-8 rs1126647 and rs2227306 did not show significant differences between the two study groups. Moreover, the genotype frequencies of IL-8 rs4073, rs1126647 and rs2227306 confirms to the HWE in controls.

Table 2. *Distributions of IL-8 rs4073, rs1126647 and rs2227306 between the two study groups.*

IL-8	CAD patients	%	Controls	%	Chi-square	P value	HWE in controls	P value
rs4073								
AA	118	42.14	148	52.86				
AT	130	46.43	114	40.71				
TT	32	11.43	18	6.43	8.35	0.02	0.41	0.52
rs1126647								
CC	244	87.14	240	85.71				
CT	31	11.07	37	13.21				
TT	5	1.79	3	1.07	1.06	0.59	1.29	0.26
rs2227306								
CC	122	43.57	118	42.14				
CT	113	40.36	123	43.93				
TT	45	16.07	39	13.93	0.92	0.63	0.58	0.45

[1]Adjusted for potential confounding factors.

By binary logistic regression analysis, we found that patients with ovarian cancer were more likely to have higher BMI (OR=1.12, 95% CI=1.06-1.17), a long term use of hormone replacement therapy (OR=3.58, 95% CI=1.28-10.01) and a habit of alcohol drinking (OR=1.47, 95% CI=1.01-2.14). Moreover, those carrying the AT (OR=1.50, 95% CI=1.05-2.16) and TT (OR=2.26, 95% CI=1.18-4.35) genotypes were associated with a higher risk of ovarian cancer when compared with those with the AA genotype (Table 3). Moreover, the AT+TT genotype was correlated with higher risk of ovarian cancer in comparison to the AA genotype (OR=1.58, 95% CI=1.12-2.24). No significant association was found between rs1126647 and rs2227306 polymorphisms and risk of ovarian cancer.

Table 3. *Association of environmental factors and IL-8 rs4073, rs1126647 and rs2227306 polymorphisms with the risk of ovarian cancer.*

Variable	B	S.E	Wald		OR (95% CI)
BMI	0.11	0.03	18.13	<0.001	1.12 (1.06-1.17)
Use of hormone replacement therapy					

No					1.0
Yes	1.28	0.53	5.91	0.02	3.58 (1.28-10.01)
Drinking habit					
Never					1.0
Ever	0.38	0.19	3.9	0.04	1.47 (1.01-2.14)
rs4073					
AA					1.0
AT	0.41	0.18	4.86	0.04	1.50 (1.05-2.16)
TT	0.82	0.33	6.02	0.01	2.26 (1.18-4.35)
AT+TT	0.46	0.18	6.82	0.01	1.58 (1.12-2.24)
rs1126647					
CC					1.0
CT	-0.09	0.27	0.12	0.73	0.91 (0.54-1.54)
TT	0.59	0.78	0.58	0.45	1.81 (0.40-8.24)
CT+TT	-0.03	0.26	0.02	0.9	0.97 (0.59-1.60)
rs2227306					
CC					1.0
CT	-0.15	0.19	0.63	0.43	0.86 (0.59-1.25)
TT	0.02	0.27	0.01	0.95	1.02 (0.60-1.71)
CT+TT	-0.1	0.18	0.31	0.58	0.91 (0.64-1.28)

A linkage disequilibrium was found between rs4073 and rs1126647 (D'=0.57, r2=0.12) (Table 4). A total of four common haplotypes (frequency>0.03) were selected into analysis, and the A-C-T haplotype showed a reduced risk of ovarian cancer, with the OR (95% CI) of 0.74 (0.56-0.99) (Table 5). The other three haplotypes were not correlated with the development of ovarian cancer.

Table 4. *Linkage disequilibrium tests for IL-8 rs4073, rs1126647 and rs2227306.*

	rs1126647	**rs2227306**
D’		
rs4073	0.572	0.003
rs1126647	-	0.09
r2		
rs4073	0.12	<0.001
rs1126647	-	<0.001

Table 5. *Haplotype analysis of rs4073-rs1126647-rs2227306.*

	Cases N=560	**%**	**Controls N=560**	**%**	**P value**	**OR (95% CI)**
A-C-C	214	38.21	236	42.14	0.15	0.84 (0.66-1.07)
A-C-T	113	20.18	141	25.18	0.04	0.74 (0.56-0.99)
T-C-C	120	21.43	89	15.89	0.17	1.43 (0.90-1.94)
T-C-T	72	12.86	50	8.93	0.25	1.50 (0.92-2.20)

Global χ^2=12.68, P=0.013.

Discussion

It is widely accepted that ovarian cancer is a multifactorial disease, and pathogenesis of ovarian cancer can be facilitated by a single dominant mutation altering expression of susceptibility genes. In the present study, we found that the AT and TT genotypes of IL-8 rs4073 increased the risk of ovarian cancer when compared with the AA genotype, and the A-C-T haplotype was associated with a reduced risk of ovarian cancer.

In agreement with our findings, previous studies reported a significant association between IL-8 rs4073 gene polymorphism and several kinds of human cancer, such as prostate cancer, thyroid cancer, oral squamous cell carcinoma, colorectal cancer and breast cancer as well as gastric cancer [10-15]. Chen et al. performed a study with 439 prostate cancer patients and 524 controls, and they did not find an association between rs4073 and risk of prostate cancer [10]. Kilic et al. conducted a study consisting of 101 patients with thyroid cancer and 109 healthy controls, and they reported that the TT genotype of IL-8 rs4073 may contribute to the risk of thyroid

carcinomas [11]. Liu et al. performed a study with 270 patients with oral squamous cell carcinoma and 350 healthy control subjects in a Taiwanese population, and they suggested that IL-8 gene polymorphisms was associated with risk of oral squamous cell carcinoma in smokers [12]. Mustapha et al. done a study in Malaysians, and they observed that IL-8 rs4073 was significantly related to the colorectal cancer susceptibility and can be considered as a high-risk variant for colorectal cancer [13]. Some studies reported contrary results [16,17]. Burada et al. did not find the association between IL-8 rs4073 and colorectal cancer risk, but no significant correlation was observed between them.

However, only one study reported the association between IL-8 polymorphisms and risk of ovarian cancer. Koensgen et al. performed a study with 268 patients with ovarian cancer and 426 matched controls in a population of German, and they investigated the association of four SNPs of IL-8 gene with the risk of ovarian cancer [9]. They found that the TT genotype of IL-8 rs2227306 was correlated with an increased frequency of ovarian cancer, but the IL-8 rs4073 did not show a significant association [9]. In contrast with previous results, we found that the IL-8 rs4073 T allele contributed to increased risk of ovarian cancer.

Moreover, we firstly reported linkage disequilibrium between rs4073 and rs1126647, and the A-C-T haplotype showed a reduced risk of ovarian cancer. It could be hypothesis that mutation linked to the IL-8 haplotypes could alter the activity and expression of IL-8, and consequently affect the susceptibility to ovarian cancer.

Two limitations should be mentioned in this study. First, as a case-control study design, the selection bias is unavoidable. Second, as the low incidence of ovarian cancer, the sample size of patients with ovarian cancer was not large, potential restricting its statistical power to distinguish difference between study groups.

Conclusions

In conclusion, our results support direct association of IL-8 rs4073 polymorphism and A-C-T haplotype with the risk of ovarian cancer, suggesting that the IL-8 may be a new biomarker for the susceptibility to ovarian cancer. Further researches with larger studies in different ethnic groups into the function of IL-8 polymorphisms and its potential biological mechanisms association are needed.

Acknowledgement

We thank nurses who helped us in collecting the blood samples for our study. We also thank patients who agreed to participate into our study.

Funding

This research was supported by the General Medicine and Health Scientific Research (06G006).

Declaration of Conflicting Interest

The authors declare no conflict of interest in preparing this article.

References

1. DeSantis CE, Lin CC, Mariotto AB. Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin 2014; 64: 252-271.
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
3. Romero I, Bast RCJ. Mini review: Human ovarian cancer: biology, current management, and paths to personalizing therapy. Endocrinol 2012; 153: 1593-602.
4. de Oliveira JG, Rossi AF, Nizato DM. Influence of functional polymorphisms in TNF-alpha, IL-8, and IL-10 cytokine genes on mRNA expression levels and risk of gastric cancer. Tumour Biol 2015; 36: 9159-9170.
5. Xie K. Interleukin-8 and human cancer biology. Cytokine Growth Factor Rev 2001; 12: 375-391.
6. Modi WS, Dean M, Seuanez HN, Mukaida N, Matsushima K, O'Brien SJ. Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily. Hum Genet 1990; 84: 185-187.
7. Gura T. Chemokine's take center stage in inflammatory ills. Sci 1996; 272: 954-956.
8. Wang N, Zhou R, Wang C. -251 T/A polymorphism of the interleukin-8 gene and cancer risk: a HuGE review and meta-analysis based on 42 case-control studies. Mol Biol Rep 2012; 39: 2831-2841.
9. Koensgen D, Bruennert D, Ungureanu S. Polymorphism of the IL-8 gene and the risk of ovarian cancer. Cytokine 2015; 71: 334-338.
10. Chen J, Ying XM, Huang XM, Huang P, Yan SC. Association between polymorphisms in selected inflammatory response genes and the risk of prostate cancer. Onco Targets Ther 2016: 223-229.
11. Kilic I, Guldiken S, Sipahi T. Investigation of VEGF and IL-8 gene polymorphisms in patients with differentiated thyroid cancer. Clin Lab 2016; 62: 2319-2325.
12. Liu CM, Yeh CJ, Yu CC. Impact of interleukin-8 gene polymorphisms and environmental factors on oral cancer susceptibility in Taiwan. Oral Dis 2012; 18: 307-314.
13. Mustapha MA, Shahpudin SN, Aziz AA, Ankathil R. Risk modification of colorectal cancer susceptibility by interleukin-8 -251T>A polymorphism in Malaysians. World J Gastroenterol 2012; 18: 2668-2673.
14. Wang Z, Liu Y, Yang L, Yin S, Zang R, Yang G. The polymorphism interleukin-8 -251A/T is associated with a significantly increased risk of cancers from a meta-analysis. Tumour Biol 2014; 35: 7115-7123.
15. Zhang Y, Zeng X, Lu H, Li Y, Ji H. Association between Interleukin-8-251A/T polymorphism and gastric cancer susceptibility: a meta-analysis based on 5286 cases and 8000 controls. Int J Clin Exp Med 2015; 8: 22393-22402.

16. Burada F, Dumitrescu T, Nicoli R, Ciurea ME, Rogoveanu I, Ioana M. Cytokine promoter polymorphisms and risk of colorectal cancer. Clin Lab 2013; 59: 773-779.
17. Yang L, Zhu X, Liang X, Ling Z, Li R. Association of IL-8-251A>T polymorphisms with oral cancer risk: evidences from a meta-analysis. Tumour Biol 2014; 35: 9211-928.

***Correspondence to**

Aiming Wang

Southern Medical University

Guangzhou

PR China