miR-146a and miR-196a2 polymorphisms in ovarian cancer risk

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ABSTRACT. We investigated the relationship between miR-146a and miR-196a2 genetic polymorphisms and development of ovarian cancer in a Chinese population. A total of 134 patients and 227 control subjects were involved in our study between January 2012 and October 2014 from China-Japan Union Hospital of Jilin University. Genotyping of miR-146a and miR-196a2 was accomplished by polymerase chain reaction coupled with restriction fragment length polymorphism analysis. Unconditional multiple-logistic regression analysis indicated that the GG genotype of miR-146a was associated with an increased risk of ovarian cancer when compared to the CC genotype, and the adjusted OR (95%CI) was 3.73 (1.79-7.80). Moreover, the CG+GG genotype of miR-146a was associated with an increased risk of ovarian cancer compared with the CC genotype (OR = 1.68, 95%CI = 1.06-2.66), and the GG genotype had a higher risk of ovarian cancer than the CC+CG genotype (OR = 3.02, 95%CI = 1.55-5.98). In conclusion, our study suggests that the miR-146a polymorphism is associated with

increased risk of ovarian cancer and could be used as a biomarker for ovarian cancer susceptibility.

Key words: miR-146a; miR-196a2; Polymorphism; Ovarian cancer

INTRODUCTION

Ovarian cancer is a common malignant tumor with high mortality in women. The etiology of ovarian cancer has been widely studied, but its pathogenesis is not clear. Many environmental and lifestyle factors are involved in the development of ovarian cancer, including early menarche, late menopause, the choice not to or inability to bear children, higher body mass index, family history of cancer, and long-term use of estrogen-replacement therapy and ovulation-inducing drugs (Brekelmans, 2003; Romero and Bast, 2015). Previous studies have reported that hereditary factors also contribute to ovarian cancer development, such as BRCA2, PALB2, X-ray repair cross-complementing group 2, MTHFR, calcium-sensing receptor, and vascular endothelial growth factor genes (Janardhan et al., 2015; Nakagomi et al., 2015; Shi and Shen, 2015; Su et al., 2015; Yan et al., 2015; Zhai et al., 2015).

In addition, numerous microRNAs (miRNAs) contribute to carcinogenesis, by regulating the expression of oncogenes and tumor suppressors (Reddy, 2015). Specifically, the roles of miRNAs in ovarian cancer include its initiation, progression, outcome, and therapeutic effect (Mitamura et al., 2013; Wuerkenbieke et al., 2015; Zhan et al., 2015). Genomic polymorphisms of the miRNA genes could influence the structure, properties and expression of the miRNA, and consequently affect function of miRNA genes and susceptibility to ovarian cancer. Two common genetic variations of the miRNAs miR-146a and miR-196a2 have been observed, and have association with pathogenesis of multiple-type diseases (Li et al., 2015; Shen et al., 2015; Xu and Tang, 2015; Nikolić et al., 2015). Up to now, only three studies have reported a relationship between miR-146a and miR-196a2 polymorphisms and susceptibility to ovarian cancer (Shen et al., 2008; Pastrello et al., 2010; Liu et al., 2015). Therefore, we investigated further the relationship of miR-146a and miR-196a2 genetic variations with the susceptibility to ovarian cancer.

MATERIAL AND METHODS

Subjects

A hospital-based case-control design was utilized, with 134 patients and 227 control subjects enrolled in our study between January 2012 and October 2014, from China-Japan Union Hospital of Jilin University. The exclusion criteria were individuals with metastasis or recurrent tumors aside from ovarian cancer, chronic and acute infection diseases, or endocrine diseases.

The healthy controls were women receiving gynecologic examinations in outpatient clinics over the same period. The control subjects were free of any history of malignant tumors, gynecological diseases, or endocrine diseases.

The clinical and baseline demographic variables of investigated subjects were derived from medical records or face-to-face interviews with a structured questionnaire. Written

informed consents of all subjects were obtained from each study subject, and the study protocol obtained the permission from the Ethics Committee of China-Japan Union Hospital of Jilin University.

DNA extraction and genotyping

Peripheral blood samples were collected, and genomic DNA was extracted using the DNA Purification Kit (Tiangen Biotech, Beijing, China). The genotyping of miR-146a and miR-196a2 was accomplished by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism analysis. The primers, restriction enzymes and lengths of digested fragments of miR-146a and miR-196a2, which were based on a previous study (Zhang et al., 2015), are summarized in Table 1.

Table 1. Primers, restriction enzymes, and lengths of digested fragments of miR-146a and miR-196a2.

miRNA	SNPs	Primers (5'-3')	Lengths of digested fragments
miR-146a	rs2910164	Forward: CATGGGTTGTGTCAGTGTCAGAGC	C: 25 bp, 122 bp
		Reverse: TGCCTTCTGTCTCCAGTCTTCCAA	G: 147 bp
miR-196a2	rs11614913	Forward: CCCTTCCCTTCTCCTCCAGATA	C: 24 bp and 125 bp
		Reverse: CGAAAACCGACTGATGTAACTCCG	T: 149 bp

SNP = single nucleotide polymorphisms.

PCR was performed in a 25-μL reaction mixture with 10X PCR buffer, 3 μL 25 mM $MgCl_2$, 1.5 μL 10 pM both primers, 2 μL 10 mM each dNTP, 2 μL (20-40 ng/μL) DNA, and 1 U Taq DNA polymerase. Reaction parameters were: initial denaturation at 94°C for 5 min; followed by 35 cycles at 91°C for 60 s, 62°C for 60 s, and 72°C for 60 s; and a final extension at 72°C for 5 min. The PCR amplification products were digested using *Sac*I and *Msp*I restriction enzymes.

Statistical analysis

Whether miR-146a and miR-196a2 genetic polymorphisms were in Hardy-Weinberg equilibrium (HWE) or not was determined using an exact chi-square test. The association of miR-146a and miR-196a2 polymorphisms to the risk of ovarian cancer was calculated using the odds ratio (OR) at 95% confidence intervals (CIs). The common genotypes of miR-146a and miR-196a2 were used as the references. The association was considered statistically significant when $P \leq 0.05$. The SPSS software Version 17.0 (SPSS Inc., Chicago, IL, USA) was used for all data analyses.

RESULTS

The mean ± SD ages of patients with ovarian cancer and control subjects were 53.33 ± 6.34 and 54.56 ± 7.04 years, respectively (Table 2). According to chi-square or *t*-test results, the patients with ovarian cancer and control subjects were comparable in age of menarche ($\chi^2 = 2.88$, $P = 0.09$), age of menopause ($\chi^2 = 0.73$, $P = 0.39$), tobacco smoking ($\chi^2 = 0.72$, $P = 0.40$), alcohol consumption ($\chi^2 = 0.50$, $P = 0.48$), and body mass index ($\chi^2 = 0.78$, P =

0.38). Notably, no significant differences were found between patients with ovarian cancer and control subjects for age (t = 1.75, P = 0.04) and family history of cancer (χ^2 = 12.32, P < 0.001). Of the patients with ovarian cancer, 52 (38.81%) cases were designated stage I-II, and 82 (61.19%) cases were diagnosed stage III-IV.

Table 2. Demographic characteristics of study subjects.

Variables	Patients (N = 134)	%	Controls (N = 227)	%	χ^2 or t-test	P value
Age (years)	53.33 ± 6.34		54.56 ± 7.04		1.75	0.04
Age of menarche (years)						
<11	62	46.27	126	55.51		
≥11	72	53.73	101	44.49	2.88	0.09
Age of menopause (years)						
<50	64	47.76	119	52.42		
≥50	70	52.24	108	47.58	0.73	0.39
Tobacco smoking						
No	110	82.09	194	85.46		
Yes	24	17.91	33	14.54	0.72	0.40
Alcohol consumption						
No	107	79.85	188	82.82		
Yes	27	20.15	39	17.18	0.50	0.48
Body mass index (kg/m^2)						
<24	55	41.04	104	45.81		
≥24	79	58.96	123	54.19	0.78	0.38
Family history of cancer						
No	125	93.28	226	99.56		
Yes	9	6.72	1	0.44	12.32	<0.001
Clinical stage						
I-II	52	38.81				
III-IV	82	61.19				

The genetic frequencies of miR-146a and miR-196a2 are described in Table 3. The genotype distributions of miR-146a (P = 0.37) and miR-196a2 (P = 0.36) in patients with ovarian cancer and controls were in agreement with HWE for the references. Using chi-square tests, no significant differences were observed between patients with ovarian cancer and controls in the genotype distributions of miR-146a (χ^2 = 15.30, P < 0.001) and miR-196a2 (χ^2 = 2.81, P = 0.25).

Table 3. Distributions of miR-146a and miR-196a2 genetic frequencies between study groups.

SNPs	Patients (N = 134)	%	Controls (N = 227)	%	χ^2 test	P value	P for HWE
							Controls
miR-146a							
CC	43	32.09	105	46.26			
CG	62	46.27	103	45.37			
GG	29	21.64	19	8.37	15.30	<0.001	0.37
miR-196a2							
TT	39	29.10	77	33.92			
TC	66	49.25	116	51.10			
CC	29	21.64	34	14.98	2.81	0.25	0.36

SNP = single nucleotide polymorphisms; HWE = Hardy-Weinberg equilibrium.

Unconditional multiple-logistic regression analysis indicated that the GG genotype of miR-146a was associated with an increased risk of ovarian cancer when compared to

the CC genotype, and the adjusted OR (95%CI) was 3.73 (1.79-7.80) (Table 4). Moreover, the CG+GG genotype of miR-146a was associated with an increased risk of ovarian cancer compared with the CC genotype (OR = 1.68, 95%CI = 1.06-2.66), and the GG genotype had a higher risk of ovarian cancer than the CC+CG genotype (OR = 3.02, 95%CI = 1.55-5.98). However, no significant relationship was found between the miR-196a2 polymorphism and ovarian cancer risk in co-dominant, dominant, and recessive models.

Table 4. Association between miR-146a and miR-196a2 genetic polymorphisms and risk of ovarian cancer.

SNPs	Patients (N = 134)	%	Controls (N = 227)	%	OR (95%CI)[1]	P value
miR-146a						
Co-dominant						
CC	43	32.09	105	46.26	1.0 (Ref.)	-
CG	62	46.27	103	45.37	1.47 (0.89-2.43)	0.11
GG	29	21.64	19	8.37	3.73 (1.79-7.80)	<0.001
Dominant						
CC	48	35.82	108	47.58	1.0 (Ref.)	-
CG+GG	91	67.91	122	53.74	1.68 (1.06-2.66)	0.02
Recessive						
CC+CG	105	78.36	208	91.63	1.0 (Ref.)	-
GG	29	21.64	19	8.37	3.02 (1.55-5.98)	<0.001
miR-196a2						
Co-dominant						
TT	39	29.1	77	33.92	1.0 (Ref.)	-
TC	66	49.25	116	51.10	1.12 (0.67-1.89)	0.64
CC	29	21.64	34	14.98	1.68 (0.86-3.30)	0.10
Dominant						
TT	39	29.1	77	33.92	1.0 (Ref.)	-
TC+CC	95	70.89	150	66.08	1.25 (0.77-2.05)	0.34
Recessive						
TT+TC	105	78.35	193	85.02	1.0 (Ref.)	-
CC	29	21.64	34	14.98	1.57 (0.87-2.81)	0.11

SNP = single nucleotide polymorphisms. [1]Adjusted for age and family history of cancer.

DISCUSSION

In the present study, we carried out a study to estimate the association of miR-146a and miR-196a2 genetic polymorphisms in the development of ovarian cancer, and we found that polymorphisms of miR-146a correlated significantly with the pathogenesis of ovarian cancer in co-dominant, dominant, and recessive models.

The polymorphism of miR-146a is a G to C substitution leading to an amino acid sequence change, and consequently influences the expression and transcriptional regulation of the miRNA. Genetic variations in miR-146a could influence the expression of mature miR-146a, as well as binding activity of target mRNA, thereby altering the gene function. A previous experimental study reported that miR-146a polymorphism was associated with its mature miRNA expression, and influenced the expression levels of the miRNA (Xiong et al., 2014).

Several studies have reported that miR-146a genetic polymorphisms could influence susceptibility to various human cancers, but the results appear to be dependent on the type of cancer. Palmieri et al. (2014) demonstrated that the miR-146a polymorphism was not correlated with the pathogenesis of oral squamous cell carcinoma in a population in Italy. On the other hand, Gao et al. (2011) carried out a meta-analysis of four studies, determining that miR-146a polymorphisms contributed to the susceptibility to breast cancer in all comparison

models. Finally, Chae et al. (2013) demonstrated that the G allele variant of miR-146a carried a higher risk of developing colorectal cancer.

Currently, only three studies analyzed the association between the miR-146a polymorphism and ovarian cancer in a Caucasian population (Shen et al., 2008; Pastrello et al., 2010; Liu et al., 2015). The C allele variant of miR-146a was associated with early familial breast and ovarian tumor development in a study conducted on 101 Italian patients with ovarian cancer and 155 controls (Pastrello et al., 2010). Shen et al. (2008) carried out a study on 82 patients with familial ovarian cancer, finding that the C allele of miR-146a may influence the onset of familial ovarian cancer. In the study described herein, we also observed that the miR-146 polymorphism could influence the development of ovarian cancers, in line with previous findings.

Our study has some limitations. First, selection bias should be considered in the study, since the subjects were recruited from only one hospital in a city of China. Second, the sample size of our study is relatively small, which may reduce the statistical power to identify the difference between the two investigated groups.

In conclusion, our study suggests that the miR-146a polymorphism is associated with increased risk of ovarian cancer. Our data also indicate that the miR-146a polymorphism could potentially be a biomarker for susceptibility to ovarian cancer. Further investigations with larger sample sizes, as well as with multiple populations are critical for the successful analysis of these interactions.

Conflicts of interest

The authors declare no conflict of interest.

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