Int J Clin Exp Pathol 2016;9(5):5706-5711
www.ijcep.com /ISSN:1936-2625/IJCEP0024141

Original Article

Role of polymorphisms in miR-146a, miR-149, miR-196a2 and miR-499 in the development of ovarian cancer in a Chinese population

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Received January 15, 2016; Accepted March 26, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Here, we carried out an investigation of the relationship between miR-146a (rs2910164), miR-149 (rs2292832), miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms and development of ovarian cancer in a Chinese population. With a hospital-based case-control design, 155 patients and 342 control subjects were involved in the First Affiliated Hospital of Harbin Medical University between March 2012 and January 2015. The genotyping of the miR-146a, miR-149, miR-196a2 and miR-499 were genotyped by polymerase chain reaction (PCR)-coupled with restriction fragment length polymorphism (RFLP). Unconditional multiple logistic regression analysis indicated that the GG genotype and G allele of miR-499 had a higher risk of ovarian cancer compared to the AA genotype, and the adjusted ORs (95% CI) for the GG genotype and G allele were 2.40 (1.13-5.08) and 1.46 (1.06-2.00), respectively. However, no significant correlation was observed between the miR-146a, miR-149 and miR-196a2 polymorphisms and the ovarian cancer risk. In conclusion, our study indicates that the miR-499 polymorphism is associated with risk of ovarian cancer, suggesting that miR-499 polymorphism could be a marker for ovarian cancer development.

Keywords: miR-146a, miR-149, miR-196a2, miR-499, polymorphism, ovarian cancer

Introduction

Ovarian cancer is one of the major causes of cancer-related death in females, largely resulting from the growth, metastasis and invasion of the cancer cells. It is estimated that there are 238719 new cases and 151917 deaths worldwide in 2012 [1]. This invasive cancer is the leading cause of death in China, with more than 151917 cases being reported in China [1]. As most of the malignant tumors, ovarian cancer is caused by multiple environmental and lifestyle factors, such as early menarche, late menopause, unbearing, lack of physical activity, higher body mass index and long-term use of estrogen replacement therapy [2, 3]. Currently studies have reported that family history of ovarian cancers is an important factor in the development of ovarian cancers, which suggests that genetic factors contribute to the susceptibility to this cancer [4, 5]. Many studies have demonstrated that genetic factors contribute to the development of this cancer, such as Nuclear factor-κB, RAD51, HER2, XRCC1; CDKN1B and ERCC2 genes [6-11].

Recently, the functions of microRNAs (miRNAs) in the carcinogenesis have attracted much interest [12]. MiRNAs are a novel class of small noncoding RNA that are responsible for the promoting messenger RNA (mRNA) degradation, inhibiting mRNA translation, and affecting transcription by binding to the 3'-untranslated region (3'-UTR) of their target mRNA [13, 14]. Lots of miRNAs play important roles in carcinogenesis by regulating the expression of oncogenes and tumor suppressors [12]. Studies about the roles of miRNA in ovarian cancer have revealed its contribution in the cancer initiation, progression, outgrowth, and drug resistance [15-17]. Single-nucleotide polymorphisms (SNPs) in the miRNA genes may influence the property and expression of the respective miRNA, and thus affect individualized susceptibility to cancers. Four common genetic variations of miR-146a, miR-149, miR-196a2 and miR-499 are identified in the miRNA, which have been shown to be correlated with the susceptibility to multiple types diseases, including digestive cancer, esophageal squamous cell carcinoma, colorectal cancer and prostate can-

Table 1. Primers, restriction enzymes and lengths of digested fragments of miR-146a, miR-149, miR-196a2 and miR-499

miRNA	SNPs	Primers (5'-3')	Restriction enzyme	Lengths of digested fragments
miR-146a	rs2910164	Forward: CATGGGTTGTGTCAGTGTCAGAGC Reverse: TGCCTTCTGTCTCCAGTCTTCCAA	sacI	C: 25 bp, 122 bp G: 147 bp
miR-149	rs2292832	Forward: TGTCTTCACTCCCGTGCTTGTCC Reverse: TGAGGCCCGAAACACCCGTA	PvuII	T: 60 bp and 194 bp C: 254 bp
miR-196a2	rs11614913	Forward: CCCTTCCCTTCTCCTCCAGATA Reverse: CGAAAACCGACTGATGTAACTCCG	MspI	C: 24 bp and 125 bp T: 149 bp
miR-499	rs3746444	Forward: CAAAGTCTTCACTTCCCTGCCA Reverse: GATGTTTAACTCCTCTCCACGTGATC	BclI	T: 26 bp and 120 bp C: 146 bp

Table 2. Demographic characteristics of patients with ovarian cancer and controls

Variables	Patients N=155	%	Controls N=342	%	χ^2 or *t* test	*P* value
Age, years	53.45±8.36		52.61±8.74		1.01	0.16
Age of menarche, years	11.72±2.73		12.58±3.01		3.04	0.001
Age of menopause, years	52.57±6.35		51.40±5.86		2.01	0.02
Tobacco smoking						
No	131	84.52	302	88.30		
Yes	24	15.48	40	11.70	1.36	0.24
Alcohol consumption						
No	117	75.48	261	76.32		
Yes	38	24.52	81	23.68	0.04	0.84
Body mass index, kg/m^2	26.24±2.42		24.63±2.85		6.11	<0.001
Family history of cancer						
No	147	94.84	335	97.95		
Yes	8	5.16	7	2.05	3.53	0.06
Clinical stage						
I-II	49	31.61				
III-IV	106	68.39				

cer [18-21]). Currently, few studies reported the relationship between miRNA miR-146a, miR-196a2 and miR-499 polymorphisms and the susceptibility to ovarian cancer [22, 23]. Therefore, we carried out an investigation of the relationship between miR-146a (rs2910164), miR-149 (rs2292832), miR-196a2 (rs11614913) and miR-499 (rs3746444) polymorphisms and development of ovarian cancer in a Chinese population.

Material and methods

Subjects

With a hospital-based case-control design, 155 patients and 342 control subjects were involved in the First Affiliated Hospital of Harbin Medical University between March 2012 and January 2015 from our hospital. Ovarian cancer was newly diagnosed and independently confirmed in all patients by two pathologists. Patients who had other malignant tumor, end-stage liver and kidney diseases, and endocrine diseases were excluded from this study. The control subjects were collected from females receiving gynecologic examination in outpatient clinics. Women with any history of malignant tumors, gynecological diseases, serious liver and kidney diseases as well as endocrine diseases were excluded as controls.

The clinical characteristics of all ovarian cancer patients and controls were selected from medi-

Table 3. Distributions of miR-146a, miR-149, miR-196a2 and miR-499 genetic frequencies between study groups

SNPs	Patients N=155	%	Controls N=342	%	χ^2 test	*P* value	P for HWE	
							Patients	Controls
miR-146a								
CC	56	36.13	131	38.30				
CG	75	48.39	161	47.08				
GG	24	15.48	50	14.62	0.23	0.89	0.89	0.96
miR-149								
CC	47	30.32	108	31.58				
TC	82	52.90	179	52.34				
TT	26	16.77	55	16.08	0.09	0.96	0.33	0.18
miR-196a2								
TT	41	26.45	100	29.24				
TC	82	52.90	176	51.46				
CC	32	20.65	66	19.30	0.43	0.81	0.44	0.46
miR-499								
AA	84	54.19	213	62.28				
AG	53	34.19	110	32.16				
GG	18	11.61	19	5.56	6.56	0.04	0.04	0.34

cal records, such as clinical stage. The demographic and lifestyle factors were collected using face-to-face interview with structured questionnaires, such as mean age, age of menarche, age of menopause, tobacco smoking, alcohol consumption, body mass index and family history of cancer. Written informed consent was obtained from all of the study subjects. The study was approved by the ethnic committee of the First Affiliated Hospital of Harbin Medical University according to the standards of the Declaration of Helsinki.

DNA extraction and genotyping

Peripheral blood samples were collected in vacuum tubes with 5% EDTA. The genomic DNA was extracted using DNA Purification Kit (Tiangen Biotech, Beijing, China) according to the instruction of the protocol. The genotyping of the miR-146a, miR-149, miR-196a2 and miR-499 were genotyped by polymerase chain reaction (PCR)-coupled with restriction fragment length polymorphism (RFLP). The primers, restriction enzymes and lengths of digested fragments of miR-146a, miR-149, miR-196a2 and miR-499 are designed based on a previous study (Zhang et al.) and summarized in **Table 1**. The PCR cycles were set as follows: an initial denaturation at 95°C for 5 min was followed by 35 cycles at 95°C for 30 s, at 64°C for 50 s, at 72°C for 20 s, and a final extension at 72°C for 10 min. The PCR products were electrophoresized on a 3% agarose gel stained with ethidium bromide, and the results were visualized under ultraviolet light. To confirm the genotyping results, 10% of the PCR-amplified DNA samples were examined using DNA sequence, and the results were 100% concordant.

Statistical analysis

Genotypic frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium (HWE) using an Chi-square test. Allele frequencies and genotype frequencies for each SNP of ovarian cancer patients and control subjects were compared using the χ^2 test. Unconditional multiple logistic regression analysis was carried out to evaluate the role of miR-146a, miR-149, miR-196a2 and miR-499 genetic variations in the susceptibility to ovarian cancer; odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. ORs and 95% CIs were calculated by unconditional logistic regression analyses adjusted for confounding factors. Statistical analyses were performed using Microsoft Excel and SPSS 16.0 statistical packages (SPSS, Chicago, IL).

Results

The mean age of ovarian cancer patients and control subjects were 53.45±8.36 and 52.61±8.74 years, respectively (**Table 2**). The mean ages of menarche of patients and controls were 11.72±2.73 and 12.58±3.01, respectively. The mean age of menopause was 52.57±6.35 and 51.40±5.86, respectively. The body mass indexes of patients and controls were 26.24±2.42 and 24.63±2.85, respectively. There were 49 (31.61%) patients with I-II stage and 106 (68.39%) patients with III-IV stage. By

Table 4. Association between miR-146a, miR-149, miR-196a2 and miR-499 genetic polymorphisms and risk of ovarian cancer

SNPs	Patients N=155	%	Controls N=342	%	OR (95% CI)[1]	*P* value
miR-146a						
CC	56	36.13	131	38.3	1.0 (Ref.)	-
CG	75	48.39	161	47.08	1.09 (0.70-1.69)	0.69
GG	24	15.48	50	14.62	1.12 (0.60-2.07)	0.69
C allele	187	60.325	423	61.84	1.0 (Ref.)	-
G allele	123	39.675	261	38.16	1.07 (0.80-1.42)	0.65
miR-149						
CC	47	30.32	108	31.58	1.0 (Ref.)	-
TC	82	52.9	179	52.34	1.05 (0.67-1.66)	0.82
TT	26	16.77	55	16.08	1.09 (0.58-2.01)	0.79
C allele	176	56.77	395	57.75	1.0 (Ref.)	-
T allele	134	43.22	289	42.25	1.04 (0.79-1.38)	0.77
miR-196a2						
TT	41	26.45	100	29.24	1.0 (Ref.)	-
TC	82	52.9	176	51.46	1.14 (0.71-1.83)	0.58
CC	32	20.65	66	19.3	1.18 (0.65-2.14)	0.56
T allele	164	52.9	376	54.97	1.0 (Ref.)	-
C allele	146	47.1	308	45.03	1.09 (0.82-1.44)	0.54
miR-499						
AA	84	54.19	213	62.28	1.0 (Ref.)	-
AG	53	34.19	110	32.16	1.22 (0.79-1.88)	0.34
GG	18	11.61	19	5.56	2.40 (1.13-5.08)	0.01
A allele	221	71.285	536	78.36	1.0 (Ref.)	-
G allele	89	28.705	148	21.64	1.46 (1.06-2.00)	0.02

[1]Adjusted for age, age of menarche, age of menopause and body mass index.

Chi-square test or student *t* test, the ovarian cancer patients were compared with the control subjects in terms of age (t=1.01, P=0.16), tobacco smoking (χ^2=1.36, P=0.24), alcohol consumption (χ^2=0.04, P=0.84) and family history of cancer (χ^2=3.53, P=0.06). There were significant differences between ovarian cancer patients and controls in terms of age of menarche (t=3.04, P=0.001), age of menopause (t=2.01, P=0.02) and body mass index (t=6.11, P<0.001).

The miR-146a, miR-149, miR-196a2 and miR-499 genetic frequencies are presented in **Table 3**. The genotype distributions of miR-146a, miR-149 and miR-196a2 in patients and controls were in agreement with the Hardy-Weinberg equilibrium in ovarian cancer patients and control subjects, but the miR-499 genotype distributions did not. Using chi-square test, there were significant differences between ovarian cancer patients and controls in terms of miR-499 genotype distribution (χ^2=6.56, P=0.04); however, the distributions of miR-146a, miR-149, miR-196a2 did not differ significantly.

Unconditional multiple logistic regression analysis indicated that the GG genotype and G allele of miR-499 had a higher risk of ovarian cancer compared to the AA genotype, and the adjusted ORs (95% CI) for the GG genotype and G allele were 2.40 (1.13-5.08) and 1.46 (1.06-2.00), respectively (**Table 4**). However, no significant correlation was observed between the miR-146a, miR-149 and miR-196a2 polymorphisms and the ovarian cancer risk.

Discussion

It is well known that miRNAs have an important role in cancer through acting as tumor suppressors or oncogenes [24]. MiRNA is a class of non-coding small RNA comprised of 18-23 nucleotides [12]. MiRNA can regulate the expression of target genes by pairing with the 3'-UTR of target genes, which ultimately controls the protein level of target genes [12]. Currently, a number of miRNAs have been identified to be involved in the growth, metastasis and drug resistance of ovarian cancer [24]. In the present study, we did a study to explore the association of miR-146a, miR-149, miR-196a2 and miR-499 polymorphisms in the susceptibility to ovarian cancer, and we observed that the GG genotype and G allele of miR-499 were correlated with an increased risk of ovarian cancer in a Chinese population.

Polymorphism of miR-499 is an A to G substitution resulting in an amino acid changing from Arginine to Glycine. Polymorphisms in miRNA play a critical role in the expression and transcriptional regulation of miRNA. Polymorphisms in miR-499 are located on the mature sequence of miR-499, which could affect the processing of miR-499 to the mature form. Therefore,

genetic variations in miR-499 could influence the expression of mature miR-499 and binding activity of target mRNA, thereby altering the gene function.

Several studies have investigated the role of miR-499 polymorphisms in the susceptibility to human cancers, but the results are inconsistent [21, 25-29]. Bansal et al. indicated that the miR-499 genetic polymorphism did not influence the risk of breast cancer in postmenopausal females [25]. Cai et al. indicated that miR-499 genetic variation may influence the risk of gastric cancer in a Chinese population [26]. Shen et al. suggested that the miR-499 polymorphism was associated with an increased esophageal cancer risk in a Chinese population [27]. Deng et al. did not observed an significant correlation between bladder cancer risk of variants of miR-146a, miR-149, miR-196a2 and miR-499 in a Chinese population [28]. Nikolić et al. indicated that G allele of miR-499 had a decreased risk of prostate cancer risk in Serbian population [21]. Liu et al. carried out a meta-analysis with 15 studies, and reported that the miR-499 polymorphism may increase the risk of colorectal cancer risk in Caucasians [30].

To date, only one study reported the correlation between miR-499 genetic variations and risk of ovarian cancer [29]. Liu et al. conducted a study in a Chinese population including 216 primary endometrial/ovarian cancer cases and 100 healthy controls, and reported that miR-499 polymorphism has potential function in reducing risk of endometrial cancer. miR-146a and miR-196a2 polymorphism can influence the susceptibility to ovarian cancer [29]. Two previous studies have reported that miR-146a genetic variations are correlated with the earlier age of onset of familial of breast and ovarian cancers [22, 23]. In our study, we only observed an significant relationship between miR-499 polymorphism and development of ovarian cancers, but no association between miR-146a, miR-149 and miR-196a2 polymorphisms and susceptibility to ovarian cancers. However, our study had some limitations. First, the study subjects were selected from only one hospital, which may induce selection bias. But our results were based on unadjusted estimates, and accurate analysis may be achieved with the adjustment of confounders such as age, age of menarche, age of menopause and body mass index. Second, our analysis might overlook the possibility of gene-gene or SNP-SNP interactions, or linkage disequilibrium between polymorphisms. Third, the sample size is not large, which may reduce the statistical power to find differences between groups. Further investigations with more sample sizes and multiple polymorphisms in different genes within the same pathway are greatly required.

In conclusion, our study indicates that the miR-499 polymorphism is associated with risk of ovarian cancer, suggesting that miR-499 polymorphism could be a marker for ovarian cancer development. Additional large-scale studies are warranted to evaluate the association between miR-146a, miR-149, miR-196a2 and miR-499 polymorphisms and ovarian cancer in different populations.

Disclosure of conflict of interest

None.

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