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Investigating the role of polymorphisms in miR-146a, -149, and -196a2 in the development of gastric cancer

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ABSTRACT. Here, we performed a case-control study to investigate the role of miR-146a, miR-149, and miR-196a2 polymorphisms in the development of gastric cancer using a hospital-based case-control design. A total of 186 gastric cancer patients and 186 control subjects were enrolled from Ren Ji Hospital between January 2012 and October 2014. MicroRNAs miR-146a, miR-149, and miR-196a2 were genotyped by polymerase chain reaction coupled with restriction fragment length polymorphism. Univariate logistic regression analysis revealed that patients with gastric cancer were more likely to be infected with *Helicobacter pylori* [odds ratio (OR) = 1.68, 95% confidence interval (CI) = 1.07-1.96]. Conditional multiple logistic regression analysis revealed that the TT genotype of miR-196a2 was associated with an increased risk of gastric cancer compared to the CC genotype (OR = 2.40; 95%CI = 1.26-4.61). Moreover, patients carrying both the TC and TT genotypes of miR-196a2 were correlated with an elevated risk of gastric cancer compared to those expressing the CC genotype alone (OR = 1.67, 95%CI = 1.01-2.75; P = 0.03). In conclusion, the results of

our study indicated that the miR-196a2 polymorphism was associated with gastric cancer development.

Key words: miR-146a; miR-149; miR-196a2; Polymorphism; Gastric cancer

INTRODUCTION

Gastric cancer accounts for approximately 13% of all deaths each year, with an incidence that is second only to those of lung, breast, colorectal, and prostate cancers. This invasive cancer is the leading cause of death in the developed world, with more than 70% of cases (677,000 cases) being reported in developing countries (456,000 men, 221,000 women), and the second leading cause of death in the developing world (Jemal et al., 2011; IARC, 2012). Half the total number of gastric cancer patients have been registered in Eastern Asia, specifically China. Some genetic and environmental factors have been indicated to play a critical role in the development of gastric cancer in addition to *Helicobacter pylori* infection (Wu et al., 2005; Dong et al., 2008; McNamara and El-Omar, 2008). However, the precise etiology of this disease remains unclear. Many studies have reported that genetic factors, such as *APE1*, *XRCC1*, *LMP2*, *LMP7*, *ILGF-1*, and *IL-17*, may be involved in the development of gastric cancer (Farahani et al., 2015; Jin et al., 2015; Long et al., 2015; Ma et al., 2015).

Previous studies have reported the role of miRNA in a variety of biological processes, including cell proliferation, differentiation, and apoptosis, by regulating approximately 60% of the human protein coding genes (Ambros, 2003; Esquela-Kerscher and Slack, 2006). SNPs in miRNA genes may affect the property of the respective miRNA. Three common variants of miR-146a, miR-149, and miR-196a2 (rs2910164, rs2292832, and rs11614913, respectively) have previously been implicated in the development of multiple types of cancers, including gastrointestinal cancer. Previous studies have also assessed the association between miR-146a, miR-149, and miR-196a2 polymorphisms and the development of gastric cancer, with inconclusive results (Ma et al., 2013; Dikeakos et al., 2014; Xu et al., 2015). Here, we carried out a case-control study to investigate the role of polymorphisms in miR-146a, miR-149, and miR-196a2in the development of gastric cancer.

MATERIAL AND METHODS

Subjects

A hospital-based case-control design was used in this study. A total of 186 hospitalized gastric cancer patients were selected from Ren Ji Hospital between January 2012 and October 2014. Gastric cancer was newly diagnosed and confirmed in all patients by two pathologists. Patients with secondary or recurrent tumors were excluded from this study.

A total of 186 individuals were randomly selected from among subjects who received regular health examinations at the Ren Ji Hospital between January 2012 and October 2014 as controls. Individuals with any digestive diseases and tumors were excluded as controls.

The *H. pylori* infection status was assessed by the urea breath test. The demographic, lifestyle, and clinical characteristics of all patients and controls, such as age, gender, smoking status, drinking status, hypertension, diabetes, *H. pylori* infection, tumor size, and TNM stage,

were collected from medical records. Written informed consent was obtained from all included patients and controls. The ethical approval of our study was in line with the standards of the Declaration of Helsinki.

DNA extraction and genotyping

Five milliliter of peripheral blood was taken from all patients and controls, and stored in EDTA tubes. DNA was extracted using the TIANamp Blood DNA Kit according to the manufacturer protocols (Tiangen, Beijing, China). miR-146a, miR-149, and miR-196a2 were genotyped by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism. The primers, lengths of digested fragments, and restriction enzymes of miR-146a, miR-149, and miR-196a2 are summarized in Table 1. The PCR cycles were set as follows: a denaturing step at 95°C for 5 min, followed by 30 cycles of denaturation at 91°C for 60s, annealing at 62°C for 60s, and extension at 72°C for 60s, and a final extension at 72°C for 5 min. The PCR products were confirmed by electrophoresing on a 3% agarose gel stained with ethidium bromide, and visualized under ultraviolet light.

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

MicroRNA	Primers (5'-3')	Restriction enzymes
miR-146a	CATGGGTTGTGTCAGTGTCAGAGCT (Forward) TGCCTTCTGTCTCCAGTCTTCCAA (Reverse)	*Sac*I
miR-149	TGTCTTCACTCCCGTGCTTGTCC (Forward) TGAGGCCCGAAACACCCGTA (Reverse)	*Pvu*II
miR-196a2	CCCCTTCCCTTCTCCTCCAGATA (Forward) CGAAAACCGACTGATGTAACTCCG (Reverse)	*Msp*I

Statistical analysis

The relationship between demographic and lifestyle characteristics and gastric cancer risk were analyzed using univariate logistic regression analysis. A chi-square test with one degree of freedom was used to analyze the conformance of the genotype distributions of miR-146a, miR-149, and miR-196a2 to the Hardy-Weinberg equilibrium (HWE). Conditional multiple logistic regression analysis was used to analyze the role of miR-146a, miR-149 and miR-196a2 gene polymorphisms in the development of gastric cancer; the results were expressed by odds ratios (ORs) and their related 95% confidence intervals (CIs). All statistical tests were two-tailed and P values less than 0.05 were considered statistically significant. SPSS v.16.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses.

RESULTS

The mean age of gastric cancer patients and control subjects were 62.43 ± 10.52 and 58.36 ± 11.47 years, respectively (Table 2). There were 58 (31.18%) female and 128 (68.82%) male patients; the controls were gender-matched. Univariate logistic regression analysis revealed that patients with gastric cancer were more likely to be infected with *H. pylori* (OR = 1.68, 95%CI = 1.07-1.96). Of the 186 patients, 72 (38.71%) were at TNM stage I-II and 114 (61.29%) were at stage III-IV; 84 (45.16%) had a tumor size ≤5 cm, while the tumor size of 102 (54.84%) was >5 cm.

Table 2. Demographic and lifestyle characteristics of gastric cancer patients and control subjects.

Variables	Patients (N = 186)	%	Controls (N = 186)	%	χ^2 test	OR (95%CI)	P value
Age (years)	62.43 ± 10.52		58.36 ± 11.47				
Gender							
Females	58	31.18	85	45.70		1.0 (Ref.)	-
Males	128	68.82	101	54.30	8.28	1.86 (1.19-2.90)	0.004
Smoking status							
No	124	66.67	133	71.51		1.0 (Ref.)	-
Yes	62	33.33	53	28.49	1.02	1.25 (0.79-2.00)	0.31
Drinking status							
No	122	65.59	128	68.82		1.0 (Ref.)	-
Yes	64	34.41	58	31.18	0.44	1.16 (0.73-1.83)	0.51
Hypertension							
No	72	38.71	89	47.85		1.0 (Ref.)	-
Yes	114	61.29	97	52.15	3.16	1.45 (0.94-2.24)	0.08
Diabetes							
No	12	6.45	9	4.84		1.0 (Ref.)	-
Yes	174	93.55	177	95.16	0.45	0.74 (0.27-1.96)	0.50
H. pylori infection							
No	109	58.60	131	70.43		1.0 (Ref.)	-
Yes	77	41.40	55	29.57	5.68	1.68 (1.07-2.65)	0.02
TNM stage							
I-II	72	38.71					
III-IV	114	61.29					
Tumor size (cm)							
≤5	84	45.16					
>5	102	54.84					

The genotype distributions of miR-146a, miR-149, and miR-196a2 polymorphisms are presented in Table 3. The genotype distributions of miR-146a, miR-149, and miR-196a2 in patients and controls did not deviate from the HWE (P values of polymorphisms in miR-146a, miR-149, and miR-196a2 in patients and controls were 0.80, 0.60, and 0.62, and 0.64, 0.47, and 0.31, respectively). The chi-square test revealed a significant difference between the genotype distribution of miR-196a2 between gastric cancer patients and controls ($\chi^2 = 8.27$, $P = 0.02$); however, the distributions of miR-146a and miR-149 did not differ significantly.

Table 3. Genotype distributions of miR-146a, miR-149, and miR-196a2 polymorphisms between patients with gastric cancer and control subjects.

SNP	Patients	%	Controls	%	Chi-square test	P value	P value for HWE	
							In patients	In controls
miR-146a								
GG	66	35.48	70	37.63				
GC	91	48.92	90	48.39				
CC	29	15.59	25	13.44	0.42	0.81	0.80	0.64
miR-149								
CC	55	29.57	60	32.26				
TC	89	47.85	87	46.77				
TT	42	22.58	39	20.97	0.35	0.84	0.60	0.47
miR-196a2								
CC	39	20.97	57	30.65				
TC	96	51.61	98	52.69				
TT	51	27.42	31	16.67	8.27	0.02	0.62	0.31

HWE = Hardy-Weinberg equilibrium.

Conditional multiple logistic regression analysis indicated that the TT genotype of miR-196a2 was associated with an increased risk of gastric cancer compared to the CC

genotype (OR = 2.40; 95%CI = 1.26-4.61; Table 4). Moreover, patients carrying both the TC and TT genotype of miR-196a2 were correlated with an elevated risk of gastric cancer, compared to those carrying only the CC genotype (OR = 1.67, 95%CI = 1.01-2.75; P = 0.03). However, we did not find any significant association between the miR-146a and miR-149 polymorphisms and the development of gastric cancer.

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

SNP	Cases	%	Controls	%	OR (95%CI)[1]	P value
miR-146a						
GG	66	35.48	70	37.63	1.0 (Ref.)	-
GC	91	48.92	90	48.39	1.07 (0.67-1.72)	0.76
CC	29	15.59	25	13.44	1.23 (0.62-2.43)	0.52
GC+CC	120	64.51	115	61.83	1.11 (0.71-1.73)	0.64
miR-149						
CC	55	29.57	60	32.26	1.0 (Ref.)	-
TC	89	47.85	87	46.77	1.12 (0.68-1.84)	0.65
TT	42	22.58	39	20.97	1.17 (0.64-2.16)	0.58
TC+TT	131	70.43	126	67.74	1.13 (0.71-1.80)	0.57
miR-196a2						
CC	39	20.97	57	30.65	1.0 (Ref.)	-
TC	96	51.61	98	52.69	1.43 (0.85-2.43)	0.15
TT	51	27.42	31	16.67	2.40 (1.26-4.61)	0.004
TC+TT	147	79.03	129	69.36	1.67 (1.01-2.75)	0.03

[1]Adjusted for age, gender, and *Helicobacter pylori* infection. OR = odds ratio; CI = confidence interval.

DISCUSSION

In this case-control study conducted in the Chinese population, we explored the role of polymorphisms in the microRNA miR-146a, miR-149, and miR-196a2 in gastric cancer susceptibility. The TT and TC+TT genotypes of miR-196a2 were found to be associated with an increased risk of gastric cancer compared to the CC genotype; however, no significant associations were found between miR-146a and miR-149 polymorphisms and gastric cancer risk.

Several previous studies have investigated the role of miR-196a2 polymorphisms in the development of several types of human cancers, such as lung cancer, colorectal cancer, breast cancer, hepatocellular carcinoma, and non-Hodgkin lymphoma (Hoffman et al., 2009; Parlayan et al., 2014; Peng et al., 2014; Li et al., 2015; Qi et al., 2015; Sodhi et al., 2015). Li et al. (2015) discovered that the miR-196a2 polymorphism could increase the risk of non-Hodgkin lymphoma by changing the expression of mature miR-196a in a Chinese population. Peng et al. (2014), in a meta-analysis comprising of 12 studies, reported a correlation between themiR-196a2 polymorphism and the development of HCC. Qi et al. (2015), in a case-control study with 321 breast cancer patients and 290 controls, suggested that the miR-196a2 polymorphism could predict breast cancer risk in the Chinese population.

So far, several studies have reported an association between the miR-196a2 polymorphism and development of gastric cancer, but with inconclusive results (Okubo et al., 2010; Ma et al., 2013; Dikeakos et al., 2014; Wei et al., 2015; Xu et al., 2015). Three studies reported a positive association between miR-196a2 polymorphism and susceptibility to gastric cancer (Ma et al., 2013; Dikeakos et al., 2014; Wei et al., 2015), while Okubo et al. (2010) did not. Wei et al. (2015), in a meta-analysis comprising eight case-control studies, reported that the miR-196a2 polymorphism could influence the development of gastric cancer.

There are three limitations in the present study. First, the patients and control subjects

were selected from only one hospital, which may bring in selection bias. However, the genetic distributions of the three SNPs were in agreement with the HWE, and thus the sample had representative of the general population. Second, some gene-gene interaction may influence the development of gastric cancer. Third, the sample size is relatively small, which may reduce the statistical power to find difference between groups.

In conclusion, our study indicated that the miR-196a2 polymorphism was associated with gastric cancer development, which can be used as the predictive biomarker for the susceptibility to gastric cancer. Further studies are required to validate our study findings.

Conflicts of interest

The authors declare no conflict of interest.

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