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Original Article

miR-124 rs531564 polymorphism influences genetic susceptibility to cervical cancer

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Abstract: Cervical cancer is the fourth most common cancer among women worldwide. It most frequently results from human papillomavirus (HPV) infection; however, recent evidence suggests that there may be underlying genetic factors, specifically in regions encoding microRNAs, dictating susceptibility to cervical cancer. This study investigated the relationship between the miR-124 rs531564 gene polymorphism and genetic susceptibility to cervical cancer in Chinese Han women. From January 2011 to July 2013, 158 Chinese Han cervical cancer patients and 260 healthy Chinese Han females were recruited to provide blood samples. The miR-124 rs531564 (C > G) polymorphism genotype was determined by polymerase chain reaction-based ligase detection reaction (PCR-LDR), and multivariate logistic regression analysis was used to deduce the relationship between the miR-124 rs531564 variant and cancer diagnosis. As expected, the incidence of HPV infection in cervical cancer patients was significantly higher than controls ($P < 0.001$). Logistic regression analysis showed that a CG genotype was associated with reduced risk of cervical cancer compared to the wildtype CC genotype (OR = 0.46, 95% CI: 0.19-0.92); the findings were similar when the variant genotypes were combined (CG + GG; OR = 0.42, 95% CI: 0.17-0.86). The G allele was associated with reduced risk of cervical cancer (OR = 0.45, 95% CI: 0.14-0.89) particularly among women over age 40 (OR = 0.31, 95% CI: 0.12-0.84), as well as reduced risk of HPV infection (OR = 0.59, 95% CI: 0.28-0.93). These results further support a role for genetic susceptibility in miR-124 rs531564 in determining the risk of cervical cancer in Chinese Han women.

Keywords: Cervical cancer, miR-124, gene polymorphism, genetic susceptibility

Introduction

Cervical cancer is the most common reproductive cancer among Chinese women. In 2012, approximately 62,000 new cases were reported in China along with about 30,000 cervical cancer-related deaths. Worldwide, roughly 528,000 new cases and 266,000 deaths were reported, making cervical cancer the fourth most common cancer in women across the globe [1]. Unfortunately, cervical cancer is becoming increasingly prevalent among young Chinese women [2]. Cervical cytology screening has made it possible to detect and treat the cancer in its early stages and has led to a decrease in overall incidence and mortality; however, cervical cancer remains one of the most deadly female-specific cancers due to its tendency to metastasize and relapse after treatment. While the majority of cervical cancers are caused by human papillomavirus (HPV) infection [3-5], genetic risk factors are thought to play a role in dictating predisposition to cervical cancer.

For example, recent findings have implicated miRNAs in the development of cervical cancer [6]. miRNAs are a class of highly conserved, endogenous, single-stranded, small RNA molecules of 20-22 nucleotides in length that play an intricate role in controlling gene expression [7]. miRNA polymorphisms have been shown to influence the processing and expression of miRNAs, thereby altering target gene expression and influencing the development of diseases [8, 9]. One such polymorphism, miR-124 rs531564 (C > G), has been shown to effect the expression of mature miR-124 [10]. This study sought to uncover the relationship between the miR-124 rs531564 polymorphism and suscep-

tibility to cervical cancer in Chinese Han women, providing a basis for early intervention and treatment of cervical cancer.

Participants and methods

Participants

This case-control study recruited 158 cervical cancer patients treated in Henan Provincial People's Hospital (Zhengzhou, China) and 260 healthy patients who received physical examinations between January 2011 and July 2013 to provide venous blood samples before treatment. Cervical cancer diagnosis was confirmed by pathologists. To be patients with cervical cancer, in whom, and no differences were detected in patient ages between the two groups. All subjects involved in this study were of Chinese Han descent with no blood relationship to each other. This study was approved by the Ethics Committee of Henan Provincial People's Hospital, The People's Hospital of Zhengzhou University (Zhengzhou, China), and all patients provided informed consent.

Methods

Biographical information collected from study participants included their age, smoking habits, knowledge of HPV infection, histological types, and tumor stages (if applicable).

Genomic DNA extraction

2Two mL of venous blood from each sample was mixed with EDTA-K2 for anticoagulation. A TIANamp Blood Genomic DNA Extraction Kit (TIANGEN Biotech, Beijing, China) was used to isolate DNA according to the manufacturer's protocol. Extracted DNA was stored at -80°C until ready for use.

Genotyping

Polymerase chain reactions (PCR) and ligase detection reactions (LDR) were used to genotype the miR-124 rs531564 polymorphism. Primers and probes for miR-124 rs531564 were synthesized by Sangon Biotech (Shanghai, China). The probe sequence for miR-124 rs531564-C was 5'-TTTTTTTTTTTTTTTTTTTTT-CTGTTT CTCTCCCTGAGTCTG-3', 5'-TTTTTTTTT-TTTTTTTTTTTTTCTGTTTCTCTCC CTGAGTCTC-3' for miR-124 rs531564-G, and 5'-P-TTTGCA-TCTCTAAGCCCC TGTTTTTTTTTTTTTTTTTTTT-FAM-3' for miR-124 rs531564-Fam, in which the length of an amplified target fragment was 240 bp. Upstream and downstream primers of miR-124 rs531564 were 5'-GA GGGGAGG-GGTCTGGAG-3' and 5'-GCCCAGA GAAAAATC-TGCAC-3', respectively. The reaction volume totaled 20 mL and went through 35 PCR cycles under the following conditions: predenaturation at 95°C for 2 minutes, denaturation at 94°C for 30 seconds, annealing at 59°C for 1 minute and 30 seconds, and extension at 72°C for 1 minute. Finally, the samples were extended at 72°C for 10 minutes. PCR reactions were carried out in a GeneAmp PCR System 9600 (PerkinElmer, Waltham, MA, USA). A 1% agarose gel electrophoresis was used to isolate PCR products. The LDR system involved a total volume of 10 mL and went through 30 cycles under the following conditions: predenaturation at 95°C for 2 minutes, denaturation at 94°C for 15 seconds, and reaction at 50°C for 25 seconds. An ABI 377 sequencer (Applied Biosystems, Foster City, CA, USA) was used to sequence the products, and GeneMapper 4.0 (Applied Biosystems, Foster City, CA, USA) was used to analyze data.

Statistical analysis

Double data entry was performed using EpiData version 3.1 (EpiData Association, Odense, Denmark) to create a data bank, and logic checks were then performed. SAS 9.2 (SAS Institute, Cary, NC, USA) was used to perform statistical analysis. We used the chi-square goodness-of-fit test to determine whether the genotype distribution at polymorphic loci was in accordance with Hardy-Weinberg equilibrium. Unconditional logistic regression analysis was used to determine significance; $P < 0.05$ was considered statistically significant.

Results

Participant information

No differences in age or smoking habits were found between cervical cancer patients and control patients. As expected, the HPV infection rate in the cervical cancer patients was markedly higher than in the control patients ($P < 0.001$). Of those with cancer, 92 patients (58.23%) presented with stage I cervical cancer, 60 (37.97%) with stage II, 4 (2.53%) with stage III, and 2 (1.27%) with stage IV. Further,

Table 1. Participant profiles [N (%)]

Variables	Controls (N = 260)	Cervical Cancer Patients (N = 158)	χ^2	P
Age (years)			0.030	0.985
< 40	133 (51.15)	82 (51.90)		
40-49	86 (33.08)	51 (32.28)		
≥ 50	41 (15.77)	25 (15.82)		
Smoking				0.268*
Yes	7 (2.69)	1 (0.63)		
No	253 (97.31)	157 (99.37)		
HPV infection			217.344	< 0.001
Yes	46 (17.69)	145 (91.77)		
No	214 (82.31)	13 (8.23)		
Stage			/	/
I	/	92 (58.23)		
II	/	60 (37.97)		
III	/	4 (2.53)		
IV	/	2 (1.27)		
Histologic type			/	/
Squamous cell carcinoma	/	137 (86.71)		
Adenocarcinomas	/	12 (7.59)		
Adenosquamous carcinoma	/	9 (5.70)		

Note: *Fisher's exact test.

Table 2. The miR-124 rs531564 polymorphism and genetic susceptibility to cervical cancer [N (%)]

	Cancer Patients (N = 158)	Control (N = 260)	*P*	OR (95% CI)
Genotype				
CC	134 (84.81)	184 (70.77)		1
CG	22 (13.92)	66 (25.38)	0.003	0.46 (0.19-0.92)
GG	2 (1.27)	10 (3.85)	0.132	0.39 (0.08-3.11)
CG + GG	24 (15.19)	76 (29.23)	0.001	0.42 (0.17-0.86)
Allele				
C	290 (91.77)	434 (83.46)		1
G	26 (8.23)	86 (16.54)	0.001	0.45 (0.14-0.89)

137 patients (86.71%) were confirmed to have squamous cell carcinoma, 12 (7.59%) had adenocarcinoma, and 9 (5.70%) had adenosquamous carcinoma.

miR-124 rs531564 genotypes

Genotyping at miR-124 rs531564 in control patients revealed the following distribution: 72.6% CC, 24.5% CG, and 9.2% GG (**Table 1**). A chi-squared test showed that, in healthy women, the distribution of genotypes at the miR-124 rs531564 locus was in accordance with Hardy-Weinberg equilibrium (P = 0.498), indicating that these women are representative of the general population.

Unconditional logistic regression analysis showed that a variant CG genotype reduced the risk of occurrence of cervical cancer in women compared to a wildtype CC genotype (OR = 0.46, 95% CI: 0.19-0.92); a GG genotype did not confer a statistically significant change in risk. Combined, the variant CG + GG genotypes were associated with reduced risk of occurrence of cervical cancer (OR = 0.42, 95% CI: 0.17-0.86); specifically, the G allele reduced the risk of occurrence of cervical cancer in women compared to the C allele (OR = 0.45, 95% CI: 0.14-0.89) (**Table 2**). Further, Patient aAge enhances the relationship between -stratified analysis of the correlation betweenthe miR-124 rs531564 polymorphism and genetic susceptibility to cervical cancerStratified analysis of the age, smoking, tumor stages and histological types showed that ilin subjects over 40 years of age, the G allele further reduced the risk of cervical cancer compared to the C allele (OR = 0.31, 95% CI: 0.12-0.84) (**Table 3**) OR.

Table 3. Increased genetic susceptibility to cervical cancer in women over 40 years of age at the miR-124 rs531564 polymorphism locus [N (%)]

	Cervical Cancer Cases (N = 76)	Controls (N = 127)	*P*	OR (95% CI)
Genotype				
CC	67 (88.16)	87 (68.50)		1
CG	7 (9.21)	35 (27.56)	0.002	0.34 (0.13-0.91)
GG	2 (2.63)	5 (3.94)	0.700	0.40 (0.11-3.42)
CG + GG	9 (11.84)	40 (31.5)	0.002	0.29 (0.09-0.88)
Allele				
C	141 (92.76)	209 (82.28)		1
G	11 (7.24)	45 (17.72)	0.003	0.31 (0.12-0.84)

Note: Results were adjusted for smoking status, stage, and histologic types.

Table 4. The miR-124 rs531564 polymorphism genotype is correlated with HPV infection [N (%)]

	HPV positive (N = 191)	HPV negative (N = 227)	*P*	OR (95% CI)
Genotype				
CC	156 (81.68)	154 (67.84)		1
CG	28 (14.66)	59 (25.99)	0.003	0.51 (0.23-0.96)
GG	7 (3.66)	14 (6.17)	0.132	0.54 (0.21-3.50)
CG + GG	35 (18.32)	73 (32.16)	0.001	0.52 (0.17-0.90)
Allele				
C	340 (89.01)	367 (80.84)		1
G	42 (10.99)	87 (19.16)	0.001	0.59 (0.28-0.93)

Correlation between miR-124 rs531564 polymorphism and risk of being infected with HPV

Unconditional logistic regression analysis showed that the CG genotype reduced the risk of occurrence of HPV infection in women compared to the CC genotype (OR = 0.51, 95% CI: 0.23-0.96); the GG genotype did not confer a significantly different risk. CG + GG genotypes reduced the risk of occurrence of HPV infection (OR = 0.52, 95% CI: 0.17-0.90), and, on its own, the G allele was associated with reduced risk of occurrence of HPV infection compared to the C allele (OR = 0.59, 95% CI: 0.28-0.93) (**Table 4**).

Discussion

Cervical cancer is the most common gynecological malignancy worldwide, and in China it is the leading cause of cancer-related death affecting the female reproductive tract. Risk factors include HPV infection as well as genetic predisposition [11].

In this study, we compared genotypes at the miR-124 rs531564 locus of women with and without a cervical cancer diagnosis. Unconditional logistic regression analysis showed that the CG genotype reduced the risk of cervical cancer compared to the CC genotype (OR = 0.46), as did the CG + GG genotypes (OR = 0.42). Specifically, the G allele was associated with reduced risk of occurrence of cervical cancer compared to the C allele (OR = 0.45). Furthermore, in subjects over 40 years of age, the G allele was closely correlated with a decrease in the development of cervical cancer.

Previous studies have shown that miR-124 is closely correlated with the development of cervical cancer [12, 13]. Mature miR-124 is composed of pre-MiR-124-1, pre-MiR-124-2, and pre-MiR-124-3, all of which exhibit decreased expression in multiple tumor tissue types [14-16]. The miR-124 rs531564 polymorphism has been shown to influence the processing of miRNAs, wherein the G allele influences the annular secondary structure of the pre-miR-124 and alters the expression of other miRNAs [9].

This study also found that the G allele of the miR-124 rs531564 polymorphism was less common in the cervical cancer patients than in the control women, which suggests that the presence of the G allele may reduce the risk of cancer development. Logistic regression analysis of HPV susceptibility revealed that the G allele reduced the risk of HPV infection (OR = 0.59), implying that this may be the mechanism by which the G allele protects women. We speculate that the G allele of the miR-124 rs531564 polymorphism promotes the expression of mature forms of miR-124 and reduces the risk of HPV infection, thus reducing the risk of cervical cancer development.

In summary, this study explored the relationship between the miR-124 rs531564 polymorphism and genetic susceptibility to cervical

cancer in Chinese Han women. Our results suggest that the G allele of the miR-124 rs531564 polymorphism may reduce the risk of occurrence of cervical cancer and provide a theoretical basis for novel methods to prevent and treat cervical cancer. Since the development of cervical cancer is a complex process involving multiple factors, additional studies are necessary to investigate the mechanisms by which certain genotypes render women more or less susceptible to cervical cancer. Such research will ultimately pave the way for new approaches to early screenings, prevention, and treatment of cervical cancer.

Disclosure of conflict of interest

None.

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