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Research Article

Association Study between miR-181b rs322931 Polymorphism and Risk of Gastric Cancer

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Abstract: ***Objective:*** To explore the relationship between miR-181b rs322931 polymorphism and the risk of gastric cancer. ***Methods:*** The peripheral venous blood of 172 patients with gastric cancer and 224 healthy controls were collected. The miR-181b rs322931 was typed by TaqMan probe method, and correlation between miR-181b rs322931 and the risk of gastric cancer was evaluated using SPSS software. ***Results:*** The frequencies of miR-181b rs322931 CC, CT, TT genotype, C, and T allele were 61.6, 33.7, 4.7, 78.5, and 21.5% in gastric cancer, and 74.1, 23.7, 2.2, 85.9, and 14.1% in controls, respectively. After the $\chi 2$ test and correction for age and gender, the risk of gastric cancer in carriers of CT and CT/TT genotypes increased by 1.71 and 1.79 times, respectively (CT vs. CC: 95% *CI*, 1.10-2.67, *P*=0.02; CT /TT compared with CC: 95%*CI*, 1.16-2.74, *P*=0.008). Moreover, compared with alleles, the risk of gastric cancer in T allele carriers increased by 1.69 times (95%*CI*, 1.16-2.44, *P*=0.006). ***Conclusion:*** miR-181b rs322931 polymorphism may be one of the susceptibility genes of gastric cancer in the Chinese Han nationality.

Key words: MicroRNA-181b; Single nucleotide polymorphism; Gastric cancer

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Gastric cancer ranks second among common malignant tumors in China, and its mortality rate ranks the third, which after lung cancer and liver cancer[1]. The incidence of gastric cancer is related to a variety of risk factors, including smoking, drinking, eating fried and preserved foods, helicobacter pylori infection and family history of tumors, etc[2]. However, not all individuals exposed to the above risk factors suffer from gastric cancer, and individuals who are not exposed to the above factors do not suffer from gastric cancer, suggesting that different individuals have different susceptibility to gastric cancer. Single nucleotide polymorphism (SNP) may affect the susceptibility of gastric cancer by affecting gene expression. Recently, studies have shown that different alleles of rs322931 may affect the expression of miR-181a and miR-181b[3], and it has been confirmed that miR-181 is closely related to the occurrence of gastric cancer[4-7]. In this study, a case-control study was conducted to investigate the relationship between the polymorphism of miR-181b rs322931 and gastric cancer in Chinese Han nationality, with the aim of identifying new susceptibility genes for gastric cancer.

1 Materials and Methods

1.1 Research object

Gastric cancer group: a total of 172 cases, inclusion criteria: (1) patients with gastric cancer diagnosed by histopathology; (2) denied family history of tumor; (3) Han nationality in Yunnan. Exclusion criteria: (1) Gastric cancer recurrence; (2) Primary cancer of other organs metastasized to the stomach; (3) Patients with severe heart, lung, liver, kidney and other organ organic diseases. The average age (mean ± standard deviation) was 58.7 ± 12.5 years, and the number of males and females was 117/55, respectively. 82 cases were clinical stage I-II, 90 cases were clinical stage III-IV; 124 cases of high-moderately differentiated carcinoma, 48 cases of poorly differentiated carcinoma; 67 cases with lymph node metastasis, 105

cases without lymph node metastasis.

Control group: a total of 224 cases, inclusion criteria: (1) healthy persons on physical examination; (2) denial of family history of tumor; (3) Han nationality in Yunnan. Exclusion criteria: patients with acute and chronic gastritis. The average age (mean ± standard deviation) was 57.7 ± 9.9 years, and the number of males and females was 157/67, respectively.

After all the study subjects signed the informed consent form, 2-3ml of peripheral EDTA anticoagulated venous blood was collected.

1.2 Main reagents and instruments

The whole blood genomic DNA extraction kit was purchased from Tiangen Biochemical (Beijing) Technology Co., Ltd., and the TaqMan probe (probe number: C_26961572_20) and 2× reaction mixture were purchased from American ABI company, and the ABI 7500 Fast fluorescent quantitative PCR instrument was also a product of American ABI company.

1.3 DNA extraction and miR-181b rs322931 typing

The whole blood genomic DNA extraction kit was used to extract DNA. The TaqMan probe method was used for genotyping miR-181b rs322931. The PCR amplification system was as follows (total volume 10μL): genomic DNA 20ng, 2×reaction mixture 5.0μL, rs322931 probe 0.25μL, sterile deionized water supplement 10μL. The PCR reaction conditions are as follows: 95℃ 10 min; 95℃ 15 s, 60℃ 60 s, a total of 40 cycles, the above procedures are completed on the ABI 7500 Fast real-time fluorescent quantitative PCR instrument.

1.4 Statistical methods

SPSS 13.0 statistical software package was used to process data. The t test or χ2 test was used to analyze the differences in age and gender distribution between the gastric cancer group and the control group. The χ^2 test was used to analyze the correlation between Hardy-Weinberg balance and miR-181b rs322931 and gastric cancer, and the relative risk was evaluated by odds ratio (OR) and 95% confidence interval (CI), $P<0.05$ The difference is statistically significant.

2 Results

2.1 General characteristics of research objects

The general characteristics of gastric cancer group and control group are shown in Table 1. There was no statistically significant difference in age and gender between the two groups. The P values were 0.55 and 0.66, respectively, indicating that the control group and the gastric cancer group were matched in age and gender.

Table 1. General situation of gastric cancer group and control group [*n* (%)]

General situation	Gastric cancer group	Control group	P value
Age (years, mean ± standard deviation)	58.7 ± 12.5	57.7 ± 9.9	0.55
Gender			
male	117 (68.0)	157 (70.1)	0.66
female	55 (32.0)	67 (29.9)	
clinical stage			
I-II	82 (47.7)		
III-IV	90 (52.3)		
Differentiation degree			
high-moderately differentiation	124 (72.1)		
Low differentiation	48 (27.9)		
Lymph node metastasis			
Has	67 (39.0)		
Hasn't	105 (61.0)		

2.2 Correlation between miR-181b rs322931 polymorphism and gastric cancer

The distribution of miR-181b rs322931 genotypes in the gastric cancer group and the control group was in accordance with the Hardy-Weinberg genetic balance, with P values of 0.99 and 0.75 respectively, indicating that there was no selection bias in the two groups, which could represent the population. The correlation between miR-181b rs322931 polymorphism and gastric cancer is shown in Table 2. The frequencies of CC, CT, TT genotypes, C and T alleles in the gastric cancer group were 61.6%, 33.7%, 4.7%, 78.5%, and

21.5%, respectively, and the frequencies in the control group were 74.1%, 23.7%, 2.2%, 85.9% and 14.1%, respectively. After the χ2 test and correction for age and gender, the risk of gastric cancer in carriers of CT and CT/TT genotypes increased by 1.71 and 1.79 times respectively (compare CT with CC, 95% *CI*, 1.10-2.67, *P*=0.02; CT /TT compared with CC, 95%*CI*, 1.16-2.74, *P*=0.008); compared with alleles, the risk of gastric cancer in T allele carriers increased by 1.69 times (95%*CI*, 1.16-2.44, *P* =0.006). The miR-181b rs322931 polymorphism has no correlation with the clinical features of gastric cancer, such as the clinical stage, degree of differentiation, and lymph node metastasis.

Table 2. Correlation between miR-181b rs322931 polymorphism and gastric cancer

rs32293polymorphism	Control group (%)	Gastric cancer group (%)	Correction *OR*(95 % *CI*)	Corrected P value
CC	166 (74.1)	106 (61.6)	1.0	
CT	53 (23.7)	58 (33.7)	1.71 (1.10-2.67)	0.02
TT	5 (2.2)	8 (4.7)	2.57 (0.81-8.13)	0.1
CT/TT	58 (25.9)	66 (38.4)	1.79 (1.16-2.74)	0.008
C allele	385 (85.9)	270 (78.5)	1.0	
T allele	63 (14.1)	74 (21.5)	1.69 (1.16-2.44)	0.006

3 Discussion

Although RNA molecules do not encode proteins, but they are involved in the pathophysiological processes of many diseases as regulatory molecules, including cell proliferation, apoptosis, differentiation, development, metabolism, invasion, migration, and angiogenesis. The miR-181 family includes miR-181a, miR-181b, and miR-181c. Among them, miR-181a and miR-181b are well studied and highly expressed in gastric cancer[4-7]. Compared with clinical stage I-II patients with gastric cancer without lymph node metastasis, the expression of miR-181a and miR-181b in patients with clinical stage III-IV gastric cancer and lymph node metastasis was significantly increased[4]. Overexpression of miR-181a can target autophagy-related genes 5 to up-regulate cell cycle regulatory proteins CDC25A, cyclin A2, and apoptosis-related protein Bcl-2 to promote cell proliferation, inhibit cell apoptosis, and increase S-phase cells[6-8]. On the contrary, low expression of miR-181a can inhibit cell proliferation, promote cell apoptosis, and arrest cells in G_0/G_1 phase[7, 9]. The above results indicate that miR-181 is expected to be used as an auxiliary diagnostic marker for gastric cancer and as a potential drug target for gastric cancer treatment.

A number of studies have confirmed that miRNA related SNPs can affect miR-181 ability to process, mature and bind target genes, resulting in individual differences in susceptibility to gastric cancer.For example, the insertion / deletion polymorphism of TTCA in the 3 'untranslated region of IL-1A, the insertion allele of TTCA, destroys the binding sites of miR-122 and miR-378, increases the transcription level of IL-1 α, and increases the susceptibility to gastric cancer[10]. Rs1056628 A / C single nucleotide mutation located in the 9 3 'untranslated region of matrix metalloproteinase, C allele has weaker ability to bind miR-491-5p, reduced transcriptional activity, and increased risk of gastric cancer[11]. Since miR-181 plays a role similar to oncogene in the evolution of gastric cancer, it is speculated that the genetic polymorphism of miR-181 may be related to the pathogenesis of gastric cancer. In order to verify this hypothesis, a case-control study was conducted to investigate the distribution of rs322931 polymorphism in 172 patients with gastric cancer and 224 healthy controls. It was found that individuals with CT, CT / TT genotype and T allele increased the risk of gastric cancer by 1.71, 1.79 and 1.69 times, respectively. As for the possible reasons, some studies have shown that the expression of miR-181b-5p in rs32293 T allele carriers is significantly increased[3], which suggests that T allele may increase the expression of miR-181b, thereby increasing the risk of gastric cancer. However, the exact mechanism needs to be further confirmed.

In summary, this study analyzed the correlation between the miR-181b rs322931 polymorphism and the incidence of gastric cancer in the Han nationality in China for the first time, and found that individuals carrying CT, CT/TT genotype and T alleles have a significantly increased risk of developing gastric cancer. It may be one of the susceptibility factors of gastric cancer in Chinese Han nationality. Since the sample is limited to the Han nationality and the sample size is small, it is necessary to carry out multi-

ethnic, large sample confirmatory research in the future.

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