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Original Article

Correlation between miRNA-196a2 and miRNA-499 polymorphisms and bladder cancer

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Abstract: MicroRNA (miRNA) polymorphisms are closely associated with cancers. This study explored the correlation between miRNA-196a2 rs11614913 and miRNA-499 rs3746444 polymorphisms and the occurrence and pathological features of bladder cancer. The case group consisted of 372 patients with bladder cancer, and the control group consisted of 372 healthy volunteers. Massarray system was used to detect single nucleotide polymorphisms (SNPs) at miRNA-499 rs3746444 and miRNA-196a2 rs11614913 sites in the two groups. Correlation between SNPs at these two sites in miRNA and the occurrence and pathological features of bladder cancer was assessed using logistic regression analysis. Genotype frequencies at rs11614913 site showed significant difference between the two groups (x^2=18.586, P<0.001). The frequency of TT genotype in the case group (18.38%) was significantly lower than that of the control group (33.35%) (P<0.05). The risk of bladder cancer among carriers of TT genotype was 0.57 times of that among carriers of CC genotype ($OR_{adjusted}$=0.57, 95% CI: 0.41~0.85). The frequency of the genotype at rs3746444 site was not significantly different between the two groups (x^2=5.107, P=0.078)). However, the frequency of GG genotype in bladder patients (7.96%) was much higher than that of the controls (3.51%) (P<0.05). The risk of bladder cancer in carriers of GG genotype was 2.33 times of that among the carriers of AA genotype ($OR_{adjusted}$=2.33, 95% CI: 1.15~5.12). The genotype frequency at rs11614913 and rs3746444 sites did not vary significantly with tumor staging, adjacent and distant lymph node metastasis (P>0.05). For patients showing different differentiation degree, the genotype frequency at these two sites differed significantly (rs11614913: x^2=18.586, P=0.005; rs3746444: x^2=11.120, P=0.025). Polymorphisms at miRNA-499 rs3746444 and miRNA-196a2 rs11614913 sites were correlated with the risk of bladder cancer as well as the differentiation degree of the cancer.

Keywords: Bladder cancer, miRNA, polymorphism

Introduction

Bladder cancer is a common cancer of the urinary system and ranks among the top 10 of all cancers in terms of mortality in China. The inducing factors of bladder cancer include smoking [1], exposure to chemical products, chronic infections, drug use (e.g., cyclophosphamide and phenacetin) and genetic factors [2-4].

Single nucleotide polymorphism (SNP) widely present in the genome is associated with various tumors including bladder cancer [5]. microRNA (miRNA) is a non-coding RNA molecule with a length of 21-23nt and is highly conservative. Targeting mRNA, miRNA can degrade the mRNA or inhibit its translation on the post-transcriptional level, thus blocking the synthesis of the target protein. miRNAs are involved in individual development, organ formation, cell proliferation, differentiation and apoptosis [6]. According to the latest research, abnormal expression of miRNA can be found in several diseases including tumors [7] and cardiovascular diseases [8]. The miR-196a2 rs11614913 T>C polymorphism is associated with the risk of several tumors, and miRNA-499 rs3746444 polymorphism is a proven contributor to tumors [9-11]. These two SNPs in miRNAs are rarely reported with respect to their correlation with bladder cancer among Chinese population, and here we attempted to test for any correlations.

Subjects and methods

Subjects

From January 2012 to December 2015, 372 patients pathologically confirmed as bladder

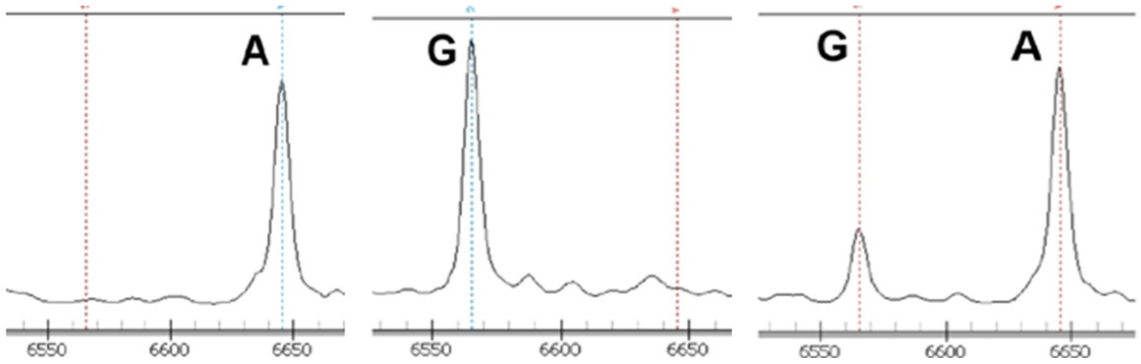

Figure 1. Genotyping of rs3746444.

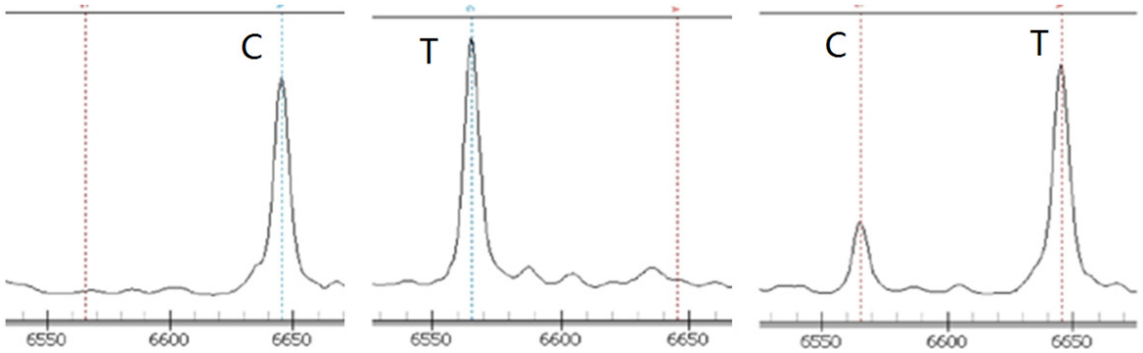

Figure 2. Genotyping of rs11614913.

cancer were recruited, including 283 males and 89 females with an average age of 66.11 ± 13.43 years. For the control group, 372 healthy cases receiving physical examination were recruited, including 279 males and 93 females with an average age of 65.73 ± 13.68 years. The control cases had no current or past history of urinary system diseases. All cases were Han people and came from Nanjing or neighboring regions. The two groups showed no significant differences in gender distribution, age, smoking status and alcohol consumption (P>0.05). The experiment protocol was approved by the ethics committee of the hospital and the informed consent was obtained from all cases.

Pathological diagnosis

Tumor samples were collected during surgery along with adjacent and distant lymph nodes for pathological examination. The patients were classified into stage I-IV by tumor extent (T), lymph node involvement (N) and lymph node metastasis (M). By differentiation degree, the tumors were divided into high, moderate and low degree of differentiation.

Genomic DNA extraction and detection

From each subject, 3 ml of peripheral blood was drawn and added with EDTA-K_2 as the anticoagulant. After centrifugation at 500×g for 5 min, the serum was discarded and 300 μL of red blood cell lysis buffer was added with oscillation for 3 min. Centrifugation was conducted again at 10000×g for 30 s to obtain the precipitate of white blood cells, which was cryopreserved at -80°C. GoldMag whole-blood genomic DNA extraction kit (Xi'an Goldmag Nano-Biotechnology Co., Ltd.) was used for genomic DNA extraction according to the manufacture's instruction. Concentration and purity of genomic DNA were analyzed using NanoDrop 2000c.

Genotyping

The sequences of the above two SNP sites were downloaded from NCBI database (http://www.ncbi.nlm.nih.gov/projects/SNP/). PCR primers and single base extension (SBE) primers were designed using Genotyping Tools and Massarray Assay Design (Sequenom, USA). The primers were synthesized by Shanghai Invi-

Table 1. Frequencies of genotypes at rs11614913 and rs3746444 sites for different pathological features of bladder cancer [n (%)]

Genotype	Rs11614913, genotype			X^2	P	rs3746444, genotype			X^2	P
	CC	CT	TT			AA	AG	GG		
Tumor stage (T)										
I~II stage	65 (23.99)	153 (56.46)	53 (19.55)	1.936	0.354	180 (66.42)	67 (24.72)	24 (8.86)	1.259	0.482
III~IV stage	32 (31.68)	54 (53.47)	15 (14.85)			69 (68.31)	26 (25.74)	6 (5.94)		
Proximal lymph node metastasis (N)										
Yes	51 (32.08)	82 (51.57)	26 (16.35)	7.647	0.095	105 (66.04)	35 (22.01)	19 (11.95)	5.235	0.183
No	45 (21.13)	124 (58.21)	44 (20.66)			143 (67.14)	63 (29.58)	7 (3.29)		
Distal lymph node metastasis (M)										
Yes	29 (29.90)	54 (55.67)	14 (14.43)	1.435	0.549	65 (67.01)	26 (26.80)	6 (6.19)	0.938	0.476
No	68 (24.73)	166 (60.36)	41 (14.91)			182 (66.18)	69 (25.09)	24 (8.73)		
Differentiation										
Well-Differentiation	45 (40.18)	54 (48.21)	13 (11.61)	18.586	0.005	86 (76.79)	21 (18.75)	5 (4.46)	14.683	0.034
Intermediate-Differentiation	27 (22.69)	63 (52.94)	29 (24.37)			74 (62.18)	39 (32.77)	6 (5.05)		
Poor-Differentiation	26 (18.44)	87 (61.70)	28 (19.86)			89 (63.12)	35 (24.82)	17 (12.06)		

Table 2. Logistic regression of Rs11614913 and rs3746444 associated with Bladder Cancer

Genotype	Control group (n=283)	Bladder Cancer Group (n=283)	OR (95% CI)
Rs11614913			
CC (%)	22.86	25.89	1
CT (%)	43.79	55.73	1.32 (0.72~1.46)
TT (%)	33.35	18.38	0.57 (0.41~0.85)
CT/TT (%)	77.14	74.11	0.82 (0.46~1.12)
Rs3746444			
AA (%)	71.86	67.23	1
GA (%)	24.63	24.81	1.25 (0.64~1.73)
GG (%)	3.51	7.96	2.33 (1.15~5.12)
GA/GG (%)	28.14	32.77	1.26 (0.76~1.68)

trogen Biotechnology Co., Ltd. SNP genotyping was performed at these two sites for all samples using MassARRAY® System through the following procedures: PCR amplification, digestion with shrimp alkaline phosphatase (SAP), SBE-PCR, resin purification, microarray spotting, mass spectrometry, and result analysis. The experiment was assisted by Shaanxi Lifegen Co., Ltd. **Figures 1** and **2** showed the genotyping results.

Statistical process

Statistical analyses were carried out using SPSS 11.0 software. Measurements were reported as mean ± standard deviation ($\bar{x} \pm s$). Intergroup comparisons were done using independent two-sample t-test. Count data were reported as frequencies and ratios, and the intergroup comparison was done using X^2 test, with $P \leq 0.05$ indicating significant difference. Correlation between genotype frequencies at SNP sites and the risk of bladder cancer was assessed using binary logistic regression. OR and 95% CI were calculated as the relative risk.

Result

Testing of Hardy-Weinberg equilibrium: Hardy-Weinberg equilibrium was tested for the control samples, and the genotype frequencies at the two SNP sites obeyed Hardy-Weinberg equilibrium ($P>0.05$). This meant the samples were representative of the population.

Correlation between SNPs and pathological features of bladder cancer: The patients were stratified by tumor staging, adjacent lymph node metastasis, distant lymph node metastasis and differentiation degree. X^2 test indicated significant differences in genotype frequencies at these two sites for different differentiation degree ($P<0.05$) (**Table 1**).

Genotype frequency distributions in the two groups: After correction for the factors of age, gender, smoking status and alcohol consumption, multiple logistic regression indicated that genotype frequencies at rs11614913 site were significantly different between the two groups ($x^2=18.586$, $P<0.001$). The frequency of TT genotype was much lower in the case group as compared to the control group ($P<0.05$). Genotype frequencies at rs3746444 site showed no significant differences between

the two groups (x^2=7.143, P=0.085). However, regression analysis indicated that the frequency of GG genotype was significantly higher in the case group as compared with the control group (P<0.05) (**Table 2**).

Discussion

The role of miRNA polymorphisms in tumors has been intensively studied in recent years, and miRNA-499 rs3746444 and miRNA-196a2 rs11614913 sites attract particular attention. Yuan et al. [12] analyzed the correlation between polymorphism at rs11614913 site and lung cancer in the Chinese population and found that C allele at this site was a contributor. Hu et al. [13] also indicated a higher risk of breast cancer among Chinese women carrying C allele at rs11614913 site. The present study focused on Han people in Nanjing and found that TT genotype at rs11614913 site was negatively correlated with the risk of bladder cancer, which agreed with previous researches. C allele was associated with downregulation of miR-196a2, thus influencing its ability to regulate target genes and response to DNA damage. He et al. [14] conducted a meta-analysis, which found that rs11614913 TT genotype was negatively correlated with the risk of tumors (TT vs CC: OR=0.80, 95% CI: 0.73~0.88; TT vs CC+CT: OR=0.85, 95% CI: 0.80~0.92).

Correlation between miRNA-499 rs3746444 polymorphism and tumors has been widely studied, and meta-analysis has confirmed their correlation [14]. This polymorphism contributes to liver cancer in the Chinese population [15]. According to some literature, polymorphism in miRNA-499 rs3746444 site was correlated with tumors in Asian population [16, 17], but not in European population [18]. Functional analysis has indicated that miRNA-499 can bind to c-MET mRNA and reduce the expression of the latter, thus promoting cell apoptosis and inhibiting cell proliferation. In the present study, rs3746444 GG genotype was a contributor to bladder cancer, consistent with the above researches.

In the present study, the patients were stratified by pathological features of bladder cancer. Genotype frequencies at SNP sites were analyzed by X^2 test. It was found that the genotype frequencies were not significantly different for patients with different pathological features (tumor staging, adjacent and distant lymph node metastasis). But the genotype frequencies varied among patients with different differentiation degree. The two SNP sites discussed above fulfill certain biological functions and contribute to tumors. However, few studies address their correlation with tumor's differentiation degree. Moreover, since the sample size was small, this finding is less convincing statistically and investigations with larger sample size are needed for clarification.

To conclude, polymorphism in miRNA-499 rs3746444 and miRNA-196a2 rs11614913 sites is correlated with the risk of bladder cancer and differentiation degree of the cancer. But we are still uncertain about the molecular mechanism.

Disclosure of conflict of interest

None.

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