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RESEARCH ARTICLE

Association between polymorphisms of microRNA-binding sites in integrin genes and gastric cancer in Chinese Han population

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Abstract Highly elevated expression of integrin has been observed in a variety of malignant tumors. Single nucleotide polymorphisms (SNPs) in the microRNA-binding sites in the 3′ UTR region of target genes may result in the level change of target gene expression and subsequently susceptible to diseases, including cancer. In this study, we aimed to investigate the association between polymorphisms of microRNA-binding sites of integrin genes and gastric cancer (GC) in Chinese Han population. Five SNPs of the microRNA-binding sites in the 3′ UTR region of integrin genes (rs1062484 C/T in ITGA3, rs17664 A/G in ITGA6, rs3809865 A/T in ITGB3, rs743554 C/T in ITGB4, and rs2675 A/C in ITGB5) were studied using high resolution melting (HRM) analysis in 1000 GC patients and 1000 unrelated controls. The polymorphism of SNP rs2675 was associated with susceptibility of GC [odds ratio (OR)=0.52, 95 % confidence interval (CI)=0.28–0.97, P=0.028]. In addition, genotype AA of rs2675 and genotype GG of rs17664 were associated with a lower chance of GC at stage 1b [OR=0.39 (0.18–0.85), P=0.009; and OR=0.37 (0.17–0.78), P=0.004, respectively]; also, the frequency of allele G of rs17664 was associated with a lower chance of stage 1b tumor [OR=0.50 (0.26–0.95), P=0.021]. Furthermore, the frequency of genotype AA and allele A of rs3809865 were associated with a higher risk of stage 4 GC [OR=1.85 (1.11–3.09), P=0.012; and OR=1.52 (0.99–2.33), P=0.043, respectively]. For rs17664, GG genotype and allele G appeared to be associated with a higher risk with GC with lymphatic metastasis 3b [OR=1.76 (1.00–3.11), P=0.036; and OR=1.64 (0.98–2.75), P=0.048, respectively]. Our data suggest that polymorphisms of the microRNA-binding sites in the 3′ UTR region of integrin are associated with GC susceptibility (rs2675), tumor stage (rs2675, rs17664, and rs3809865), and lymphatic metastasis (rs17664) in Chinese Han population.

Keywords MicroRNA · Single nucleotide polymorphism · Integrin · Gastric cancer

Introduction

Gastric cancer (GC) is an aggressive disease, which remains a serious public health problem, especially in Eastern Asian and Western Europe [1]. It is the fourth most common malignancy and the second leading cause of cancer mortality worldwide [2]. It was estimated that approximately 4.6 million new cases of GC occurred in 2008 in China, accounting for 42 % of the total cases around the world [3]. Although the current treatments, including surgery, chemotherapy, radiation, and immunotherapy, have significantly improved the survival of GC patients without metastasis, the prognosis of patients with metastatic GC is still poor and the 5-year overall survival rate remains only 10 to 15 % [4, 5]. Both environmental and genetic factors have been thought to contribute to the development of GC [6]. Classic epidemiological studies have determined that *Helicobacter pylori* (HP) infection, salted and nitrated food, heavy alcohol drinking, and smoking are major risk factors associated with GC [7]. In the meantime, different individuals in the same environment have significant variance of the risk in tumorigenesis, invasion, and metastasis. Such

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variations have been postulated to be associated with gene polymorphism [8–10].

MicroRNAs are short, endogenous, single-stranded, and non-coding RNAs consisting of about 22 nucleotides [11]. MicroRNAs regulate gene expression during the translation process and functionally act as a translational inhibitor of the specific mRNAs by targeting the untranslated 3′ UTR region [12]. It was reported that microRNAs are involved in a variety of biological processes, including cell differentiation, proliferation, stress responses, fat metabolism, apoptosis, and development. Therefore, microRNAs play important roles in the pathogenesis of various diseases including cancer [13, 14].

Integrins are a family of cell adhesion receptors that are critical to maintain the integrity of complex tissues and organs in animals [15]. They interact with the extracellular matrix and bind to intracellular linker proteins that are connected with the cytoskeleton, thereby mediating cell adhesion [16, 17]. The genetic polymorphisms of microRNA-binding region within 3′ UTR region of integrin gene are thought to be the cause of the variation of the expression of integrin, which may become a risk factor of many diseases. Several studies have been conducted to investigate the relationship between SNPs in microRNA-binding site in integrin genes and the risk in developing of cancer [18–20]. A previous research reported that the C807T polymorphism in integrin alpha 2 (ITGA2) gene might be associated with risk of GC development, differentiation, and invasion [21]. Scartozzi et al. [22] found that polymorphism of rs2269772 (targeting ITGA3) and rs11902171 (targeting ITGV) may be used to define high-risk GC patients for peritoneal carcinomatosis among those who relapsed after curative resection. However, the association between the polymorphisms of microRNA-targeting region within integrin gene and the susceptibility to GC has not been well studied.

In the current study, we selected five SNPs of the microRNA-binding sites in 3′ UTR region of integrin gene (rs1062484 C/T in ITGA3, rs17664 A/G in ITGA6, rs3809865 A/T in ITGB3, rs743554 C/T in ITGB4, and rs2675 A/C in ITGB5) and determined the relevance of these SNPs with GC susceptibility as well as its other clinical characteristics in Chinese Han ethnic population.

Materials and methods

Study populations

We enrolled 1000 GC patients in Chinese Han population who visited West China Hospital of Sichuan University from Sept. 2010 to Nov. 2013. The diagnosis of GC was made in accordance with the clinical criteria and pathological confirmation. A total of 1000 unrelated healthy Han controls were selected with comparable age and sex with GC cases. All of controls had not a history of any major medical illness. These healthy individuals were recruited from the routine physical examination during the same period and in the same regions of GC patients. The participants with blood relationship were excluded. All participants gave written consents, and this study was approved by the ethical committee of West China Hospital of Sichuan University.

Genomic DNA extraction and high resolution melting (HRM) analysis

Genomic DNA was extracted from the whole blood using QIAamp® DNA blood mini kit (Qiagen, Düsseldorf, Germany) following the manufacturer's protocol. The isolated DNA was stored at −80 °C. Genotypes of the five SNPs were determined using HRM method. Polymerase chain reaction (PCR) amplifications were carried out in the LightCycler® 480 (Roche Diagnostics, Bavaria, Germany). Three DNA samples with known genotypes were run concurrently in every experiment as references. The PCR reaction mixture (20 μL) contained the following: 10.8 μL H_2O, 2 μL 10× buffer, 2 μL $MgCl_2$ (25 mM), 1 μL 20×EVA-GREEN, 2 μL dNTP (10 mM), 0.5 μL forward primer (10 μM), 0.5 μL reverse primer (10 μM), 0.2 μL Hot Star *Taq*® Plus DNA Polymerase (5 U/μL), and 1 μL genomic DNA (10 ng/μL). Real-time PCR was performed with the following conditions: an initial denaturation at 95 °C for 15 min, followed by 50 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s, extension at 72 °C for 25 s. Following the completion of the cycle program, PCR products were denatured at 95 °C for 1 min and cooled to 40 °C for 1 min to form double-strand DNA. Then, the HRM analysis was conducted by gradually increasing the temperature from 65 to 95 °C at the rate of 0.01 °C/s. The data were analyzed by the LightCycler®480 Gene Scanning software (v1.2, Roche Diagnostics, Bavaria, Germany).

Statistical analysis

Statistical analyses were performed using SPSS statistical software package (version 17.0, SPSS Inc., USA). The Hardy-Weinberg equilibrium (HWE) was determined using the goodness-of-fit chi-squared test (χ^2). The genotype and allelic frequencies of the SNP loci were calculated by direct count. Differences of categorical variables such as genotype and allele frequencies between GC patients and controls were tested by Pearson's chi-squared test. Odds ratio (OR) and 95 % confidence intervals (CIs) were calculated using logistic regression. A *P*-value of less than 0.05 was considered to be statistically significant.

Results

Basic demographic data of subjects

A total of 2000 subjects (1000 cases and 1000 controls) were enrolled in the current study. All of them were from Chinese Han population, and there were no significant differences between case and control groups in terms of the distribution of sex, age, smoking status, and drinking status, as summarized in Table 1.

The relevance between SNPs of microRNA-binding sites in integrin genes and GC susceptibility

All individuals of patients and controls had been successfully genotyped for the five SNPs: rs1062484 C/T, rs17664 A/G, rs3809865 A/T, rs743554 C/T, and rs2675 A/C. No deviation from HWE was detected for genotype distributions of the five SNPs in either patient or control groups ($P>0.05$). The genotype distributions and allele frequencies of the five SNPs were counted, calculated, and summarized in Table 2.

Subjects carrying a genotype of CC in rs2675 showed a significant decrease in risk for GC than individuals carrying AA genotype [OR=0.52, 95 % CI=0.28–0.97, $P=0.028$]. It was suggested that genotype AA of rs2675 may be associated with a higher risk of GC. No significant differences of genotype distributions or allelic frequencies of the other four examined SNPs were found between patient and healthy control groups.

Stratified analysis for the association between SNPs and GC clinical characteristics

To determine whether there is an association between SNPs in the microRNA-binding sites in 3′ UTR region of integrin genes and certain clinical parameters of GC, we further divided GC patients into subgroups according to the tumor size, tumor stage, differentiation degree, and lymphatic metastasis. The SNPs rs1062484 C/T and rs743554 C/T were not included in the stratified analysis since there were only two genotypes and their dominant genotype accounted for more than 90 % of the overall genotype. Using the lowest level subgroup in each parameter as the reference, we determined the relevance of genotypes and alleles of rs2675, rs17664, and rs3809865 with tumor size, tumor stage, differentiation degree, and lymphatic metastasis. The comparison results for each clinical parameter at different subgroup are summarized in Tables 3, 4, and 5, respectively. As shown in Table 3, genotype AA of rs2675 was significantly lower in stage 1b than stage 1a, suggesting an association with a lower chance with GC at this stage 1b [OR=0.39 (0.18–0.85), $P=0.009$]. However, the frequency of allele A did not show significant association with this stage. Regarding rs17664, as shown in Table 4, the genotype GG at stage 1b was significantly lower than stage 1a, suggesting an association with a lower chance for this stage GC [OR=0.37 (0.17–0.78), $P=0.004$]. Also, the frequency of allele G appeared to be associated with a lower chance of this stage tumor

Table 1 Basic demographic data of subjects and clinical characteristics of GC cases

Parameters	Case		Control		*P*
	n	Frequencies (%)	*n*	Frequencies (%)	
Age (year, mean±SD)	56.6±12.1		57.4±12.4		0.151
Gender					
Male	667	66.7	629	62.9	0.075
Female	333	33.3	371	37.1	
Smoking status					
Smokers	432	43.2	408	40.8	0.277
Nonsmokers	568	56.8	592	59.2	
Drinking status					
Drinker	313	31.3	307	30.7	0.772
Nondrinker	687	68.7	693	69.3	
Tumor size (diameter)					
<5 cm	319	31.9			
5–10 cm	345	34.5			
≥10 cm	90	9.0			
N.A.	246	24.6			
Tumor stages					
1a	93	9.3			
1b	61	6.1			
2a	75	7.5			
2b	108	10.8			
3a	91	9.1			
3b	119	11.9			
3c	190	19.0			
4	263	26.3			
Degree of differentiation					
Low	639	63.9			
Medium	201	20.1			
High	160	16.0			
Lymphatic metastasis					
0	236	23.6			
1	157	15.7			
2	169	16.9			
3a	194	19.4			
3b	121	12.1			
N.A.	123	12.3			

N.A. data not available

Table 2 Comparisons of gene polymorphisms between case and control groups

SNP	Cases		Controls		OR (95 % CI)*	*P**
	N	%	*N*	%		
rs1062484						
Genotype						
CC	992	99.2	987	98.7	1.00 (reference)	
CT	8	0.8	13	1.3	0.66 (0.25–1.75)	0.367
Allele						
C	1992	99.6	1987	99.4	1.00 (reference)	
T	8	0.4	13	0.7	0.61 (0.25–1.48)	0.274
rs2675						
Genotype						
AA	708	70.8	681	68.1	1.00 (reference)	
AC	274	27.4	287	28.7	0.92 (0.75–1.13)	0.414
CC	18	1.8	32	3.2	0.52 (0.28–0.97)	**0.028**
Allele						
A	1690	84.5	1649	82.5	1.00 (reference)	
C	310	15.5	351	17.6	0.86 (0.73–1.02)	0.081
rs17664						
Genotype						
GG	732	73.2	741	74.1	1.00 (reference)	
AG	252	25.2	248	24.8	1.04 (0.84–1.27)	0.736
AA	16	1.6	11	1.1	1.57 (0.69–3.60)	0.245
Allele						
G	1716	85.8	1730	86.5	1.00 (reference)	
A	284	14.2	270	13.5	1.06 (0.89–1.27)	0.522
rs743554						
Genotype						
CC	965	96.5	969	96.9	1.00 (reference)	
CT	35	3.5	31	3.1	1.17 (0.70–1.96)	0.534
Allele						
C	1965	98.3	1969	98.5	1.00 (reference)	
T	35	1.8	31	1.6	1.13 (0.70–1.84)	0.620
rs3809865						
Genotype						
AA	631	63.1	654	65.4	1.00 (reference)	
AT	331	33.1	314	31.4	1.09 (0.90–1.33)	0.351
TT	38	3.8	32	3.2	1.27 (0.76–2.13)	0.331
Allele						
A	1593	79.7	1622	81.1	1.00 (reference)	
T	407	20.4	378	18.9	1.10 (0.94–1.28)	0.248

*Adjusted by sex, age, smoking status, and drinking status

[OR=0.50 (0.26–0.95), *P*=0.021]. However, GG genotype and allele G of rs17664 appeared to be associated with a higher risk with lymphatic metastasis 3b [OR=1.76 (1.00–3.11), *P*=0.036; and OR=1.64 (0.98–2.75), *P*=0.048, respectively]. For rs3809865, the frequency of genotype AA and allele A in patients at tumor stage 4 is significantly higher than those in stage 1a and appeared to be associated with a higher risk with this stage GC [OR=1.85 (1.11–3.09), *P*=0.012; and OR=1.52 (0.99–2.33), *P*=0.043, respectively]. Further study is needed to determine the mechanisms underlying these associations.

Discussion

Genetic factors play an important role in the tumorigenesis of GC. In the present study, we examined the possible association between five SNPs of the microRNA-binding site in 3′ UTR region of integrin genes (rs1062484 in ITGA3, rs17664 in ITGA6, rs3809865 in ITGB3, rs743554 in ITGB4, and rs2675 in ITGB5) and GC in Chinese Han population. The main finding of the study was that polymorphism of SNP rs2675 was associated with susceptibility to GC. Genotype AA of rs2675 as well as genotype GG and allele G of rs17664 were associated with a lower chance with GC at stage 1b. The frequency of genotype AA and allele A of rs3809865 were associated with a higher risk with GC at stage 4. In addition, the frequency of genotype GG and allele G of rs17664 were associated with a higher risk of GC with lymphatic metastasis 3b.

MicroRNAs are involved in a variety of important biological processes such as cell differentiation, proliferation, and apoptosis [23]. Recent studies have confirmed that microRNAs play an important role in a variety of pathways related to cell proliferation and apoptosis and some of them are associated with tumorigenesis. In addition, abnormal expression of microRNAs was observed in cancer patients [24–26]. Extensive researches have been done to investigate the relationship between the polymorphisms within or near the microRNA-binding sites and phenotypic variation including disease susceptibility [27–29]. It has been shown that the genetic mutation within 3′ UTR region of MDM4 gene (SNP rs34091) results in a binding site for has-miR-191. Because of has-miR-191-mediated inhibition, ovarian cancer patients with this genetic mutation have a lower MDM4 expression and slower progression [30]. In addition, patients with the genotype CC of the SNP rs8126, which is in the binding sites of MiR-184 on the TNFAIP2 gene, have a relatively higher susceptibility to GC than those with genotypes TT and TC [31].

Integrins are a family of transmembrane glycoproteins consisting of noncovalent heterodimers. Previous studies demonstrated that integrins are highly expressed in many malignant tumors. Because certain integrins can hydrolyze the basement membrane protein, their expression on tumor cells promotes metastasis by accelerating cell migration and adhesion [32–35]. Consistently, the

Table 3 Stratified analysis for the association between rs2675 and GC clinical characteristics

Clinical characteristics	Genotype			OR (95 % CI)*	*P*	Allele		OR (95 % CI)	*P*
	AA	AC	CC			A	C		
Tumor size									
<5 cm	233	79	7	1.00 (reference)		545	93	1.00 (reference)	
5–10 cm	236	102	7	0.80 (0.57–1.14)	0.204	574	116	0.84 (0.62–1.15)	0.264
≥10 cm	65	23	2	0.97 (0.56–1.69)	0.899	153	27	0.97 (0.59–1.58)	0.888
Tumor stages									
1a	72	18	3	1.00 (reference)		162	24	1.00 (reference)	
1b	37	24	0	0.39 (0.18–0.85)	**0.009**	98	24	0.60 (0.31–1.17)	0.109
2a	58	16	1	0.93 (0.42–2.07)	0.857	132	18	1.09 (0.54–2.19)	0.803
2b	73	31	4	0.57 (0.29–1.13)	0.083	177	39	0.67 (0.37–1.21)	0.157
3a	68	23	0	0.81 (0.39–1.69)	0.546	159	23	1.02 (0.53–1.97)	0.940
3b	86	32	1	0.68 (0.35–1.35)	0.241	204	34	0.89 (0.49–1.61)	0.681
3c	134	52	4	0.66 (0.35–1.35)	0.155	320	60	0.79 (0.46–1.35)	0.364
4	180	78	5	0.58 (0.32–1.05)	0.057	438	88	0.74 (0.44–1.23)	0.218
Degree of differentiation									
Low	447	180	12	1.00 (reference)		1074	204	1.00 (reference)	
Medium	251	92	6	1.14 (0.85–1.54)	0.354	594	104	1.10 (0.85–1.44)	0.447
High	10	2	0			22	2		
Lymphatic metastasis									
0	173	59	4	1.00 (reference)		405	67	1.00 (reference)	
1	111	44	2	0.92 (0.57–1.47)	0.705	266	48	0.92 (0.60–1.40)	0.671
2	115	51	3	0.81 (0.51–1.28)	0.337	281	57	0.82 (0.55–1.22)	0.298
3a	140	50	4	0.99 (0.63–1.54)	0.945	330	58	0.94 (0.63–1.40)	0.755
3b	86	32	3	0.93 (0.56–1.56)	0.783	204	38	0.89 (0.56–1.40)	0.590

*Adjusted by tumor size, tumor stages, degree of differentiation, and lymphatic metastasis properly

monoclonal antibody of α3β1 integrin can inhibit the spreading and migration of GC cells in matrix [36]. The elevated expression of β1 integrin in GC tissue is considered as an indication of high degree of malignancy, including increase of invasive tendency, infiltrating growth, low degree of differentiation, liver metastasis, and late clinical stage. It has been shown that synthetic antibodies against the β1 subunit of integrin not only inhibit cell adhesion to the extracellular matrix (ECM) but also completely inhibit tumor cells to degrade collagen, laminin, and fibronectin. Thus, the application of antibodies against integrin significantly reduces the distant metastasis and migration of tumor cells [37].

The SNPs in the 3′ UTR region of integrin gene may also affect microRNA-mediated regulatory function, which, in turn, changes the level of integrin expression [38]. These SNPs may have association with gastric cancer susceptibility. In the current study, we chose five SNPs of the microRNA-binding sites in 3′ UTR region of integrin gene (rs1062484 C/T, rs17664 A/G, rs3809865 A/T, rs743554 C/T, and rs2675 A/C) to investigate whether there is a difference of the genotypic distribution as well as the allele frequency of these SNPs between GC patients and the controls. Our data indicated that the subjects carrying rs2675 genotype CC had a significantly decreased risk of GC than individuals carrying genotype AA [OR=0.52 (0.28–0.97), *P*=0.028]. In addition, the genotype AA of rs2675 and genotype GG of rs17664 appeared to be associated with a lower chance with stage 1b GC [OR=0.39 (0.18–0.85), *P*=0.009; and OR=0.37 (0.17–0.78), *P*=0.004, respectively]; also, the frequency of allele G of rs17664 showed a lower chance with stage 1b GC [OR=0.50 (0.26–0.95), *P*=0.021]. Furthermore, the frequency of genotype AA and allele A of rs3809865 were associated with a higher risk with stage 4 GC [OR=1.85 (1.11–3.09), *P*=0.012; and OR=1.52 (0.99–2.33), *P*=0.043, respectively]. Lastly, patients with genotype GG and allele G of rs17664 showed a higher risk with lymphatic metastasis 3b [OR=1.76 (1.00–3.11), *P*=0.036; and OR=1.64 (0.98–2.75), *P*=0.048, respectively]. Regarding the association between the five SNPs examined in the current study and other tumors, the A allele of rs3809865 has been shown to be associated with the risk of oral squamous cell carcinoma (OSCC) (*P*<0.05) and

Table 4 Stratified analysis for the association between rs17664 and GC clinical characteristics

Clinical characteristics	Genotype			OR (95 % CI)*	*P*	Allele		OR (95 % CI)	*P*
	GG	AG	AA			G	A		
Tumor size									
<5 cm	236	77	6	1.00 (reference)		549	89	1.00 (reference)	
5–10 cm	240	103	2	0.78 (0.55–1.11)	0.151	583	107	0.88 (0.64–1.21)	0.424
≥10 cm	68	18	4	1.07 (0.60–1.91)	0.808	154	26	0.96 (0.59–1.58)	0.866
Tumor stages									
1a	71	20	2	1.00 (reference)		162	24	1.00 (reference)	
1b	35	24	2	0.37 (0.17–0.78)	**0.004**	94	28	0.50 (0.26–0.95)	**0.021**
2a	52	22	1	0.62 (0.29–1.30)	0.171	126	24	0.78 (0.40–1.50)	0.420
2b	78	30	0	0.76 (0.38–1.51)	0.399	186	30	0.92 (0.50–1.70)	0.773
3a	72	19	0	1.11 (0.52–2.36)	0.780	163	19	1.27 (0.64–2.53)	0.462
3b	92	26	1	0.99 (0.49–1.99)	0.985	210	28	1.11 (0.60–2.07)	0.723
3c	128	57	5	0.59 (0.32–1.08)	0.067	313	67	0.69 (0.41–1.18)	0.150
4	204	54	5	1.01 (0.55–1.84)	0.977	462	64	1.07 (0.63–1.81)	0.793
Degree of differentiation									
Low	480	149	10	1.00 (reference)		1109	169	1.00 (reference)	
Medium	240	103	6	0.78 (0.58–1.05)	0.087	583	115	0.80 (0.62–1.05)	0.096
High	12	0	0			24	0		
Lymphatic metastasis									
0	168	64	4	1.00 (reference)		400	72	1.00 (reference)	
1	110	45	2	0.94 (0.59–1.49)	0.777	265	49	0.96 (0.64–1.46)	0.893
2	131	37	1	1.42 (0.88–2.31)	0.129	299	39	1.38 (0.89–2.14)	0.129
3a	132	56	6	0.88 (0.57–1.36)	0.542	320	68	0.85 (0.58–1.24)	0.369
3b	98	22	1	1.76 (1.00–3.11)	**0.036**	218	24	1.64 (0.98–2.75)	**0.048**

*Adjusted by tumor size, tumor stages, degree of differentiation, and lymphatic metastasis properly

the C allele of rs2675 was related with the progression of OSCC, but other SNPs had no association with OSCC [39]. Ma et al. [40] reported that patients with AT genotype of rs3809865 and GC genotype of rs1190271 tended to have a higher chance of regional lymph node metastasis ($P<0.01$) and proposed as new biological markers in predicting lymph node metastasis of OSCC. Zhang et al. [41] reported that rs3809865 had a significant association with asthma due to its effect on the binding of hsa-miR-124 to ITGB3 ($P=0.004$). Ma et al. [42] demonstrated that there was an association between rs3809865 and autism ($P=0.040$) within FH+ families (family-history positive, $P=0.031$). Another research revealed that there was a significant association between the A allele of the SNP rs743554 in the ITGB4 gene and susceptibility to estrogen receptor-negative tumors (OR=2.09, CI= 1.19–3.67) as well as patient survival [20]. The AA genotype of rs743554 in the ITGB4 gene had been reported to be associated with the risk of colorectal cancer in females [43]. The current study for the first time revealed an association between rs2675 and GC susceptibility. In addition, we found that rs2675, rs17664 and rs3809865 are also associated with GC stage and lymphatic metastasis. However, the mechanism underlying these associations remains to be elucidated.

SNPs in the microRNA-binding sites within 3′ UTR region of a variety of genes other than integrin gene have also been studied for their association with the susceptibility and progression of GC. Li et al. [44] reported that the T allele of the SNP rs712 in let-7 microRNA-binding site within 3′ UTR region of KRAS gene was associated with a significantly increased risk of GC, suggesting that the rs712 polymorphism in KRAS gene might be another new genetic marker for GC in Chinese population. Lin et al. reported that miR-181a was significantly upregulated in GC tissues, and the SNP within miR-181a-binding sites (rs12537) was associated with susceptibility and prognosis of GC. It was suggested that miR-181a and its target gene MTMR3 may play important roles in the development of GC [45]. Because the SNPs in the microRNA target genes may be used as tumor susceptibility biomarkers as well as the therapeutic targets, it is of importance to fully elucidate the relationship between these SNPs and human diseases, including tumor. In addition, because integrins are not only the critical transmembrane proteins for

Table 5 Stratified analysis for the association between rs3809865 and GC clinical characteristics

Clinical characteristics	Genotype			OR (95 % CI)*	*P*	Allele		OR (95 % CI)	*P*
	AA	AT	TT			A	T		
Tumor size									
<5 cm	196	114	9	1.00 (reference)		506	132	1.00 (reference)	
5–10 cm	225	105	15	1.19 (0.86–1.66)	0.275	555	135	1.07 (0.81–1.42)	0.609
≥10 cm	56	29	5	1.05 (0.63–1.75)	0.851	141	39	0.94 (0.62–1.44)	0.776
Tumor stages									
1a	52	38	3	1.00 (reference)		142	44	1.00 (reference)	
1b	38	22	1	1.36 (0.67–2.78)	0.340	98	24	1.27 (0.70–2.30)	0.410
2a	49	23	3	1.55 (0.79–3.55)	0.168	121	29	1.29 (0.74–2.27)	0.339
2b	67	37	4	1.35 (0.74–2.46)	0.301	171	45	1.18 (0.72–1.94)	0.497
3a	52	34	5	1.10 (0.59–2.05)	0.753	138	44	0.97 (0.59–1.61)	0.907
3b	68	48	3	1.10 (0.61–1.97)	0.737	184	54	1.06 (0.65–1.70)	0.815
3c	123	56	11	1.51 (0.88–2.59)	0.108	302	78	1.20 (0.77–1.86)	0.395
4	182	73	8	1.85 (1.11–3.09)	**0.012**	437	89	1.52 (0.99–2.33)	**0.043**
Degree of differentiation									
Low	408	208	23	1.00 (reference)		1024	254	1.00 (reference)	
Medium	213	121	15	0.93 (0.71–1.23)	0.605	547	151	0.92 (0.73–1.16)	0.483
High	10	2	0			22	2		
Lymphatic metastasis									
0	138	89	9	1.00 (reference)		365	107	1.00 (reference)	
1	87	63	7	0.90 (0.59–1.38)	0.605	237	77	0.90 (0.64–1.28)	0.548
2	112	50	7	1.42 (0.92–2.19)	0.094	274	64	1.26 (0.87–1.80)	0.199
3a	126	59	9	1.34 (0.89–2.02)	0.144	311	77	1.18 (0.84–1.67)	0.315
3b	80	36	5	1.41 (0.87–2.29)	0.140	196	46	1.25 (0.83–1.87)	0.259

*Adjusted by tumor size, tumor stages, degree of differentiation, and lymphatic metastasis properly

cell adhesion but also serving as cell surface receptors, they sense and mediate signals from the extracellular environment [46], subsequently involved in many aspects of cancer progression including proliferation, cell survival, invasion, and metastasis [47–49].

Taken together, our data suggest that polymorphisms of the microRNA-binding sites in the 3′ UTR region of integrin genes (ITGA3, ITGA6, ITGB3, ITGB4, and ITGB5) are associated with GC susceptibility (rs2675), tumor stage (rs2675, rs17664, and rs3809865), and lymphatic metastasis (rs17664) in Chinese Han population. Thus, the SNPs of rs2675, rs17664, and rs3809865 might be useful parameters to evaluate susceptibility, tumor stage, and lymphatic metastasis of GC. Further larger population-based prospective and functional studies are required to validate our conclusion and elucidate the roles of integrin in the pathogenesis of GC.

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Conflict of interest The authors declare that they have no conflict of interest.

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