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ORIGINAL ARTICLE

The rs2839698 Single Nucleotide Polymorphism of lncRNA H19 is Associated with Post-Operative Prognosis in T3 Gastric Adenocarcinoma

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SUMMARY

Background: **It has been widely demonstrated that long non-coding RNA H19 (lncRNA H19) plays an important role in the progression of various human cancers. However, the associations of common genetic variations with recurrence and survival in gastric adenocarcinoma in this lncRNA remain largely unknown.**
Methods: **The rs2839698 single nucleotide polymorphism (SNP) of H19 was genotyped in tissue samples from 441 patients with T3 gastric adenocarcinoma who had surgical operations between 2004 to 2009, and the relationships between the different genotypes and recurrence and survival after surgery alone (n = 156) or surgery plus chemotherapy (n = 285) were assessed using 3 different statistical-methods.**
Results: **Based on the final day of investigation (November 2014), the GA genotype was significantly associated with recurrence and survival in patients treated with surgery alone, but not in patients treated with surgery plus chemotherapy. In patients treated with surgery alone, individuals with the GA genotype had significantly lower risks of recurrence and death [adjusted hazard ratio (HR) 0.57, 95% CI 0.37 - 0.88; adjusted HR: 0.58, 95% CI 0.38 - 0.88] than the GG genotype (p = 0.010 and p = 0.010), respectively. More importantly, patients treated with surgery alone who carried the GA genotype achieved significantly longer median disease-free survival time and overall survival than carriers of the GG genotype (45 vs. 26 months, p = 0.010; 44 vs. 23 months, p = 0.013).**
Conclusions: **The rs2839698 SNP of H19 may have potential as a novel prognostic factor for survival in T3 gastric adenocarcinoma after surgery alone; these finding have special relevance to patients who are not suitable for post-operative chemotherapy.**
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KEY WORDS

lncRNA H19, single nucleotide polymorphisms, rs2839698, gastric adenocarcinoma, T3 category, survival

INTRODUCTION

Gastric cancer is one of the most commonly diagnosed malignant tumors worldwide [1] and the survival rates for patients with advanced disease are poor [2]. Surgery or surgery plus chemotherapy are currently the major therapeutic options for gastric cancer; however, some patients are not suitable for chemotherapy after surgery due to various factors, such as old age, poor condition or the risk of serious side-effects. It is necessary to better evaluate the prognosis of these patients so that the value of other post-operative therapies can be determined, such as individualized treatments or therapies targeting the oncogenic characteristics of the tumor that are generally regarded as having fewer side-effects. Assessment of biomarkers is widely used to evaluate the prognosis of patients with cancer [3,4]. The higher the number of prognostic factors assessed, the more accurate the prognosis. Long non-coding (lnc) RNAs are a recently identified type of RNA over 200 nucleotides long that do not have the ability to encode proteins [5]. Numerous studies have shown lncRNAs are widely involved in the initiation and progression of various human cancers [6,7]. Aberrant lncRNA expression and abnormal function are closely related to oncogenesis and the development of cancer, and common genetic variations within lncRNAs - mainly single nucleotide polymorphisms (SNPs) - are also associated with the risk of cancer and clinical outcomes (recurrence and survival) of patients with cancer [8,9]. H19, one of the earliest lncRNAs to be discovered, is one of the most widely investigated lncRNAs related to human cancer. H19 is frequently overexpressed in a diverse range of cancers and can alter the biological behavior of cancer cells via a number of molecular mechanisms [10,11]. For example, H19 is reported to increase AGS gastric cancer cell proliferation and migration by regulating miR-675 expression, and the H19/miR-675 complex activates the Akt/mTOR signaling pathway, a major pathway that regulates cancer cell proliferation and survival [12]. Several similar examples have been reported, and, while some results are conflicting [13], all findings indicate that H19 is a cancer-related lncRNA and is essential to the initiation and development of human cancer. Recent genome wide association studies have indicated that a SNP in H19 may be related to breast cancer risk [14]. The rs217727 SNP of H19 is associated with significantly increased risk of bladder cancer [15]. The rs2839698 SNP of H19 has been related to susceptibility to bladder cancer, colorectal cancer and gastric cancer and is also associated with the serum H19 mRNA concentration in patients with gastric cancer [16-18], indicating this SNP is functionally important. However, the relationship between the rs2839698 SNP of H19 and the prognosis of patients with gastric cancer is still unclear. With respect to histopathological type, more than 90% of cases of gastric cancer are adenocarcinoma, and T3 gastric adenocarcinoma is one of the commonly diagnosed gastric cancers. Therefore, in this study, we assessed the association between the rs2839698 SNP of H19 and the prognosis of patients with T3 gastric adenocarcinoma after surgery alone or surgery plus chemotherapy.

MATERIALS AND METHODS

Patients

This retrospective study included 441 patients with histopathologically-confirmed T3 category gastric adenocarcinoma who underwent surgery between 2004 and 2009 at Fuzhou General Hospital, Fujian, China. Recurrence was diagnosed according to previous standards [19], and survival was measured from the day of surgery to the day of the death or last day of follow-up. Among this group of 441 patients, 156 patients were treated with surgery alone, whereas the other 285 patients were treated with surgery plus post-operative chemotherapy based on fluorouracil, cisplatin, epirubicin, or various combinations of these drugs. This study was approved by the Ethics committee of Fuzhou General Hospital and all of the study participants provided informed consent for the use of their materials and records.

SNP selection and genotyping

The methods, reagents, and instruments used for extracted genomic DNA from paraffin embedded gastric cancer tissue and genotyping the rs2839698 SNP of H19 in the 441 T3 gastric adenocarcinoma tissues were the same as previously described [19] and included four main procedures for genotyping: (1) amplification of the products by PCR, (2) extension of the products using a SPA-iPlex systems, (3) genotyping of the products using a MassARRAY SpectroCHIP, and (4) analysis of the results.

Statistical analysis

The relationships between the clinicopathological characteristics of the patients treated with surgery alone and treated with surgery plus post-operative chemotherapy were compared using the chi-square (χ^2) test. The relationships between clinicopathological characteristics among the carriers with different genotypes of the rs2839698 SNP as well as between recurrence and no recurrence, and dead and alive were also evaluated using chi-square tests. The differences in survival and recurrence rate between different genotypes groups as well as between different genotype groups and in all patients treated with surgery alone or surgery plus chemotherapy were evaluated using the chi-squared partition

Table 1. Clinicopathological features of the patients with T3 gastric adenocarcinoma stratified by surgery alone vs. surgery plus chemotherapy.

Characteristics	Surgery plus chemotherapy n (%)	Surgery alone n (%)	p *
Patient number	285 (64.6)	156 (35.4)	
Age (years)			0.010
< 60	147 (51.6)	55 (35.3)	
≥ 60	138 (48.4)	101 (64.7)	
Gender			0.177
Male	216 (75.8)	109 (69.9)	
Female	69 (24.2)	47 (30.1)	
Differentiation grade			0.408
Well	13 (4.6)	8 (5.1)	
Medium	138 (48.4)	85 (54.5)	
Poor	134 (47.0)	63 (40.4)	
Lymph node metastasis			0.218
Positive	218 (76.5)	111 (71.2)	
Negative	67 (23.5)	45 (28.8)	
Tumor size			0.577
< 5cm	174 (61.1)	91 (58.3)	
≥ 5cm	111 (38.9)	65 (41.7)	
Genotype			0.528
G/G	145 (50.9)	75 (48.1)	
G/A	110 (38.6)	68 (43.6)	
A/A	30 (10.5)	13 (8.3)	

NOTE: Underlined values indicate significant results. * - Two-sided Chi-square test.

test. Cox model analyses with adjusted hazard ratio (HR) and 95% CIs were applied to examine the relationship between different genotypes and the risks of recurrence and death, respectively. Disease-free and overall survival times (in months) for the genotypes were compared using Kaplan-Meier survival curve analysis combined with the log-rank test. All statistical tests were carried out using SPSS software (version 17.0, SPSS Inc., Chicago, IL, USA); $p < 0.05$ was regarded as statistically significant.

RESULTS

Clinical characteristics of the patients

Among the entire group of 441 patients with T3 gastric adenocarcinoma, 156 patients underwent surgery only and the other 285 patients received surgery plus chemotherapy (Table 1). In terms of the clinical characteristics of these two groups, only age (< 60 vs. ≥ 60 years) was significantly different ($p = 0.01$). No other differences in any factor were identified between these groups, including gender ($p = 0.177$), tumor grade (well, medium, and poor differentiation; $p = 0.408$), lymph node metastasis ($p = 0.218$) and tumor size ($p = 0.577$; Table 1). Three different genotypes of the rs2839698 H19 SNP were detected: GG, GA, and AA; 145 (50.9%) and 75 (48.1%) patients in the surgery plus chemotherapy and surgery alone groups carried the GG genotype, 110 (38.6%) and 68 (43.6%) patients in surgery plus chemotherapy and surgery alone groups carried the GA genotype, and 30 (10.5%) and 13 (8.3%) patients in the surgery plus chemotherapy and surgery alone groups carried the AA genotype, respectively. No significant differences in genotype frequency were detected between the two treatment groups ($p = 0.528$; Table 1). Moreover, no significant differences in genotype frequency were observed between patients stratified by age (< 60 vs. ≥ 60 years), gender (male vs. female), tumor grade (well, medium, and poor differentiation), lymph node metastasis (positive vs. negative), and tumor size (< 5 vs. ≥ 5 cm) as well as whether they received chemotherapy (yes vs. no) (Table 2).

Table 2. Clinicopathological features for the patients with T3 gastric adenocarcinoma stratified by the individual genotypes of the rs2839698 SNP of H19.

Characteristics	Genotype				Recurrence			Overall Survival		
	G/G, n (%)	G/A, n (%)	A/A, n (%)	p *	No, n (%)	Yes, n (%)	p *	Dead, n (%)	Alive, n (%)	p *
Age (years)				0.944			0.079			0.023
< 60	99 (45.0)	83 (46.6)	20 (46.5)		84 (51.2)	118 (42.6)		114 (41.6)	88 (52.7)	
≥ 60	121 (55.0)	95 (53.4)	23 (53.5)		80 (48.8)	159 (57.4)		160 (58.4)	79 (47.3)	
Gender				0.234			0.847			0.987
Male	170 (77.3)	125 (70.2)	30 (69.8)		120 (73.2)	205 (74.0)		202 (73.7)	123 (73.7)	
Female	50 (22.7)	53 (29.8)	13 (30.2)		44 (26.8)	72 (26.0)		72 (26.3)	44 (26.3)	
Tumor grade				0.249			0.028			0.077
1	7 (3.2)	11 (6.2)	3 (7.0)		5 (3.0)	16 (5.8)		16 (5.8)	5 (3.0)	
2	121 (55.0)	83 (46.6)	19 (44.2)		96 (58.5)	127 (45.8)		128 (46.7)	95 (56.9)	
3	92 (41.8)	84 (47.2)	21 (48.8)		63 (38.5)	134 (48.4)		130 (47.5)	67 (40.1)	
Lymph node				0.189			0.000			0.000
Positive	172 (78.2)	128 (71.9)	29 (67.4)		99 (60.4)	230 (83.0)		227 (82.8)	102 (61.1)	
Negative	48 (21.8)	50 (28.1)	14 (32.6)		65 (39.6)	47 (17.0)		47 (17.2)	65 (38.9)	
Chemotherapy				0.528			0.039			0.004
Yes	145 (65.9)	110 (61.8)	30 (69.8)		116 (70.7)	169 (61.0)		163 (59.5)	122 (73.1)	
No	75 (34.1)	68 (38.2)	13 (30.2)		48 (29.3)	108 (39.0)		111 (40.5)	45 (26.9)	
Tumor size				0.810			0.273			0.351
< 5cm	129 (58.6)	109 (61.2)	27 (62.8)		104 (63.4)	161 (58.1)		160 (58.4)	105 (62.9)	
≥ 5cm	91 (41.4)	69 (38.8)	16 (37.2)		60 (36.6)	116 (41.9)		114 (41.6)	62 (37.1)	

NOTE: Underlined values indicate significant results. * - Two-sided Chi-square test.

Table 3. Partitioned chi-squared test analysis of the associations between the individual genotypes of the rs2839698 SNP of H19 and disease-free survival in T3 gastric adenocarcinoma.

A. Non-chemotherapy group					
Genotype	n	Recurrence (n)/non-recurrence (n)	Recurrence rate (%)	Case-total *	Case-case **
				p < 0.00833	p < 0.0125
Total	156	108/48	69.2		
G/G	75	59/16	78.7	0.133	1 vs. 2:0.004
G/A	68	38/30	55.9	0.054	2 vs. 3:0.052
A/A	13	11/2	84.6	0.349	3 vs. 1:1.000
B. Chemotherapy group					
Genotype	n	Recurrence (n)/non-recurrence (n)	Recurrence rate (%)	Case-total *	Case-case **
				p < 0.00833	p < 0.0125
ALL	285	169/116	59.3		
G/G	145	85/60	58.6	0.893	1 vs. 2: 0.940
G/A	110	65/45	59.1	0.970	2 vs. 3: 0.674
A/A	30	19/11	63.3	0.668	3 vs. 1: 0.632

NOTE: Underlined values indicate significant results. * - Case-total: p-values for the comparison between the different genotypes' groups and the entire group of patients, ** - Case-case: p-values for the pairwise comparisons between the different genotypes' groups.

Table 4. Association between the individual genotypes of the rs2839698 SNP of H19 and disease-free survival and recurrence risk in T3 gastric adenocarcinoma.

A. Non-chemotherapy group					
Genotype	**n**	**disease-free survival, n (%)**	**Recurrence, n (%)**	**HR [95% CI] (p)**	**Adjusted HR [95%CI] (p) ***
G/G	75	16 (33.3)	59 (54.6)	1.0	1.0
G/A	68	30 (62.5)	38 (35.2)	0.54 [0.36 - 0.82] (0.003)	0.57 [0.37 - 0.88] (0.010)
A/A	13	2 (4.2)	11 (10.2)	1.10 [0.57 - 2.10] (0.769)	1.33 [0.69 - 2.57] (0.402)
B. Chemotherapy group					
Genotype	**n**	**Disease-free survival, n (%)**	**Recurrence, n (%)**	**HR [95% CI] (p)**	**Adjusted HR [95% CI] (p) ***
G/G	145	60 (51.7)	85 (50.3)	1.0	1.0
G/A	110	45 (38.8)	65 (38.5)	1.04 [0.75 - 1.44] (0.800)	1.10 [0.79 - 1.52] (0.578)
A/A	30	11 (9.5)	19 (11.2)	1.23 [0.75 - 2.03] (0.407)	1.51 [0.91 - 2.51] (0.111)

NOTE: Underlined values indicate significant results. * - Adjusted for gender, tumor grade, node status, tumor size and age.

Table 5. Partitioned chi-squared test analysis of the associations between individual genotypes of the rs2839698 SNP of H19 and overall survival in T3 gastric adenocarcinoma.

A. Non-chemotherapy group					
Genotype	**n**	**Alive (n)/Dead (n)**	**Survival rate (%)**	**Case-total ***	**Case-case ****
				p < 0.00833	**p < 0.0125**
Total	156	45/111	28.8		
G/G	75	14/61	18.7	0.097	1 vs. 2:0.002
G/A	68	29/39	42.6	0.043	2 vs. 3:0.117
A/A	13	2/11	15.4	0.519	3 vs. 1:1.000
B. Chemotherapy group					
	n	**Alive (n)/Dead (n)**	**Survival rate (%)**	**Case-total ***	**Case-case ****
				p < 0.00833	**p < 0.0125**
ALL	285	122/163	42.8		
G/G	145	64/81	44.1	0.792	1 vs. 2:0.822
G/A	110	47/63	42.7	0.989	2 vs. 3:0.550
A/A	30	11/19	36.7	0.517	3 vs. 1: 0.452

NOTE: Underlined values indicate significant results. * - Case-total: p-values for the comparison between the different genotypes' groups and the entire group of patients, ** - Case-case: p-values for the pairwise comparisons between the different genotypes' groups.

Associations between genotype and recurrence and disease-free survival

Chi-square tests indicated tumor grade (p = 0.028), lymph node metastasis (p = 0.000) and post-operative chemotherapy (p = 0.039) were significantly associated with the recurrence rate (Table 2). However, age, gender, and tumor size were not associated with the recurrence rate (Table 2). Further analysis using the chi-squared partition test showed patients carrying the GA genotype had a significantly lower rate of recurrence than GG or AA carriers in the group of patients treated with surgery alone (55.9% vs. 78.7%, p = 0.004), but not in the group of patients treated with surgery plus chemotherapy (Table 3). The multivariate Cox model analysis adjusted for gender, tumor grade, lymph node metastasis, tumor size, and age confirmed that patients carrying the GA genotype had a significantly lower risk of recurrence compared with GG or AA carriers in the

Table 6. Association between the individual genotypes of the rs2839698 SNP of H19 and overall survival and death risk in T3 gastric adenocarcinoma.

A. Non-chemotherapy group					
Genotype	**n**	**Alive, n (%)**	**Dead, n (%)**	**CHR [95% CI] (p)**	**Adjusted HR [95%CI] (p) ***
G/G	75	14 (31.1)	61 (55.0)	1.0	1.0
G/A	68	29 (64.4)	39 (35.1)	0.54 [0.36 - 0.81] (0.003)	0.58 [0.38 - 0.88] (0.010)
A/A	13	2 (4.5)	11 (9.9)	1.07 [0.56 - 2.03] (0.842)	1.27 [0.66 - 2.46] (0.471)
B. Chemotherapy group					
Genotype	**n**	**Alive, n (%)**	**Dead, n (%)**	**CHR [95% CI] (p)**	**Adjusted HR [95% CI] (p) ***
G/G	145	64 (52.5)	81 (49.7)	1.0	1.0
G/A	110	47 (38.5)	63 (38.6)	1.08 [0.78-1.50] (0.646)	1.13 [0.81-1.58] (0.459)
A/A	30	11 (9.0)	19 (11.7)	1.29 [0.78-2.12] (0.321)	1.58 [0.95-2.64] (0.078)

NOTE: Underlined values indicate significant results. * - Adjusted for gender, tumor grade, node status, tumor size and age.

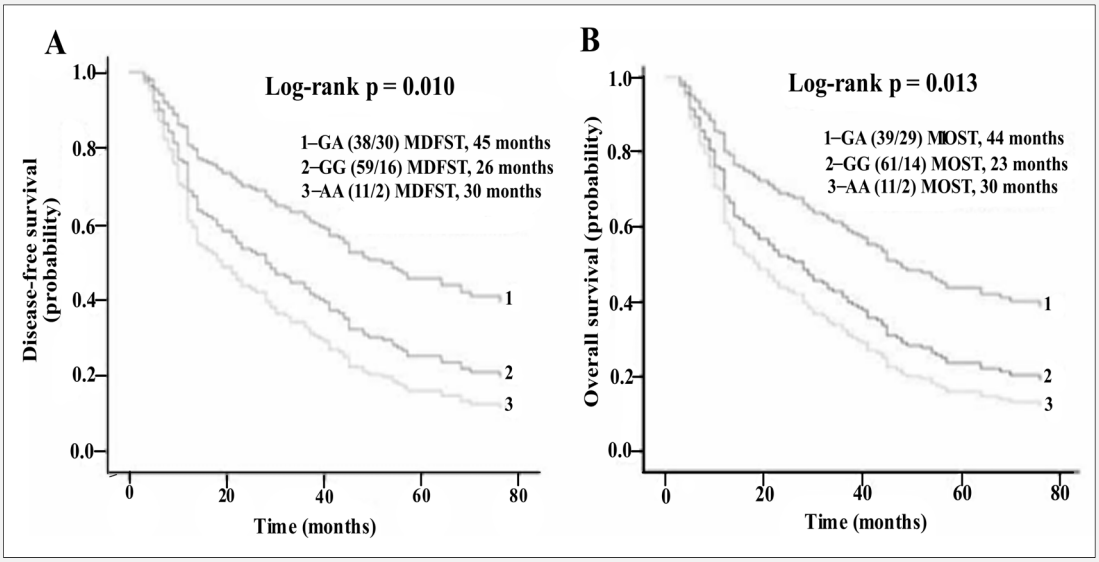

Figure 1. Kaplan-Meier survival curves for the group of patients treated with surgery alone stratified by the individual genotypes of the rs2839698 SNP of H19.

A: MDFST: median disease-free survival time in months; B: MOST: median overall survival time in months. *P*-values were calculated using the log-rank test.

group treated with surgery alone (adjusted HR: 0.57, 95% CI: 0.37 - 0.88, p = 0.010), but not in the group treated with surgery plus chemotherapy (Table 4). Kaplan-Meier survival curve analysis combined with the log-rank test demonstrated patients carrying the GA genotype had significantly longer median disease-free survival time in months (MDFST) than GG or AA carriers in the group treated with surgery alone (45 vs. 26 months and 45 vs. 30 months, respectively, p = 0.010; Figure 1A). Collectively, these results indicate that the GA genotype of the rs2839698 SNP of H19 may be associated with a better prognosis in patients with T3 gastric adenocarcinoma treated with surgery alone.

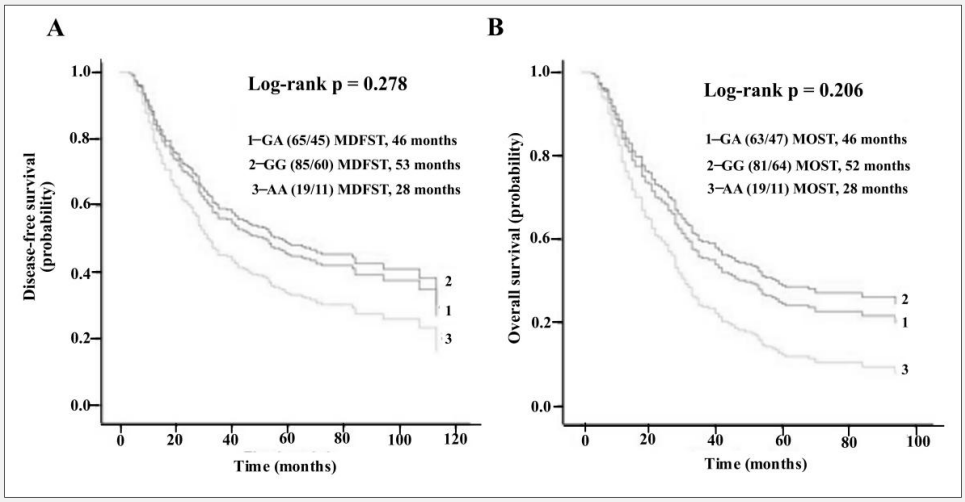

Figure 2. Kaplan-Meier survival curves for the group of patients treated with surgery plus chemotherapy stratified by the individual genotypes of the rs2839698 SNP of H19.

A: MDFST - median disease-free survival time in months, B: MOST - median overall survival time in months. P-values were calculated using the log-rank test.

Associations between genotype and the risk of death and overall survival

Chi-square tests indicated that age (p = 0.023), lymph node metastasis (p = 0.000) and postoperative chemotherapy (p = 0.004) were significantly associated with the overall survival rate (Table 2). GA genotype carriers exhibited significantly better overall survival than GG had (42.6% vs. 18.7%, p = 0.002; chi-squared partition test; Table 5) and exhibited a significantly reduced risk of death than GG had: adjusted HR: 0.58, 95% CI: 0.38 - 0.88, p = 0.010; Table 6) and significantly longer MOST than GG or AA carriers in the group treated with surgery alone (44 months vs. 23 months, and 44 months vs. 30 months, respectively, p = 0.013; Figure 1B). These results further indicate that the GA genotype may be associated with a favorable prognosis in patients with T3 gastric adenocarcinoma treated with surgery alone, but not in patients treated with surgery plus chemotherapy.

DISCUSSION

In terms of the treatments for patients with gastric adenocarcinoma, surgery alone is one of the most commonly used clinical methods. Therefore, prognostic biomarkers with the value for indicating the recurrence or survival for this therapeutic mean may have important clinical significance. However, until now, few such prognostic biomarkers have been identified to be associated with this type of cancer patient treated with surgery alone. LncRNA H19, an intergenic ncRNA that is 2300 nucleotides long located on human chromosome 11p15.5 [17,18], has been found to mainly exert an oncogenic role to promote the development and progression of cancer and has the potential to serve as a cancer biomarker, including in gastric cancer. Li et al. provided the evidence that H19 is upregulated in gastric cancer tissues, and elevated H19 levels in gastric cancer cells led to increased proliferation and metastasis [20]. Arita et al. showed plasma H19 levels are significantly increased in patients with gastric cancer compared to healthy controls and were also significantly higher at pretreatment than in postoperative samples [21]. Though there is strong evidence that H19 plays a role in gastric cancer and the SNP located in the H19 gene is found to be associated with different human cancers as described above [14-18], as far as we are aware, no studies have previously investigated the relationship between the rs2839698 SNP of H19 and the prognosis of patients with gastric cancer. In the present study, the relationships between the individual genotypes of the rs2839698 SNP of the H19 gene and the prognosis of patients with gastric cancer were evaluated. Our results showed that patients with the GA genotype in the group treated with surgery alone had significantly lower risks of recurrence and death and significantly increased survival, disease-free survival, and overall survival time. These results suggest this common genetic variant in H19 may have prognostic value in gastric cancer, espe-

cially for patients with T3 gastric adenocarcinoma treated with surgery alone.

Gastric adenocarcinoma is the predominant type of gastric cancer. Many patients with T3 gastric adenocarcinoma have advanced disease and many cannot tolerate the side-effects of post-operative chemotherapy. Therefore, the discovery of biomarkers that indicate the prognosis of patients with T3 gastric adenocarcinoma who cannot receive post-operative chemotherapy could help to identify subgroups of patients who may benefit from other molecular-targeted therapies with fewer side effects such as trastuzumab for HER-2 positive patients [22].

CONCLUSION

In conclusion, this study provides evidence that the rs2839689 SNP of the H19 gene is associated with recurrence and survival in patients with T3 gastric adenocarcinoma treated with surgery alone. The rs2839689 SNP may have potential as a novel prognostic biomarker for this group of patients.

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Declaration of Interest:

The authors declare that they have no conflicts of interest.

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