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ORIGINAL ARTICLE

The clinicopathological and genetic differences among gastric cancer patients with no recurrence, early recurrence, and late recurrence after curative surgery

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Abstract

Background: To date, few reports have investigated the genetic alterations and clinicopathological features among gastric cancer (GC) patients with no tumor recurrence, early recurrence, and late recurrence following curative surgery.

Methods: A total of 473 GC patients undergoing curative surgery were included. The clinicopathological characteristics, patient prognosis, recurrence patterns, and genetic alterations were compared between GC patients with early recurrence and late recurrence.

Results: Among the 473 GC patients, 119 had early recurrence (<2 years) and 45 had late recurrence (≥2 years). Patients with early recurrence had tumor size larger than 5 cm, fewer superficial-type tumors, more lymphovascular invasion, more advanced pathological T and N categories and Tumor, Node, Metastasis (TNM) stages, and worse 5-year overall survival than patients with late recurrence and no recurrence. For intestinal-type GC, patients with no tumor recurrence had more Helicobacter pylori infection than patients with early recurrence and late recurrence; for diffuse-type GC patients, the frequency of *PIK3CA* amplification was the highest in early recurrence, followed by late recurrence and no recurrence. GC patients with single-site recurrence had more *ARID1A* mutations than those with multiple-site recurrence. Multivariate analysis demonstrated that age, tumor recurrence, and pathological N categories were independent prognostic factors.

Conclusion: *PIK3CA* amplifications were more common in diffuse-type GC with early recurrence, whereas *ARID1A* mutations were more common in patients with single-site recurrence. Targeted therapy and immunotherapy might be helpful for these patients.

Keywords: ARID1A; Early recurrence; Gastric cancer; Genetic alteration; Late recurrence; *PIK3CA* amplification

1. INTRODUCTION

Gastric cancer (GC) is the sixth most common cancer and is associated with the third most common cancer-related deaths worldwide.[1] Radical gastrectomy with lymphadenectomy remains the mainstay of curative intent for GC.

Despite curative surgery, a proportion of patients experienced tumor recurrence and most of them died of GC. The majority of tumor recurrence of GC following curative surgery are within 2 years. Consequently, 2 years has been defined as the cutoff value for early and late recurrence.[2,3] In these studies, patients with early recurrence tended to have a more advanced stage and worse survival than those with late recurrence. It was reported that the most common recurrence pattern was hematogenous metastasis in the early recurrence group, whereas locoregional

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and peritoneal recurrence was the most common recurrence pattern in the late recurrence group.[4]

Although the clinical features between early and late recurrence have been reported by other studies,[5,6] there are few studies comparing the difference in genetic alterations between early and late recurrence. The loss expression of some tumor suppressor genes was associated with GC recurrence, such as *ARID1A*, *XRCC1*, and *JWA*.[7,8] To date, it is unclear whether genetic mutations are associated with early recurrence in GC, which deserves more investigation.

The aim of the current study was to compare the clinicopathological characteristics, patient prognosis, and genetic alterations between no tumor recurrence, early recurrence, and late recurrence of GC patients after curative surgery.

2. METHODS

Between 2005 and 2015, a total of 473 GC patients with adenocarcinoma who underwent curative surgery were enrolled. Collection of the tumor and normal gastric mucosa tissues and analysis of the genetic alteration for all 473 GC patients were performed. Among them, 119 patients with tumor recurrence within 2 years after surgery were defined as having an early recurrence, whereas 45 patients with tumor recurrence ≥2 years after surgery were defined as having a late recurrence. The study was approved by the Institutional Review Board of Taipei Veterans General Hospital (No. 2022-01-015AC), and all samples used in the present study were anonymized and had been previously collected from the biobank of Taipei Veterans General Hospital. An informed consent form was signed by all patients enrolled before sample collection.

For early GC, at least D1+ lymph node dissection was performed, whereas D2 dissection was performed for advanced GC.[9] As described in a previous study,[10] follow-up examinations were performed postoperatively every 3 months during the first 3 years and then every 6 months thereafter. The definition of single-site recurrence was tumor recurrence in one organ, whereas multiple-site recurrence was defined as tumor recurrence in more than one organ. For example, patients with multiple liver metastases only were considered as single-site recurrence. Patients diagnosed with tumor recurrence could receive 5-fluorouracil (FU)-based chemotherapy. Before surgery, none of the patients in the present study received chemotherapy. Since 2008, S-1 has been used as adjuvant chemotherapy for stage II or III disease after curative surgery at our institute based on its proven survival benefit.[11]

2.1. Analysis of microsatellite instability and genetic mutations

Five reference microsatellite markers, D5S345, D2S123, D17S250, BAT25, and BAT26, were used to determine microsatellite instability (MSI) status.[12] MSI-high (MSI-H) was defined as ≥2 loci showing instability, whereas one locus showing instability or no MSI loci was defined as MSI-low/stable (MSI-L/S).[12]

Identification of 68 mutation hotspots in eight GC-related genes using a MassARRAY system (Agena, San Diego, CA, USA) was performed, including *PIK3CA*, *AKT1*, *AKT2*, *AKT3*, *PTEN*, *ARID1A*, *TP53*, and B-Raf proto-oncogene.[13] Mutations in *PTEN*, *PIK3CA*, *AKT1*, *AKT2*, or *AKT3* were defined as *PI3K/AKT* pathway genetic mutations.

2.2. Detection of HP and Epstein-Barr virus infection

Helicobacter pylori (HP) infection was detected using the polymerase chain reaction (PCR) method.[14] The reference sequence of the HP reference genome (GenBank: AE000511.1) was used to design PCR forward (AAGCTTACTTTCTAACACTAACGC) and reverse (AAGCTTTTAGGGGTGTTAGGGGTTT) primers. The PCR method was the same as in a previous report.[14] Both tumor tissue and normal tissues were checked for HP infection. The PCR results were shown in Fig. 1. Epstein-Barr virus (EBV) infection was detected as EBV-encoded small RNAs (EBERs) in formalin-fixed paraffin-embedded tissue samples using the in situ hybridization technique.[15] Positive EBER immunohistochemical staining result using the in situ hybridization technique is shown in Fig. 2.

2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, USA). The χ^2 test with Yates correction or Fisher's exact test was used to compare categorical data between groups. The data of the follow-up period and survival time was presented as mean ± SD. Overall survival (OS) was defined from the surgery date until the patient's death or the last follow-up. Postrecurrence survival was defined from the date of GC recurrence to the date of death or the last follow-up. The Kaplan–Meier method was performed for the survival analysis of OS and postrecurrence survival. Multivariable analysis with a Cox proportional hazards model was used to analyze the

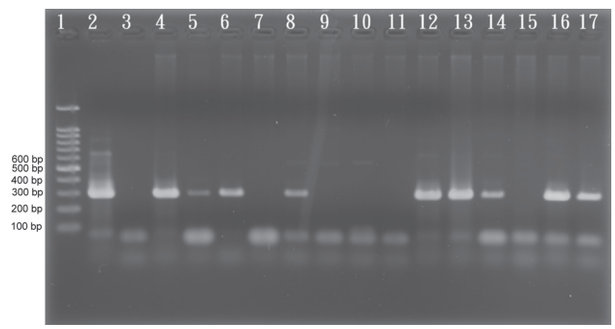

Fig. 1 Agarose gel electrophoresis of the PCR product. 100 bp DNA ladder was used. Lane 1: size marker (100 bp); lane 2: Helicobacter pylori (positive control); lane 3: ddH_2O (negative control); lane 4, 6, 8, 10, 12, 14, and 16: the normal stomach tissue DNA of patient No. 1 to No.7; lane 5, 7, 9, 11, 13, 15, and 17: tumor tissue DNA of patient No.1 to No.7. PCR=polymerase chain reaction.

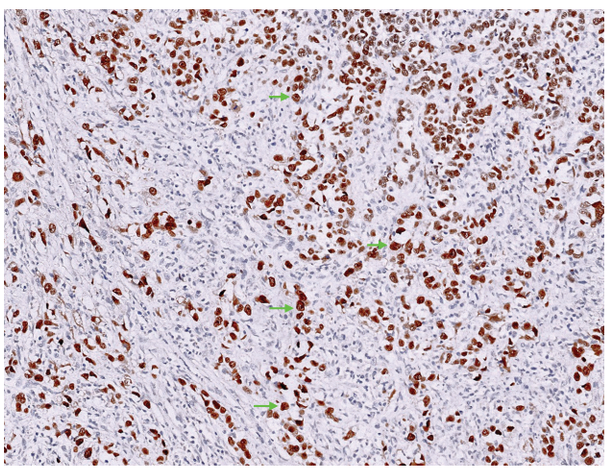

Fig. 2 Positive EBV-encoded small RNA in situ hybridization (EBER ISH) result is stained with brown color and pointed with green arrows. EBER=EBV-encoded small RNAs; EBV=Epstein-Barr virus.

Table 1

Clinical profiles between early and late recurrence in gastric cancer patients after curative surgery

	No recurrence n = 309 n (%)	Early recurrence n = 119 n (%)	Late recurrence n = 45 n (%)	*p*
Age (years old)				0.216
<65	123 (39.8)	43 (36.1)	23 (51.1)	
≥65	186 (60.2)	76 (63.9)	22 (48.9)	
Sex				0.632
Male	214 (69.3)	88 (73.9)	32 (71.1)	
Female	95 (30.7)	31 (26.1)	13 (28.9)	
Tumor size (cm)				**<0.001**
<5	135 (43.7)	22 (18.5)	17 (37.8)	
≥5	174 (56.3)	97 (81.5)	28 (62.2)	
Tumor location				0.775
Upper third	52 (16.8)	27 (22.7)	7 (15.6)	
Middle third	112 (36.2)	27 (22.7)	15 (33.3)	
Lower third	136 (44.0)	61 (51.3)	23 (51.1)	
Whole stomach	9 (2.9)	4 (3.4)	0	
Extent of lymphadenectomy				0.190
D1+	85 (27.5)	26 (21.8)	16 (35.6)	
D2	224 (72.5)	93 (78.2)	29 (64.4)	
Gross appearance				**<0.001**
Superficial type	56 (18.1)	2 (1.7)	5 (11.1)	
Borrmann type 1&2	105 (34.0)	21 (17.6)	6 (13.3)	
Borrmann type 3&4	148 (47.9)	96 (80.7)	33 (75.6)	
Lauren's classification				0.783
Intestinal-type	166 (53.7)	61 (51.3)	22 (48.9)	
Diffuse-type	143 (46.3)	58 (48.7)	23 (51.1)	
Adjuvant chemotherapy	42 (13.6)	16 (13.4)	6 (13.3)	0.998
Lymphovascular invasion	201 (65.0)	110 (92.4)	32 (71.1)	**<0.001**
Pathological T category				**<0.001**
T1	58 (18.8)	2 (1.7)	6 (13.3)	
T2	62 (20.1)	8 (6.7)	2 (4.4)	
T3	104 (33.7)	42 (35.3)	14 (31.1)	
T4	85 (27.5)	67 (56.3)	23 (51.1)	
Pathological N category				**<0.001**
N0	112 (36.2)	13 (10.9)	12 (26.7)	
N1	59 (19.1)	13 (10.9)	7 (15.6)	
N2	68 (22.0)	32 (26.9)	18 (40.0)	
N3	70 (22.7)	61 (51.3)	8 (17.8)	
Pathological TNM stage				**<0.001**
I	81 (26.2)	2 (1.7)	5 (11.1)	
II	92 (29.8)	23 (19.3)	10 (22.2)	
III	136 (44.0)	94 (79.0)	30 (66.7)	

Bold values indicate statistically significant ($p < 0.05$)

Table 2

The initial recurrence pattern between early and late recurrence of gastric cancer patients after curative surgery

	All gastric cancer			Intestinal-type gastric cancer			Diffuse-type gastric cancer		
Recurrence pattern	**Early recurrence n=119n (%)**	**Late recurrenc en=45n (%)**	***p***	**Early recurrence n=61n (%)**	**Late recurrenc en=22n (%)**	***p***	**Early recurrence n=58n (%)**	**Late recurrenc en=23n (%)**	***p***
Locoregional recurrence	48 (40.3)	16 (35.6)	0.575	25 (41.0)	6 (27.3)	0.254	23 (39.7)	10 (43.5)	0.752
Distant metastasis	107 (89.9)	34 (75.6)	**0.018**	55 (90.2)	19 (86.4)	0.623	52 (89.7)	15 (65.2)	**0.009**
Peritoneal dissemination	54 (45.4)	16 (35.6)	0.256	24 (39.3)	7 (31.8)	0.532	30 (51.7)	9 (39.1)	0.306
Hematogenous metastasis	48 (40.3)	15 (33.3)	0.411	29 (47.5)	10 (45.5)	0.867	19 (32.8)	5 (21.7)	0.327
Liver	35 (29.4)	8 (17.8)		23 (37.7)	6 (27.3)		12 (20.7)	2 (8.7)	
Lung	8 (6.7)	3 (6.7)		5 (8.2)	2 (9.1)		3 (5.2)	1 (4.3)	
Bone	7 (5.9)	5 (11.1)		4 (6.6)	2 (9.1)		3 (5.2)	3 (13.0)	
Brain	1 (0.8)	0		0	0		1 (1.7)	0	
Adrenal	2 (1.7)	1 (2.2)		0	1 (4.5)		2 (3.4)	0	
Skin	2 (1.7)	1 (2.2)		1 (1.6)	1 (4.5)		1 (1.7)	0	
Distant lymphatic recurrence	32 (26.9)	6 (13.3)	0.066	17 (27.9)	2 (9.1)	0.072	15 (25.9)	4 (17.4)	0.417
Virchow's lymph node	7 (5.9)	1 (2.2)		5 (8.2)	1 (4.5)		2 (3.4)	0	
Inguinal lymph node	1 (0.8)	0		1 (1.6)	0		0	0	
Paraaortic lymph node	26 (21.8)	6 (13.3)		13 (21.3)	2 (9.1)		13 (22.4)	4 (17.4)	

Some patients had more than one recurrence pattern

Bold values indicate statistically significant ($p < 0.05$)

independent prognostic factors of OS. A p value less than 0.05 was defined as statistically significant.

3. RESULTS

3.1. Clinicopathological features

Among the 473 GC patients who underwent curative surgery, 164 (34.7%) experienced tumor recurrence, including 119 with early recurrence and 45 with late recurrence. Regarding the clinicopathological characteristics, as shown in Table 1, patients with early recurrence had larger tumor sizes, fewer superficial-type tumors, more lymphovascular invasion, and earlier pathological T and N categories and TNM stages than patients with no recurrence and patients with late recurrence.

3.2. Initial recurrence patterns

As demonstrated in Table 2, among the 164 patients with tumor recurrence, patients with early recurrence had more distant metastases than patients with late recurrence. For intestinal-type GC, there was no significant difference in the initial recurrence pattern between patients with early recurrence and late recurrence. For diffuse-type GC, patients with early recurrence had more distant metastases than patients with late recurrence.

3.3. Analysis of genetic mutations

As shown in Table 3, there was no significant difference in genetic mutations among patients with no recurrence, early recurrence, and late recurrence. For intestinal-type GC, patients with no recurrence had more HP infection than patients with early recurrence and late recurrence. For diffuse-type GC, the frequency of *PIK3CA* amplification was the highest in patients with early recurrence, followed by late recurrence and no recurrence (62.1% vs. 56.5% vs. 43.4%; p = 0.043). As shown in Table 4, regarding the number of tumor recurrence sites, patients with single-site recurrence had more *ARID1A* mutations than patients with multiple-site recurrence (21.7% vs. 7.4%; p = 0.008).

3.4. Survival analysis

The follow-up period of all the patients included in the study was 79.8 ± 78.5 months. The time to recurrence after curative surgery was 11.1 ± 5.6 months in patients with early recurrence and 48.6 ± 32.1 months in patients with late recurrence. The OS for patients with early recurrence was significantly shorter than that for patients with late recurrence and patients with no recurrence (25.4 ± 30.1 vs. 66.5 ± 47.0 vs. 102.8 ± 84.2 months; p < 0.001)

We further analyzed the survival rates for patients with tumor recurrence. As shown in Fig. 3A, patients with early recurrence had significantly worse 5-year OS rates than patients with late recurrence and patients without recurrence (4.2% vs. 42.2% vs. 70.0%; p < 0.001). Patients with single-site recurrence had a better 5-year OS rate than patients with multiple-site recurrence (24.6% vs. 7.4%; p < 0.001). For patients with single-site recurrence, the 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (7.1% vs. 51.9%; p < 0.001, Fig. 3B). For patients with multiple-site recurrence, the 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (2.6% vs. 27.8%; p < 0.001, Fig. 3C).

For intestinal-type GC, the 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (6.6% vs. 36.4%; p < 0.001). For diffuse-type GC, the 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (1.7% vs. 47.8%; p < 0.001).

Table 3

The molecular features in gastric cancer patients after curative surgery

	All GC patients				Intestinal-type gastric cancer				Diffuse-type gastric cancer			
Variables	**No recurrence n = 309 n (%)**	**Early recurrence n = 119 n (%)**	**Late recurrence n = 45 n (%)**	***p***	**No recurrence n = 166n (%)**	**Early recurrence n = 61n (%)**	**Late recurrence n = 22n (%)**	***p***	**No recurrence n = 143n (%)**	**Early recurrence n = 58n (%)**	**Late recurrence n = 23n (%)**	***p***
MSI status				0.436				0.349				0.107
MSI-L/S	281 (90.9)	106 (89.1)	43 (95.6)		11 (6.6)	10 (16.4)	1 (4.5)		126 (88.1)	55 (94.8)	22 (95.7)	
MSI-H	28 (9.1)	13 (10.9)	2 (4.4)		4 (7.7)	3 (11.1)	0		17 (11.9)	3 (5.2)	1 (4.3)	
HP infection	123 (39.8)	38 (31.9)	14 (31.1)	0.220	65 (39.2)	13 (21.3)	6 (27.3)	**0.032**	58 (40.6)	25 (43.1)	8 (34.8)	0.789
EBV infection	41 (13.3)	19 (16.0)	8 (17.8)	0.614	18 (10.8)	13 (21.3)	4 (18.2)	0.080	23 (16.1)	6 (10.3)	4 (17.4)	0.712
PIK3CA amplification	127 (41.0)	61 (51.7)	20 (44.4)	0.136	64 (38.6)	26 (42.6)	7 (31.8)	0.661	62 (43.4)	36 (62.1)	13 (56.5)	**0.043**
Genetic mutations												
PI3K/AKT pathway	45 (14.6)	25 (21.0)	5 (11.1)	0.173	30 (18.1)	20 (32.8)	4 (18.2)	0.216	15 (10.5)	5 (8.6)	1 (4.3)	0.351
TP53	39 (12.6)	18 (15.1)	5 (11.1)	0.724	23 (13.9)	9 (14.8)	2 (9.1)	0.704	16 (11.2)	9 (15.5)	3 (13.0)	0.550
ARID1A	33 (10.7)	16 (13.4)	6 (13.3)	0.677	18 (10.8)	13 (21.3)	4 (18.2)	0.080	15 (10.5)	3 (5.2)	2 (8.7)	0.428
BRAF	2 (0.6)	0	0	0.587	2 (1.2)	0	0	0.357	0	0	0	-

Bold values indicate statistically significant (p <0.05) and place it before abbreviation list.

BRAF=B-Raf proto-oncogene; EBV=Epstein-Barr virus; HP=Helicobacter pylori; MSI=microsatellite instability; MSI-H=MSI-high; MSI-L/S=MSI-low/stable.

The 5-year postrecurrence survival rates were not significantly different between GC patients with early recurrence and those with late recurrence (4.2% vs. 6.1%; $p = 0.076$). For patients with single-site recurrence, the 5-year postrecurrence survival rates were not significantly different between patients with early recurrence and those with late recurrence (7.1% vs. 8.1%; $p = 0.548$). For patients with multiple-site recurrence, the 3-year postrecurrence survival rates were not significantly different between patients with early recurrence and those with late recurrence (2.6% vs. 7.4%; $p = 0.460$).

Univariate analysis demonstrated that age, sex, tumor recurrence, gross appearance, lymphovascular invasion, and pathological T and N categories were significantly associated with OS. The above-mentioned seven factors were included in the multivariable analysis. The Cox proportional hazards model demonstrated that age, tumor recurrence, and pathological N categories were independent prognostic factors of OS (Table 5).

Table 4

The molecular features in gastric cancer patients after curative surgery

	Gastric cancer patients with recurrence		
Variables	Single-site recurrence n = 69 n (%)	Multiple-site recurrence n = 95 n (%)	*p*
MSI status			0.705
MSI-L/S	62 (89.9)	87 (91.6)	
MSI-H	7 (10.1)	8 (8.4)	
HP infection	18 (26.1)	34 (35.8)	0.187
EBV infection	14 (20.3)	13 (13.7)	0.260
PIK3CA amplification	34 (49.3)	48 (50.5)	0.874
Genetic mutations			
PI3K/AKT pathway	16 (23.2)	14 (14.7)	0.167
TP53	8 (11.6)	15 (15.8)	0.445
ARID1A	15 (21.7)	7 (7.4)	**0.008**
BRAF	0	0	-

Bold values indicate statistically significant ($p < 0.05$) and place it before abbreviation list.
BRAF=B-Raf proto-oncogene; EBV=Epstein-Barr virus; HP=Helicobacter pylori; MSI=microsatellite instability; MSI-H=MSI-high; MSI-L/S=MSI-low/stable.

4. DISCUSSION

Although the clinical features between early and late recurrence have been reported by other studies, the novel findings of the present study are the molecular difference among GC patients with no recurrence, early recurrence, and late recurrence. In the present study, our results showed that GC patients with early recurrence had more unfavorable clinicopathological features and worse 5-year OS rates than patients with no recurrence and late recurrence. For diffuse-type GC, *PIK3CA* amplifications

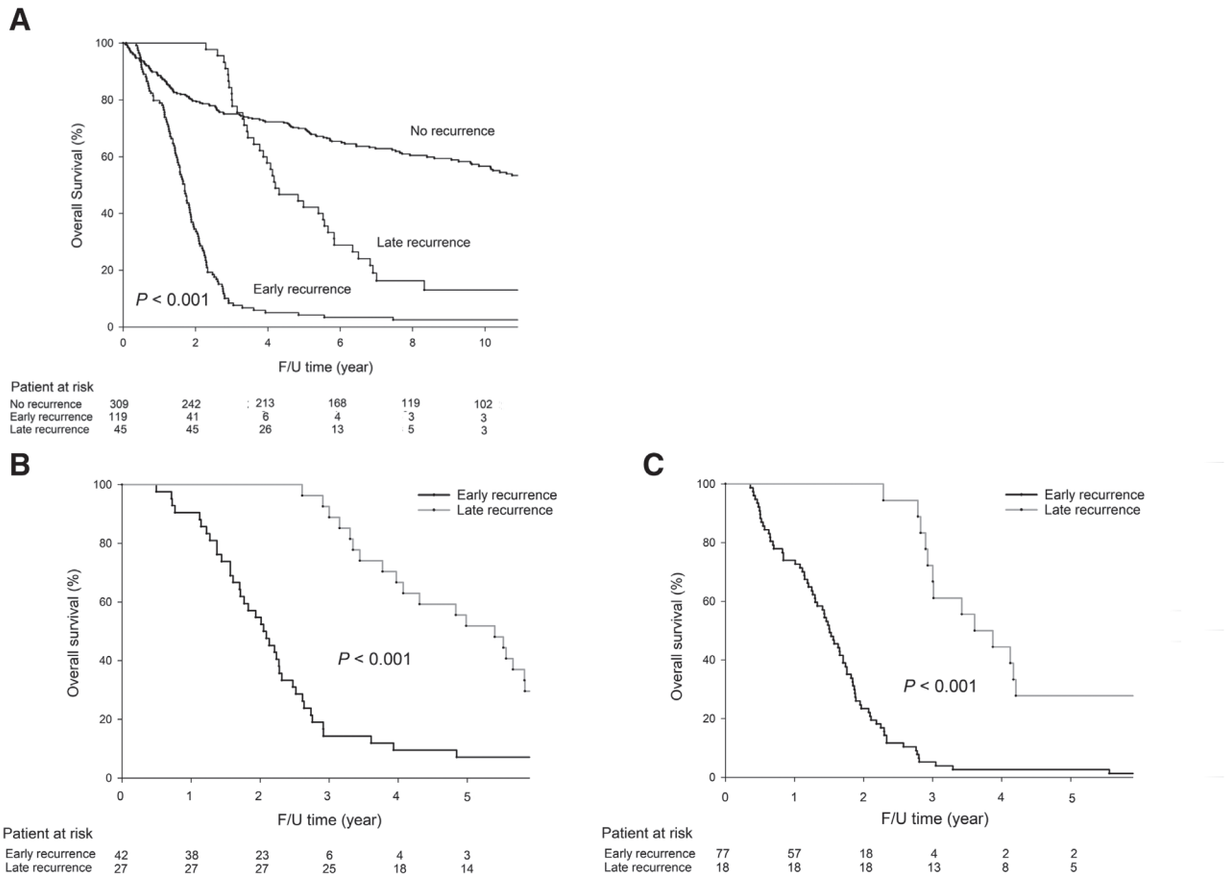

Fig. 3 5-year OS rates were significantly lower in gastric cancer patients with early recurrence than in those with late recurrence and those without recurrence (4.2% vs. 42.2% vs. 70.0%; $p < 0.001$). For gastric cancer with single-site recurrence, 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (7.1% vs. 51.9%; $p < 0.001$). For gastric cancer with multiple-site recurrence, 5-year OS rates were significantly lower in patients with early recurrence than in those with late recurrence (2.6% vs. 27.8%; $p < 0.001$). The survival curves shown are as follows: (A) all gastric cancer patients, (B) single-site recurrence gastric cancer patients, and (C) multiple-site recurrence gastric cancer patients. OS=overall survival.

Table 5

Univariate and multivariate analysis of factors affecting overall survival of all GC patients after curative surgery

	Univariate analysis			Multivariate analysis		
Variables	**Hazard ratio**	**Confidence interval**	***p***	**Hazard ratio**	**Confidence interval**	***p***
Age (years old)			**<0.001**			**<0.001**
<65	1.000			1.000		
≥65	1.682	1.323–2.140		1.790	1.383–2.317	
Sex			**0.001**			0.545
Male	1.000			1.000		
Female	0.634	0.487–0.826		0.916	0.689–1.217	
Tumor recurrence			**<0.001**			**<0.001**
No recurrence	1.000			1.000		
Early recurrence	6.798	5.183–8.915		4.141	3.087–5.556	
Late recurrence	2.248	1.576–3.207		1.913	1.318–2.777	
Extent of lymphadenectomy			0.131			
D1+	1.000					
D2	0.829	0.652–1.058				
Gross appearance			**<0.001**			0.143
Superficial type	1.000			1.000		
Bormann type 1&2	2.332	1.498–3.630		1.562	0.942–2.590	
Bormann type 3&4	3.180	2.110–4.794		1.654	1.003–2.728	
Lymphovascular invasion	2.487	1.861–3.325	**<0.001**	1.098	0.768–1.570	0.610
Lauren's classification			0.157			
Intestinal type	1.000					
Diffuse type	1.175	0.940–1.468				
Adjuvant chemotherapy	0.924	0.644–1.324	0.665			
Pathological T category			**<0.001**			0.065
T1	1.000			1.000		
T2	1.684	1.007–2.815		0.919	0.505–1.672	
T3	2.617	1.673–4.095		0.943	0.529–1.683	
T4	4.501	2.920–6.936		1.310	0.732–2.344	
Pathological N category			**<0.001**			**<0.001**
N0	1.000			1.000		
N1	1.050	0.706–1.563		0.936	0.620–1.414	
N2	1.959	1.414–2.716		1.403	0.985–1.997	
N3	5.169	3.801–7.030		3.407	2.375–4.885	
MSI status			0.885			
MSI-L/S	1.000					
MSI-H	0.970	0.645–1.460				
PIK3CA amplification	1.051	0.840–1.315	0.662			
Genetic mutation						
PI3K/AKT pathway	0.928	0.689–1.251	0.625			
TP53	1.220	0.892–1.669	0.213			
ARID1A	0.783	0.552–1.110	0.169			
BRAF	1.001	0.249–4.026	0.999			

Bold values indicate statistically significant ($p < 0.05$) and place it before abbreviation list.

BRAF=B-Raf proto-oncogene; MSI=microsatellite instability; MSI-H=MSI-high; MSI-L/S=MSI-low/stable.

were more common in patients with early recurrence; for intestinal-type GC, HP infections were more common in patients with no tumor recurrence. GC patients with single-site recurrence had more *ARID1A* mutations and better survival than GC patients with multiple-site recurrence.

GC with early recurrence was associated with larger tumor sizes, more extensive lymph node metastasis, more advanced TNM stages, more distant metastasis, and worse survival than GC with late recurrence,[2,16] which is consistent with the results of the present study. Furthermore, our results demonstrated that postrecurrence survival was poor in patients with early and late recurrence, regardless of whether recurrence is early or late.

It was reported that *PIK3CA* amplifications were associated with diffuse-type GC, poor differentiation, and peritoneal recurrence.[17] In addition, *PIK3CA* amplifications were associated with poor survival in GC.[18] GC with early recurrence was more likely to develop distant metastasis than GC with late recurrence.[4] In the present study, *PIK3CA* amplifications were associated with early recurrence in diffuse-type GC. In addition, diffuse-type GC patients with early recurrence were more likely to develop distant metastasis than those with late recurrence. According to the results of other reports and the present study, *PIK3CA* amplification may serve as a prognostic biomarker for early recurrence and poor prognosis in GC, especially diffuse-type GC. *PIK3CA* amplifications could activate the *PI3K/AKT* pathway, which may be targeted by *mTOR* and *AKT* inhibitors.[19] Consequently, in addition to chemotherapy, combination therapy with *mTOR* or *AKT* inhibitor might be applicable for diffuse-type GC patients with early recurrence.

The correlation between HP infection and patient prognosis in GC is still controversial.[20,21] HP infection was associated with better survival, especially in intestinal-type GC.[22] In the

present study, for intestinal-type GC, HP infection was more common in patients with no tumor recurrence compared with those with early recurrence and late recurrence. Although HP infection can increase the incidence of GC, HP infection may also improve patient's outcome by inducing a tumor-specific immune response. It seems that HP infection might play a protective role and induce an immune response and further decrease tumor recurrence in GC. Further in vivo and in vitro studies are required to validate our hypothesis.

ARID1A, a key component of the *SWI/SNF* chromatin remodeling complex, is considered as a tumor suppressor gene.[23] However, the relationship between loss expression of *ARID1A* and prognosis in GC is controversial. Some studies reported a poor prognosis,[24] and some studies demonstrated a good prognosis.[25] It was reported that GC with *ARID1A* mutations was associated with two molecular subtypes, MSI-H and EBV-associated tumors, which were correlated with a favorable prognosis.[25] In our study, GC patients with single-site recurrence had more *ARID1A* mutations and better OS than those with multiple-site recurrence, which has not yet been reported. Among GC with recurrence, patients with *ARID1A* mutations were associated with more MSI-H GCs than those without *ARID1A* mutations (27.3% vs. 6.3%; $p = 0.002$), and this factor might play a role in the better survival of patients with single-site recurrence than in those with multiple-site recurrence. PD-L1 expression was reported to be associated with EBV infection and MSI-H in GC,[26] indicating that MSI-H GC was a potential predictor of response to immunotherapy. It was reported that gastrointestinal tract cancer with high enrichment of immune signatures had frequent *ARID1A* mutations and a higher response rate to immunotherapy.[27] Since *ARID1A* mutations were associated with MSI-H tumors, immunotherapy might be beneficial for this subgroup of GC patients.

There are limitations in the present study. First, this is a retrospective and single-center study, which may cause selection bias in the present study. The enrollment of more patients from different countries and with different races is needed for the validation of our results. We hope our study will provide useful information for treating GC in the near future. The hotspots of the eight genes selected in the present study are based on the mutation prevalence in GC from the COSMIC database, indicating those can be used as good DNA biomarkers for us to investigate the molecular profiles in GC. Although RNA sequencing provides more information, especially in expression levels, splicing error, gene fusion, and so on, the RNA quality of our GC formalin-fixed paraffin-embedded (FFPE) samples might be still a great challenge for us to perform RNA-seq and have the final good data using the FFPE RNA. Further application of RNA-seq in GC study for testing more mutations is required in the future.

In conclusion, GC with early recurrence was associated with unfavorable clinicopathological features, distant metastases, and poor survival. *PIK3CA* amplifications were associated with early recurrence in diffuse-type GC, whereas GC patients with single-site recurrence had more *ARID1A* mutations and a better prognosis than those with multiple-site recurrence. Targeted therapy and immunotherapy might be applicable for these patients.

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