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Original Article

Down-regulated expression of Rap1GAP predicts unfavorable prognosis in diffuse type gastric cancer and may be correlated with EMT

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Abstract: Aim: To investigate the correlation between Rap1 GTPase-activating protein (Rap1GAP) expression and clinicopathological outcome in patients with gastric cancer. Methods: A total of 219 patients with gastric cancer who were underwent surgery were enrolled in this study. The expression of Rap1GAP, MMP9, MMP2 and E-cadherin was examined by immunohistochemistry. The data of Rap1GAP expression, clinicopathological characteristics and prognosis were analyzed. Associations between Rap1GAP expression with Helicobacter pylori (Hp), MMP9, MMP2 and E-cadherin were also assessed. Results: The rate of down-regulated expression was present in 64.1% of diffuse type gastric cancer and in 35.5% of intestinal type gastric cancer. Interestingly, the results suggested a significant difference of its expression with histological type. In diffuse type gastric cancer, down-regulated expression of Rap-1GAP was significantly correlated with the vascular invasion, histological differentiation, depth of invasion, nodal involvement, advanced tumor-node-metastasis (TNM) stage and poor prognosis. In multivariate analysis, down-regulated expression of Rap1GAP was an independent prognostic factor. Interesting, those statistically significant relationships were not found in intestinal type. Furthermore, Rap1GAP expression was associated with MMP9, MMP2 and E-cadherin with a significant difference. Conclusion: Down-regulated expression of Rap1GAP is present predominantly in diffuse type gastric cancer, not in intestinal type. And, down-regulated expression of Rap1GAP is associated with poor prognosis in diffuse type gastric cancer. Furthermore, down-regulated expression of Rap1GAP may be correlated with epithelial-mesenchymal transition (EMT).

Keywords: Rap1GAP, gastric cancer, diffuse type, intestinal type, prognosis

Introduction

Gastric cancer is one of the most common malignant tumors worldwide, particularly in China, South Korea and Japan. And it remains the second most common mortality throughout the world [1-3]. Because of complexity and heterogeneity of gastric mucosa carcinogenesis, diagnostic techniques and therapeutic methods are improved in the limited situation [4]. Therefore, The identification of novel biomarkers for gastric cancer progression and prognosis would contribute to the development of individualized and refinement treatments.

Rap1 GTPase-activating protein (Rap1GAP), a member of a family of GTPase activation proteins that can work with Rapl-GTP, make the latter becomes inactive Rapl-GDP, thus make Rapl inactivation as a negative regulator [5-7]. Previous reports demonstrated that expression of Rap1GAP was down-regulated in various tumors including pancreatic cancer [8], thyroid tumor [9-12], head and neck squamous cell carcinoma [13, 14], renal carcinoma [15, 16], colorectal carcinoma [17, 18], cervical cancer [19], melanoma [20], acute myeloid leukemia [21], B-cell lymphomas [22], and prostate cancer [23]. Moreover, studies in vivo and in vitro suggested that Rap1GAP, as a kind of tumor suppressor genes, played an important role in tumor cell proliferation, invasion, migration, and metastasis [5, 10, 14, 24, 25]. Only a few studies have reported that expression of Rap-1GAP was associated with clinical significance of Rap1GAP and poor prognostic in endometrial cancer [26] and gastric cancer [27].

Table 1. Clinicopathological characteristics of patients with gastric cancer

Clinicopathological characteristics	N/Mean ± SD	%
Age (year)	61.16±10.41	
≤ 65	146	66.7
> 65	73	33.3
Gender		
Male	151	68.9
Female	68	31.1
Diameter of tumor (cm)	5.02±2.38	
≤ 5	116	53.0
> 5	103	47.0
Vascular invasion		
No	78	35.6
Yes	141	64.4
Differentiation		
High	27	12.3
Moderate	66	30.1
Poor	126	57.5
Depth of invasion		
T1	35	16.0
T2	34	15.5
T3	47	21.5
T4	103	47.0
Nodal involvement		
No	46	21.0
Yes	173	79.0
TNM stage		
I	33	15.1
II	50	22.8
III	121	55.3
IV	15	6.8
Lauren's classification		
Intestinal type	141	64.4
Diffuse type	78	35.6

The purpose of the present study was to investigate the expression of Rap1GAP in gastric cancer, and to retrospectively analyzed the relationship between expression of RaplGAP and clinicopathological characteristics and patients prognosis by histological subtype classifications. To our knowledge, this is the first report of the correlation of Rap1GAP with two histological subtypes of gastric cancer. Furthermore, associations between Rap1GAP with Helicobacter pylori (Hp), MMP9, MMP2 and E-cadherin were also assessed.

Materials and methods

Clinicopathological data

Paraffin embedded sections of 219 gastric cancers and tumor-adjacent tissues were obtained from the Department of General Surgery, Huzhou central Hospital from January 2008 to December 2012. The criteria for enrollment included the following aspects: (1) newly diagnosed gastric cancer by histopathology; (2) no history of other tumors; (3) no neoadjuvant or adjuvant chemotherapy before the operation; (4) no radiotherapy before the operation; (5) no anti-Hp treatment within 1 year before the surgery; (6) the patients who died within 3 months after surgery were not included; (7) patients aged over 80 years were excluded; (8) the patients with severe heart disease or lung disease or liver disease or kidney disease were also excluded.

Clinicopathological data is described in detail in **Table 1**. Of these patients, 151 were males and 68 were females; Age from 21 to 79 years, the median age was 61.16±10.41 years. Cancer staging relies on the tumor node metastasis (TNM) system designed by the American Joint Committee on Cancer (AJCC). Overall survival was measured from the date of surgery to the date of death. The study protocols were approved by the ethics committee and the human research review committee of Huzhou central Hospital and met the guidelines of the responsible governmental agency.

Immunohistochemistry

Immunohistochemical analysis was used to evaluate expression of all samples. 4-μm serial sections from the paraffin blocks were deparaffinized in xylene, dehydrated with gradient ethanol and rehydrated in Tris-buffered saline (TBS). Then, sections were autoclaved at 121°C for 10 min and treated with 3% hydrogen peroxide at room temperature for 15 min. Then, sections were incubated in 3% normal goat serum for 60 min and incubated with primary antibody (Rap-1GAP, 1:200; Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. After washing with TBS, sections were subsequently incubated with the second antibody (goat antirabbitIgG, 1:2000; zhongshan Biotechnology, Beijing, China) for 45 min at room temperature. Finally

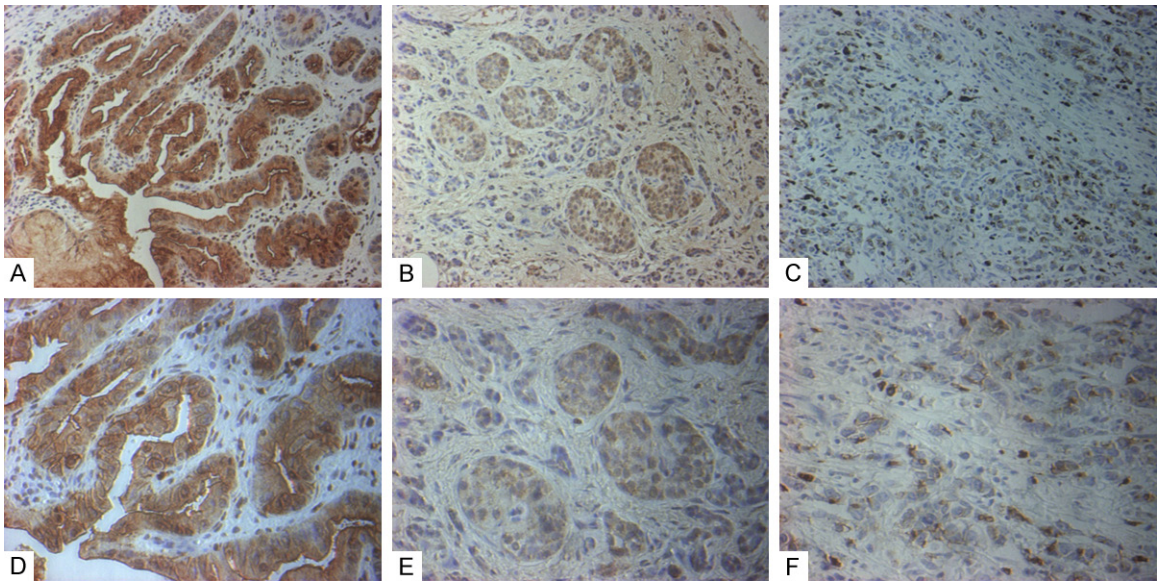

Figure 1. Immunohistochemical expression of Rap1GAP in gastriccancer and para-carcinoma tissues. A, D. Rap1GAP expression was observed in the cytoplasm of para-carcinoma tissues. B, E. Rap1GAP expression was observed in the cytoplasm of intestinal type gastric cancer tissues. C, F. Rap1GAP expression was observed in the cytoplasm of diffuse type gastric cancer tissues. Original magnification: A, B, C × 200; D, E, F × 400.

sections were stained with 3,3-diaminobenzidine (DAB) at room temperature for 60 sec, counter-stained with hematoxylin, dehydrated with gradient ethanol and mounted with neutral resin. Negative controls were made by the omission of the primary antibody during the procedure of immunohistochemistry. MMP9, MMP2 and E-cadherin were also performed immunohistochemical staining.

Two pathologists blinded to the clinical features of gastric cancer patients, independently examined the sections and were in agreement with the final effective immunostaining under a light microscope. The staining was scored by the sum of two parts: (1) staining intensity (negative, 0; weak, 1; moderate, 2; high, 3). (2) percentage of positive cells (≤ 5%% tumor cells with staining, 0; 6-20% tumor cells with staining, 1; 21-50 % tumor cells with staining, 2; 51-75% tumor cells 3; > 75% tumor cells with staining, 4). Histochemistry score = (1) × (2). Rap1GAP and E-cadherin expression was defined as decreased when the score ≤ 4, and the expressions were defined as no down-regulated when the score > 4. The tumors were regarded as having MMP9 and MMP2 high expression when the tumor sample reached a score ≥ 6, and tumor samples with a score < 6 were considered as having low expression.

Statistical analysis

All data were processed using SPSS version 17.0 statistical software. The association between Rap1GAP expression and the patients' clinicopathological characteristics was analyzed with a chi-square test. Overall survival curves were calculated by Kaplan-Meier method and intergroup differences were examined with log-rank test. The prognostic factors were determined by the multivariate survival analysis using Cox proportional hazards model. The association of the Rap1GAP expression with E-cadherin, MMP9, MMP2 and Hp was analyzed by the Spearman's rank correlation coefficient. *P* values < 0.05 was considered to represent statistical significance.

Results

Down-regulated expression of RapIGAP in diffuse type gastric cancer, and decreased expression of E-cadherin and increased expression of MMP9 and MMP2 in gastric cancer

As shown in **Figure 1**, RapIGAP was observed to be mainly expressed in the cytoplasm of gastric cancer cells. The expression of RapIGAP was lower in gastric cancer tissue than normal gastric tissue mucosa. As shown in **Table 2**, the

Table 2. Association between Rap1GAP expression and clinicopathological characteristics of gastric cancer

Clinicopathological characteristics	n	Down-regulated of diffuse type		p	n	Down-regulated of intestinal type		p	n	Down-regulated of total		p
		Yes	No			Yes	No			Yes	No	
Age (year)												
≤ 65	51	32 (62.7%)	19 (37.3%)	0.731	95	35 (36.8%)	60 (63.2%)	0.622	146	67 (45.9%)	79 (54.1%)	0.924
> 65	27	18 (66.7%)	9 (33.3%)		46	15 (32.6%)	31 (67.4%)		73	33 (45.2%)	40 (54.8%)	
Gender												
Male	52	31 (59.6%)	21 (40.4%)	0.243	99	33 (33.3%)	66 (66.7%)	0.417	151	64 (42.4%)	87 (57.6%)	0.147
Female	26	19 (73.1%)	7 (26.9%)		42	17 (40.5%)	25 (59.5%)		68	36 (52.9%)	32 (47.1%)	
Diameter of tumor (cm)												
≤ 5	37	23 (62.2%)	14 (37.8%)	0.734	79	24 (34.4%)	55 (69.6%)	0.155	116	47 (40.5%)	69 (59.5%)	0.105
> 5	41	27 (65.9%)	14 (34.1%)		62	26 (41.9%)	36 (58.1%)		103	53 (51.5%)	50 (48.5%)	
Vascular invasion												
No	28	13 (46.4%)	15 (56.3%)	0.015	50	15 (30.0%)	35 (70.0%)	0.315	78	28 (35.9%)	50 (64.1%)	0.031
Yes	50	37 (74.0%)	13 (26.0%)		91	35 (38.5%)	56 (61.5%)		141	72 (51.1%)	69 (48.9%)	
Differentiation												
High	10	3 (30.0%)	7 (70.0%)	0.001	17	5 (29.4%)	12 (70.6%)	0.128	27	8 (29.6%)	19 (70.4%)	0.001
Moderate	22	10 (45.5%)	12 (54.5%)		44	11 (25.0%)	33 (75.0%)		66	21 (31.8%)	45 (68.2%)	
Poor	46	37 (80.4%)	8 (19.6%)		34	34 (42.5%)	46 (57.5%)		126	71 (56.3%)	55 (43.7%)	
Depth of invasion												
T1	13	4 (30.8%)	9 (69.2%)	0.009	22	8 (36.4%)	14 (63.6%)	0.346	35	12 (34.3%)	23 (65.7%)	0.029
T2	12	7 (58.3%)	5 (41.7%)		22	6 (27.3%)	16 (72.7%)		34	13 (38.2%)	21 (61.8%)	
T3	16	9 (56.2%)	7 (43.8%)		31	8 (25.8%)	23 (74.2%)		47	17 (36.2%)	30 (63.8%)	
T4	37	30 (81.1%)	7 (18.9%)		66	28 (42.4%)	38 (57.6%)		103	58 (56.3%)	45 (43.7%)	
Nodal involvement												
No	17	7 (41.2%)	10 (58.8%)	0.026	29	6 (20.7%)	23 (23.0%)	0.062	46	13 (28.3%)	33 (71.7%)	0.008
Yes	61	43 (70.5%)	18 (29.5%)		112	44 (39.3%)	68 (60.7%)		173	87 (50.3%)	86 (49.7%)	
TNM stage												
I	13	4 (30.8%)	9 (69.2%)	0.036	20	4 (20.0%)	16 (80.0%)	0.144	33	8 (24.2%)	25 (75.8%)	0.016
II	18	11 (61.1%)	7 (38.9%)		33	10 (30.3%)	23 (69.7%)		51	21 (41.2%)	30 (58.8%)	
III	40	30 (75.0%)	10 (25.0%)		78	30 (28.5%)	48 (61.5%)		118	60 (50.8%)	58 (49.2%)	
IV	7	5 (71.4%)	2 (28.6%)		10	6 (60.0%)	4 (40.0%)		17	11 (64.7%)	6 (35.3%)	

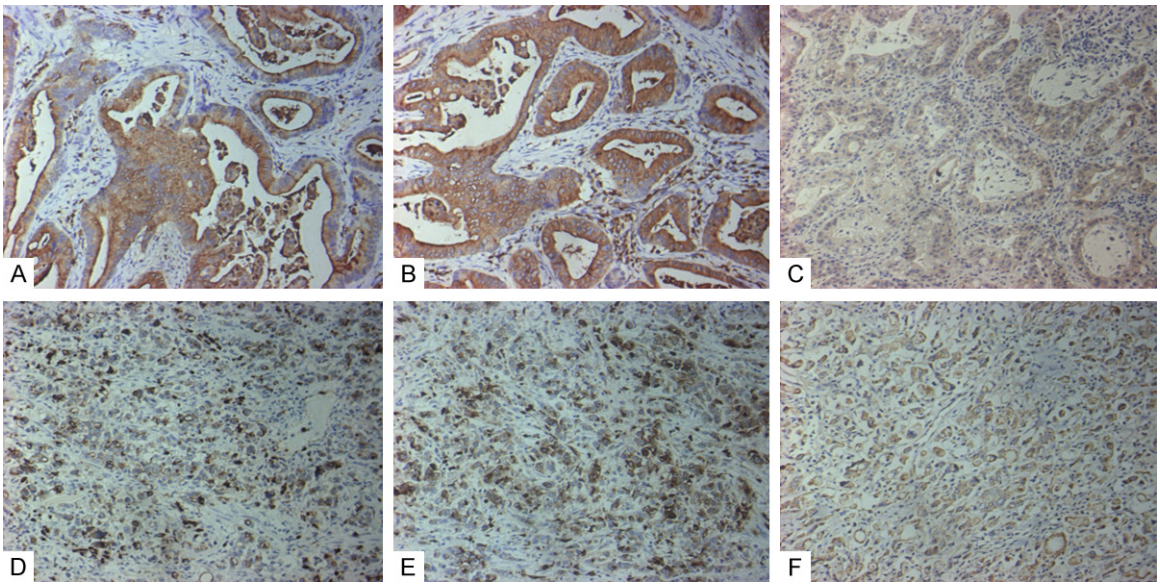

Figure 2. Immunohistochemical expression of MMP9, MMP2 and E-cadherin in gastric cancer tissues. A. MMP9 expression was observed in the cytoplasm of intestinal type gastric cancer tissues. D. MMP9 expression was observed in the cytoplasm of diffuse type gastric cancer tissues. B. MMP2 expression was observed in the cytoplasm of intestinal type gastric cancer tissues. E. MMP2 expression was observed in the cytoplasm of diffuse type gastric cancer tissues. C. E-cadherin expression was observed in the plasma membrane of intestinal type gastric cancer tissues. F. E-cadherin expression was observed in the plasma membrane of diffuse type gastric cancer tissues. Original magnification, × 200.

Table 3. Univariate analysis using log-rank testfor overall survival

Variables	Overall survival of diffuse type		p	Overall survival of intestinatype		p	Overall survival of total		p
	Median ± SE	95% CI		Median ± SE	95% CI		Median ± SE	95% CI	
Rap1 GAP expression									
Down-regulatd	24.00±1.72	20.64-27.36	< 0.001	32.00±3.34	25.45-38.55	0.095	27.00±1.62	23.83-30.17	< 0.001
No down-regulatd	42.00±6.61	29.04-54.96		38.00±2.68	32.76-43.24		39.00±2.14	34.80-43.20	
Age									
≤ 65 year	28.00±2.91	22.31-33.70	0.700	35.00±2.05	30.98-39.02	0.529	34.00±1.50	31.06-36.94	0.492
> 65 year	41.82±4.33	23.52-40.48		37.00±5.03	27.15-46.85		34.00±3.76	26.64-41.36	
Gender									
Male	30.00±3.89	22.37-37.63	0.595	40.00±2.83	34.45-45.55	0.145	36.00±2.86	30.39-41.61	0.128
Female	25.00±4.46	16.26-33.75		33.00±1.86	29.35-36.65		31.00±1.83	27.41-34.59	
Diameter oftumor									
≤ 5 cm	32.00±4.56	23.05-40.95	0.576	33.00±2.91	27.29-38.71	0.373	32.00±1.94	28.20-35.80	0.794
> 5 cm	28.00±3.19	21.74-34.26		38.00±3.21	31.70-44.30		34.00±2.07	29.95-38.05	
Vascular invasion									
No	36.00±7.93	20.44-51.56	0.002	42.00±7.44	27.42-56.58	0.180	42.00±6.29	29.66-54.34	0.004
Yes	24.00±1.72	20.64-27.36		35.00±2.16	30.77-39.23		33.00±1.51	30.05-35.95	
Differentiation									
High	50.00±10.28	29.86-70.14.	0.006	34.00±6.86	20.56-47.45	0.023	42.00±12.12	18.25-65.75	< 0.001
Moderate	36.00±4.43	27.32-44.68		44.00±5.71	32.80-55.20		42.00±4.14	33.89-50.11	
Poor	24.00±2.35	19.40-28.60		33.00±2.41	28.29-37.71		31.00±1.57	27.93-34.08	
Depth of invasion									
T1	44.00±11.38	21.69-66.31	0.061	46.00±7.57	31.16-60.84	0.101	46.00±7.59	34.65-57.36	0.006
T2	32.00±3.46	25.21-38.79		46.00±10.42	25.58-66.42		42.00±4.36	33.45-50.55	
T3	29.00±2.00	25.08-32.92		40.00±5.73	28.77-51.23		36.00±3.80	28.56-43.44	
T4	23.00±1.98	19.12-26.88		34.00±1.48	31.10-36.90		31.00±2.24	26.62-35.38	
Nodal involvement									
No	28.00±6.55	15.17-40.83	0.911	37.00±5.93	25.38-48.63	0.898	33.00±3.65	25.84-40.16	0.972
Yes	30.00±2.74	24.63-35.37		36.00±2.51	31.09-40.91		34.00±1.52	31.02-36.98	
TNM stage									
I	55.00	0.00	0.010	33.00±15.65	2.32-63.68	0.016	50.00	0.00	< 0.001
II	29.00±1.41	26.23-31.77		48.00±2.64	42.83-53.18		44.00±7.35	29.59-58.41	
III	25.00±2.44	20.21-29.79		34.00±2.08	29.93-38.07		32.00±1.48	29.09-34.91	
IV	21.00±1.31	18.43-23.57		30.00±9.24	11.90-48.10		22.00±1.94	18.21-25.79	

rate of down-regulated expression was 64.1%% (50 of 78) in diffuse type gastric cancer, 35.5% (50 of 141) in intestinal type and 45.7% (100 of 219) in total. Interestingly, the results suggested a significant difference of its expression with histological type (64.1%% vs 35.5%; P < 0.001).

As shown in **Figure 2**, MMP9, MMP2 was diffusely detected in the cytoplasm of gastric cancer cells. The expression of MMP9, MMP2 was higher in gastric cancer tissue compared with normal gastric tissue mucosa. On the contrary, E-cadherin was observed to be mainly expressed in the plasma membrane of gastric cancer cells. The expression of E-cadherin was lower in gastric cancer tissue compared to normal gastric tissue mucosa.

Rap1GAP expression was associated with clinicopathological characteristics of diffuse type gastric cancer

As shown in **Table 2**, there were no correlations between the expression of Rap1GAP and gender, age ordiameter of tumor (all Ps > 0.05). The down-regulated expression of Rap1GAP was correlated with the vascular invasion (P = 0.031), histological differentiation (P = 0.001), depth of invasion (P = 0.029), nodal involvement (P = 0.008) and advanced TNM stage (P = 0.016).

When diffuse type gastric cancer was examined separately, there were also no correlations between the expression of Rap1GAP and gen-

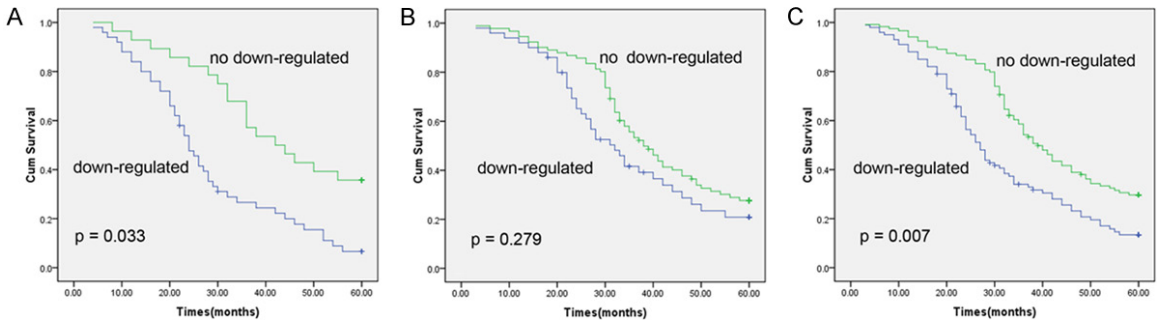

Figure 3. Overall survival curves according to Rap1GAP expression in gastric cancer. Patients with Rap1GAP expression in diffuse typegastric cancer and total had significantly poorer prognosis. A. Overall survival curves according to Rap1GAP expression in diffuse type. B. Overall survival curves according to Rap1GAP expression in intestinal type. C. Overall survival curves according to Rap1GAP expression in total.

Table 4. Multivariate Cox Proportional Hazards analysis for overall survival

Variables	Overall survival of diffusetype		p	Overall survival of intestinaltype		p	Overall survival of total		p
	HR	95% CI		HR	95% CI		HR	95% CI	
Rap1GAP expression									
(down-regulated vs nodown-regulatd)	2.114	1.063-4.204	0.033	1.271	0.823-1.963	0.279	1.581	1.133-2.207	0.007
Age									
(≤ 65 year vs > 65 year)	1.281	0.688-2.386	0.435	0.887	0.561-1.401	0.607	0.965	0.684-1.362	0.839
Gender									
(male vs female)	1.144	0.636-2.058	0.654	0.712	0.450-1.127	0.147	0.840	0.594-1.189	0.325
Diameter oftumor									
(≤ 5 cm vs > 5 cm)	0.785	0.431-1.428	0.428	1.499	0.984-2.283	0.059	1.211	0.877-1.673	0.245
Vascular invasion									
(no vs yes)	1.067	0.443-2.570	0.884	1.616	0.792-3.295	0.187	1.217	0.711-2.082	0.474
Differentiation									
(high+moderate vs poor)	0.566	0.292-1.096.	0.092	0.555	0.337-0.916	0.021	0.627	0.426-0.923	0.018
Depth of invasion									
(T1+T2 vs T3+T4)	1.163	0.489-2.762	0.733	0.800	0.462-1.384	0.424	0.829	0.525-1.310	0.421
Nodal involvement									
(no vs yes)	0.787	0.378-1.640	0.523	1.092	0.653-1.827	0.737	0.945	0.630-1.417	0.784
TNM stage									
(I+II vs III+IV)	0.514	0.221-1.198	0.023	0.635	0.310-1.299	0.213	0.627	0.408-1.185	0.182

der, age ordiameter of tumor (all Ps > 0.05). Meanwhile, a statistically significant correlation was also found with the down-regulated expression of Rap1GAP and the vascular invasion (P = 0.015), histological differentiation (P = 0.001), depth of invasion (P = 0.009), nodal involvement (P = 0.026) and advanced TNM stage (P = 0.036) (**Table 2**).

On the other hand, interestingly, no statistically significant association was observed between the down-regulated expression of Rap1GAP and clinicopathological characteristics in intestinal type gastric cancer (all Ps > 0.05) (**Table 2**).

Down-regulated expression of Rap1GAP was related with poor prognosis in diffuse type gastric cancer

As shown in **Table 3**, univariate analysis suggested that the down-regulated expression of Rap1GAP (P < 0.001), vascular invasion (P = 0.004), histological differentiation (P < 0.001), depth of invasion (P = 0.006) and advanced TNM stage (P < 0.001) were significantly associated with a poorer prognosis. As shown in **Figure 3C**, Kaplan-Meier analysis indicated that the overall survival of the down-regulated Rap1GAP group was significantly poorer than that of the no down-regulated Rap1GAP group

Table 5. Correlation between Rap1GAP and HP infection, MMP9, MMP2 and E-cadherin expression

Variables	n	Rap1GAP		r	p
		Down-regulated	No down-regulated		
Hp					
No infection	122	49 (40.2%)	73 (59.8%)	-0.124	0.067
Infection	97	51 (52.6%)	46 (47.4%)		
MMP9					
No up-regulated	59	14 (23.7%)	45 (76.3%)	-0.267	< 0.001
Up-regulated	160	86 (53.8%)	74 (46.2%)		
MMP2					
No up-regulated	61	17 (27.9%)	44 (72.1%)	-0.222	0.001
Up-regulated	158	83 (52.5%)	75 (47.5%)		
E-cadherin					
Down-regulated	147	78 (53.1%)	69 (46.9%)	0.168	0.013
No down-regulated	72	22 (30.6%)	50 (69.4%)		

(P < 0.001). As shown in **Table 4**, multivariate analysis revealed that Rap1GAP expression and histological differentiation were independent predictive factors for poor prognosis (P = 0.007 and P = 0.018, respectively).

When diffuse type gastric cancer was examined separately, univariate analysis suggested that the down-regulated expression of Rap1GAP (P < 0.001), vascular invasion (P = 0.002), histological differentiation (P < 0.006) and advanced TNM stage (P = 0.010) were significantly associated with a poorer prognosis (**Table 3**). However, no statistically significant association was found between the depth of invasion and a poor prognosis (P = 0.061) (**Table 3**). As shown in **Figure 3A**, Kaplan-Meier analysis indicated that the overall survival of the down-regulated Rap1GAP group was significantly poorer than that of the no down-regulated Rap1GAP group (P < 0.001). Furthermore, multivariate analysis revealed that Rap1GAP expression and advanced TNM stage were independent prognostic factors for patients with diffuse type gastric cancer (P = 0.033 and P = 0.023, respectively) (**Table 4**).

On the other hand, when intestinal type gastric cancer was examined separately, univariate analysis suggested that the histological differentiation (P = 0.023) and advanced TNM stage (P = 0.016) were significantly associated with a poorer prognosis (**Table 3**). As shown in **Figure 3B**, interestingly, Kaplan-Meier analysis indicated that no statistically significant association was found between the down-regulated expression of Rap1GAP and a poor prognosis (P = 0.095). Meanwhile, multivariate analysis revealed that histological differentiation was an independent prognostic factor for patients with intestinal type gastric cancer (P = 0.021). Interestingly, Rap1GAP expression was not an independent prognostic factor (P = 0.279) (**Table 3**).

Rap1GAP was correlated with MMP9, MMP2 and E-cadherin expression

As shown in **Table 5**, down-regulated expression of Rap1GAP was negatively correlated with MMP9 and MMP2 expression (r = -0.267, P < 0.001 and r = -0.222, P = 0.001, respectively) and positively associated with E-cadherin expression (r = 0.168, P = 0.013). Moreover, no statistically significant correlation was found between the down-regulated expression of Rap1GAP and HP infection (r = -0.124, P = 0.067).

Discussion

Gastric cancer, which has high morbidity, difficult early diagnosis, and poor treatment results, is a type of malignant tumor in the digestive system. And it remains the second most common mortality worldwide [1-3]. To reduce mortality of patients with gastric cancer, it is particularly important to diagnosis patients of poor outcome by an appropriate prognostic factor.

Rap1GAP is a member of the GTPase activation protein family, which promotes hydrolysis of GTP to GDP by specific stimulation as a negative regulator of Rap1 activity [5-7]. Recently, various studies have suggested that Rap1GAP, as a tumor suppressor marker, play an important role in the inhibition of proliferation, invasion, and progression [10, 18, 20, 14]. On the contrary, other reports showed up-expression of Rap1GAP facilitated tumor invasion [8, 21]. And, Rap1GAP expression and its clinical significance in gastric cancer is lesser unknown. In our present study, the expression of Rap1GAP was down-regulated in gastric cancer. Moreover, our results showed the down-regulated expres-

sion is present predominantly in the diffuse type gastric cancer and clinical significance of Rap1GAP expression in diffuse type. For all we know, this is the first report of the correlation of Rap1GAP with gastric cancer by histological subtype classifications.

Based on Lauren's classification, there are two major histological subtypes in gastric cancer including diffuse type and intestinal type [28]. Peritoneal dissemination is more likely to occur in diffuse type gastric cancer by the reason of the propensity for intramural and transmural spread [29, 30]. Liver metastasis is more inclined to occur in intestinal type gastric cancer because of the influence of environmental factors such as intestinal metaplasia and Hp infection [31, 32]. Previous study demonstrated that the expression of Rap1GAP was down-regulated in various tumors including pancreatic cancer [8], thyroid tumor [9-12], head and neck squamous cell carcinoma [13, 14], renal carcinoma [15, 16], colorectal carcinoma [17, 18], cervical cancer [19], melanoma [20], acute myeloid leukemia [21], B-cell lymphomas [22], and prostate cancer [23]. In this study, we detected that the rate of down-regulated expression was 64.1%% in diffuse type gastric cancer, 35.5% in intestinal type and 45.7% in total. Interestingly, the results suggested a significant difference of its expression with histological type. One of the major reason for this phenomenon could be that diffuse type gastric cancer means a greater likelihood of malignant behavior, such as a higher tendency of lymph node metastasis, extracapsular extension and neurovascular invasion compared with intestinal type. That is, diffuse type tumor is more aggressive and progress compared to intestinal type.

The down-regulated expression of Rap1GAP was significantly associated with the vascular invasion, histological differentiation, depth of invasion, nodal involvement and advanced TNM stage, findings that is similar to a recent report [27]. When diffuse type gastric cancer was examined separately, a statistically significant correlation was found with the down-regulated expression of Rap1GAP and the vascular invasion, histological differentiation, depth of invasion, nodal involvement and advanced TNM stage. That is, the lower tumor histological grade, the deeper tumor invasion depth, the higher tendency of lymph node metastasis and vascular invasion, the advanced TNM stage, the higher rate of Rap1GAP down-regulated expression. Our results indicated that RaplGAP may be involved in the tumorigenesis and progression of gastric cancer. Meanwhile, Kaplan-Meier analysis and log-rank test showed that patients with down-regulated expression of Rap1GAP had shorter overall survival than without down-regulated expression. Furthermore, multivariate Cox analysis demonstrated that Rap1GAP was an independent prognostic factor in diffuse type gastric cancer. Our results suggested that Rap1GAP could be used to identify high-risk individual patients with diffuse type gastric cancer who have an increased risk of tumor recurrence and death. Those patients with down-regulated expression may be good candidates for receiving more aggressive treatment.

On the other hand, interestingly, no statistically significant association was observed between the down-regulated expression of Rap1GAP and clinicopathological characteristics in intestinal type gastric cancer. Moreover, no statistically significant correlation was found between the down-regulated expression of Rap1GAP and prognosis. Our results indicated that subtype stratification is likely to be important as the down-regulated expression of Rap1GAP is present predominantly in diffuse type gastric cancer, not in intestinal type. Two subtype compositions of gastric cancer were analyzed together, which may have an influence on accuracy in reporting the association of the down-regulated expression and clinicopathological characteristics and prognosis.

The molecular mechanism of the down-regulated expression of Rap1GAP is still not fully understood [27, 29, 30]. As one of the most common pathogens, Hp is a Gram negative microaerophilic bacterium, which is closely associated with gastric cancer and even has a synergistic contribution on colorectal cancer [33, 34]. However, in this study, we observed that there is not a notable correlation of Rap1GAP expression with Hp infection. We think one of the reasons could be that Hp infection is one of the major etiological factors of intestinal type gastric cancer [31, 32], while down-regulated expression of Rap1GAP is present predominantly in diffuse type gastric cancer, not in intestinal type.

Matrix metalloproteinases (MMPs) can degrade extracellular matrix and the basal membrane,

thus induce tumor progression through increased migration, invasion, metastasis, and angiogenesis [35-39]. It has been reported that up-regulated expression of MMP9 and MMP2 was closely associated with tumor aggressiveness and poor prognosis in various cancers including gastric cancer [37, 38, 40]. In head and neck squamous cell carcinoma, Rap1GAP inhibited tumor growth and promoted MMP9 secretion via Rap1-mediated effects on MMP9 protein and mRNA [41]. In leukemia, up-regulated expression of Rap1GAP enhanced the invasion ability of the leukemic cells through increased MMP9 expression and secretion [21]. However, to date, no study has reported the relation between Rap1GAP expression and MMP9 in gastric cancer, and we found a significant negative correlation between Rap1GAP and MMP9 expression. We consider a reason for the difference could be that the biomarker was studied in different kinds of tumor cell. Meanwhile we also observed a significant negative correlation between Rap1GAP and MMP2, findings that are similar to this study [27].

The down-regulated expression of E-cadherin can promote tumor cell invasion and metastasis in many human malignancies including gastric cancer [15, 42, 43]. It was reported that down-regulated expression of Rap1GAP did not induce E-cadherin secretion [44], andup-expression of Rap1GAP did not suppress the E-cadherin secretion [15]. Another report showed that the up-expression of Rap1GAP decreased the expression of E-cadherin [5]. However, we observed a significant positive correlation between Rap1GAP and E-cadherin, findings that are similar to this study [27]. Our results suggested that down-regulated expression of Rap1GAP may be correlated with epithelial-mesenchymal transition (EMT). Therefore, our future investigations will focus on the EMT molecular mechanism of the effect of Rap1GAP in gastric cancer.

Data from this study should be interpreted with some caution. The major limitation is the relatively small number of patients and the short follow-up period. Therefore, this issue should be investigated in larger studies that include more number of patients and longer follow-up periods.

In conclusion, down-regulated expression of Rap1GAP is present predominantly in diffuse type gastric cancer, not in intestinal type. And, down-regulated expression of Rap1GAP closely associated with some of the clinicopathological characteristics and poor prognosis in diffuse type gastric cancer. Furthermore, we found that expression of Rap1GAP was correlated with the MMP9, MMP2 and E-cadherin. Our results suggested that RaplGAP may be involved in the tumorigenesis and progression of gastric cancer, finding that might be helpful to the treatment of patients with diffuse type gastric cancer as a prognosis biomarker. Meanwhile, down-regulated expression of Rap1GAP may be correlated with EMT.

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Disclosure of conflict of interest

None.

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