

Original Article

Is the signet ring cell histological type a positive prognostic factor for gastric adenocarcinoma after D2 radical gastrectomy?

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Abstract: Background: Previous studies indicated the histological type might have prognostic value in gastric adenocarcinoma patients. The aim of this study was to clarify whether the signet ring cell (SRC) histological type of gastric adenocarcinoma is associated with worse prognosis than pure gastric adenocarcinoma without any mixed histological component (PGA) for patients underwent D2 radical gastrectomy. Method: By the database of 6205 surgical patients with gastric adenocarcinoma at our department between September 2008 and May 2015, we investigated 133 SRC patients and 2847 PGA patients who underwent D2 radical gastrectomy. The clinic and pathologic data, especially tumor and pathology molecular markers, and 5-year overall survival rate were compared between SRC and PGA. The univariate and multivariate analysis were used to testify the prognosis significance of SRC. Results: SRC was more frequently found in younger (<50), female patients, lower part of stomach and easy to metastasize lymph nodes. And more positive CA19-9 and less positive EGFR were obtained in SRC. The 5-year survival rate was not different between SRC and PGA after D2 radical gastrectomy, even assessed by T stage. Multivariate analysis showed age, T and N stage, CEA, CA19-9 and CA125 were the independent prognostic factors, not included the SRC histological type. Conclusion: Although SRC histological type of gastric adenocarcinoma after D2 radical gastrectomy had its own clinic-pathologic characters, especially in molecular tumor and pathological markers, the histological type of SRC was a negative prognostic factor in gastric adenocarcinoma patients after D2 radical gastrectomy.

Keywords: Signet ring cell adenocarcinoma (SRC), pure gastric adenocarcinoma without any mixed histological component (PGA), D2 radical gastrectomy, clinic-pathologic characters, prognosis

Introduction

Despite the development in diagnosis and treatment, gastric adenocarcinoma (GA) remains one of the most leading causes of tumor related death worldwide [1, 2], especially in China [3]. Because of the mixed histological patterns in GA [4], the World Health Organization (WHO) defined signet ring cell adenocarcinoma (SRC) as a special histological type in which the predominant component consists of isolated or small groups of malignant cells containing intracytoplasmic mucins [5-7]. Some studies indicated SRC had worse prognosis than non-SRC [8-10]; according to others, SRC and non-SRC had equivalent prognosis [11-13]. Thus, in

view of the studies on prognostic value of histological variables in SRC and their conflicting results, we performed a retrospective single-center study to compare the difference of clinic-pathologic characteristics, especially tumor and pathology molecular markers, and 5-year overall survival rate between SRC and PGA patients after D2 radical gastrectomy to test the hypothesis that SRC was an independent factor with worse prognosis.

Patient enrollment

During the period between September 2008 and May 2015 patients diagnosed as gastric adenocarcinoma at Division of Digestive

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Table 1. Clinic-pathological features of SRC versus PGA

Variables	SRC N=133	PGA N=2847	P value
Age			0.000
<30	4 (3.0%)	32 (1.1%)	
31-40	19 (14.3%)	153 (5.4%)	
41-50	33 (24.8%)	524 (18.4%)	
51-60	43 (32.3%)	913 (32.1%)	
61-70	23 (17.3%)	857 (30.1%)	
71-80	11 (8.3%)	350 (12.3%)	
>80	0 (0.0%)	18 (0.6%)	
Gender			0.000
Male	87 (65.4%)	2244 (78.8%)	
Female	46 (34.6%)	603 (21.2%)	
Blood type			0.758
A	43 (33.9%)	855 (30.5%)	
B	32 (25.2%)	806 (28.8%)	
AB	12 (9.4%)	295 (10.5%)	
O	40 (31.5%)	848 (30.2%)	
Smoking history			0.104
Yes	118 (88.7%)	2374 (83.4%)	
No	15 (11.3%)	473 (16.6%)	
Drinking history			0.192
Yes	11 (8.3%)	342 (12.0%)	
No	122 (91.7%)	2505 (88.0%)	
Location			0.000
Upper	21 (17.5%)	998 (35.1%)	
Middle	26 (21.7%)	513 (18.0%)	
Lower	68 (56.7%)	1294 (45.5%)	
Whole	5 (4.2%)	41 (1.4%)	
Macroscopic type			0.399
Early GC	20 (16.4%)	518 (19.0%)	
Borrmann I	9 (7.4%)	207 (7.6%)	
Borrmann II	28 (23.0%)	686 (25.2%)	
Borrmann III	48 (39.3%)	1070 (39.3%)	
Borrmann IV	17 (13.9%)	240 (8.8%)	
Depth on invasion			0.598
T1	22 (16.5%)	550 (19.1%)	
T2	23 (17.3%)	451 (16.0%)	
T3	44 (33.1%)	1030 (36.2%)	
T4	44 (33.1%)	816 (28.7%)	
Lymph node metastasis			0.037
N0	41 (30.8%)	1057 (37.2%)	
N1	18 (13.5%)	551 (19.5%)	
N2	31 (23.3%)	491 (17.4%)	
N3	43 (32.3%)	727 (25.7%)	
CEA			0.454
Positive	23 (20.4%)	450 (17.6%)	

Surgery of Xijing Digestive Hospital were enrolled in as candidates. Every patient was assessed carefully for the safety of anesthesia and operation. A signed formal informed consent was obtained from all participants before anesthesia and surgery. And this study adhered to the guideline established by the Declaration of Helsinki and was approved by the institutional review board. All patients enrolled in had undergone D2 curative resection surgery as the standard approach, and pathologic diagnosis was made by at least two experienced trained gastrointestinal pathologists.

Collection of patients' clinic-pathologic characters

Histological types were classified by using the WHO criteria [14, 15]. Data collected for each patient included age, gender, blood type, history of drinking and smoking. The tumor-specific information included tumor location, Borrmann type, tumor biomarkers (AFP, CEA, CA-125, CA19-9), depth of gastric wall invasion, lymph nodes metastasis and features of molecular pathological markers (S-100, EGFR) were obtain from anesthetic and surgical records, nursing and medical charts and post-operative histopathology reports.

Follow-up and statistical analysis

Patients between SRC and PGA were selected by using gmatch methods based on same gender, tumor location, T and N stage in order to reduce the differences of clinic-pathological characteristics. And the gmatch macro of SAS 9.2 (SAS institute, Cary, NC, USA) in this study was written by Erik Bergstrahl and Jon Kosanke (<http://www.mayo.edu/research/departments-divisions/department-health-sciences-research/division-biomedical-statistics-informatics/software/locally-written-sas-macros>) and used to execute this pair matching process. The principle of this macro is computerized matching of cases to controls using the greedy matching algorithm with a fixed number of controls per case. Controls may be matched to cases using one or more factors.

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Negative	90 (79.6%)	2106 (82.4%)	0.068
Positive	2 (1.9%)	151 (6.1%)	
Negative	105 (98.1%)	2312 (93.9%)	0.021
Positive	29 (26.6%)	446 (17.9%)	
Negative	80 (73.4%)	2048 (82.1%)	0.645
Positive	4 (3.8%)	116 (4.8%)	
Negative	101 (96.2%)	2312 (95.2%)	0.627
Positive	61 (74.4%)	481 (23.4%)	
Negative	21 (25.6%)	1585 (76.6%)	0.000
Positive	34 (40.5%)	1341 (60.5%)	
Negative	50 (59.5%)	875 (39.5%)	

The selected patients were followed up according the standard protocol (3 months intervals for the first two years, every six months for the subsequent three years and yearly thereafter). The overall survival rate was estimated by the Kaplan-Meier methods and values were compared by long-rank test. Covariates that remained significant through univariate analysis were selected for multivariate analysis. The COX proportional hazard model was employed for a multivariate analysis. All statistical analyses were performed by SPSS software (version 22.0). *P* values less than 0.05 were considered as statistically significant, and all tests were 2-sided.

Results

Patients' characters

Among 6205 gastric adenocarcinoma patients, 2980 patients were enrolled (including 133 SRC and 2847 PGA). The clinicopathologic characteristics of SRC and PGA patients are showed in **Table 1**. Compared with PGA, SRC occurred more frequencies in female, younger patients (≤ 50), distal stomach and lymph nodes metastasis compared to that of PGA ($P < 0.05$). As for the tumor specific and pathological molecular markers, the positive expression of CA19-9 (26.6% vs 17.9%, $P = 0.021$) in SRC was higher than that of PGA ($P < 0.05$), while the positive rate of EGFR (40.5% vs 60.5%, $P = 0.000$) was lower in SRC. And there were no significant differences were obtained in blood type, smok-

ing and drinking history, depth of gastric wall invasion, positive expression of CEA, AFP, CA125 and S-100 between SRC and PGA ($P > 0.05$) (**Table 1**).

Survival analysis

By gmatch methods, 120 pairs of patients between SRC and PGA were selected and analyzed based on same gender, tumor location, T and N stage. The follow-up data ranged from 1 to 75 months with an overall rate of visits available for analysis at 100%.

The 5-year survival rate for SRC patients after D2 radical gastrectomy had no significant difference compared to PGA (41% vs 61.6%, $P = 0.592$). When 5-year overall survival rates were compared by T stage, no significant differences were also found between SRC and PGA (except for T1 stage because of small quantity) (**Figure 1**).

Eleven factors (including age, tumor location, Borrmann type, T and N stage, CEA, AFP, CA19-9, CA125, S-100 and SRC histological type) evaluated in the univariate analysis had a significant influence on the long-term survival (**Table 2**). And the multivariate analysis indicated that independent prognostic factors were age, T and N stage, Borrmann type, CEA, CA19-9 and CA125, and the histological type of SRC was not a prognostic factor for gastric adenocarcinoma patients after D2 radical gastrectomy (**Table 2**).

Discussion

The WHO defined signet ring cell (SRC) as special histological subtype in which a poorly cohesive carcinoma composed predominantly of cancer cells with prominent cytoplasmic mucin and a crescent shaped nucleus eccentrically placed [16].

Although there were already numerous researches on SRC, the prognosis of SRC in gastric adenocarcinoma is still controversial. Kwon reported a study included 769 gastric adenocarcinoma, which showed a better prognosis of SRC in early gastric adenocarcinoma patients and worse prognosis in advanced gastric adenocarcinoma [17]. In Gronnier's study, SRC had similar prognosis compared to non-

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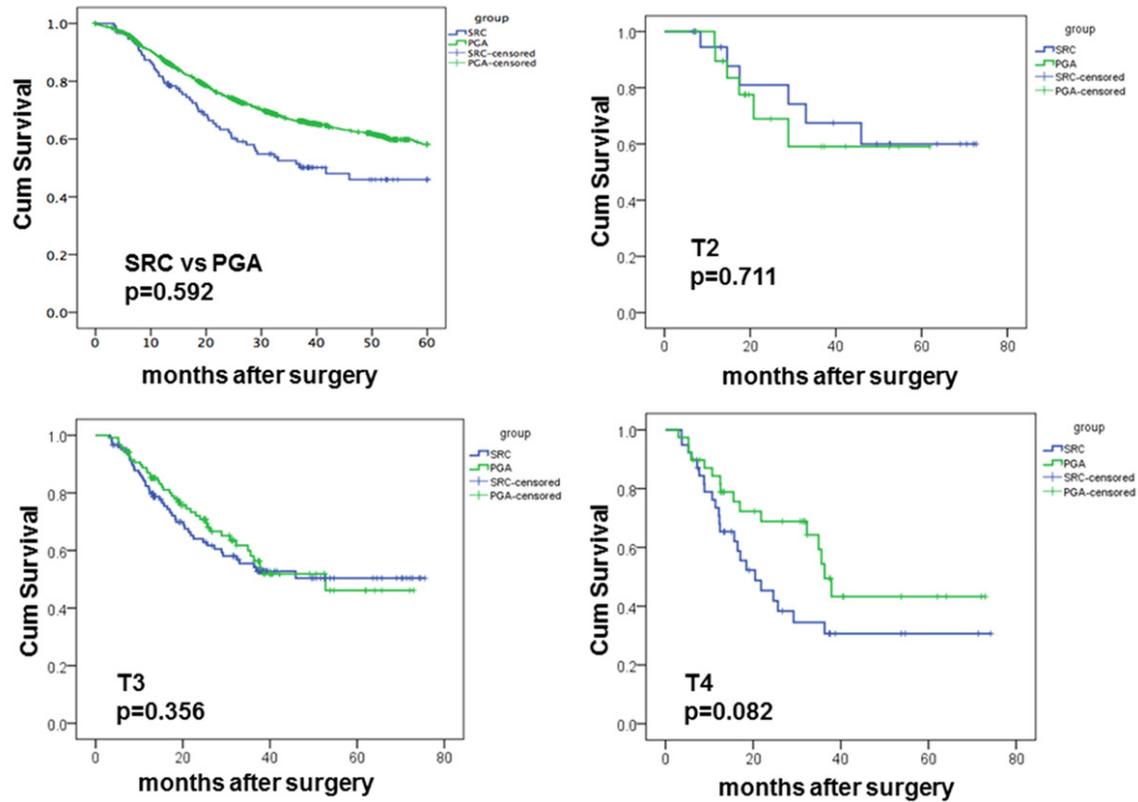


Figure 1. Survival curve for patients of SRC and PGA.

Table 2. Univariate and Multivariate analyses of prognostic factors between SRC and PGA

Characteristics	β	Hazard ratio (95% CI)	P value
Univariate analyses			
Age	0.114	1.120 (1.055-1.189)	0.000
T stage	0.803	2.233 (2.056-2.426)	0.000
N stage	0.702	2.018 (1.897 -2.146)	0.000
Location	-0.102	0.903 (0.839-0.971)	0.006
Borrmann type	0.625	1.869 (1.737-2.011)	0.000
CEA	0.864	2.373 (2.018 -2.790)	0.000
AFP	0.747	2.111 (1.654-2.696)	0.000
CA19-9	0.797	2.218 (1.886-2.610)	0.000
CA125	1.266	3.548 (2.749-4.578)	0.000
S-100	1.133	3.106 (2.405-4.011)	0.000
SRC histological type	-0.422	0.656 (0.500-0.860)	0.002
Multivariate analyses			
Age	0.116	1.123 (1.029-1.224)	0.009
T stage	0.454	1.575 (1.346-1.844)	0.000
N stage	0.571	1.771 (1.591-1.970)	0.000
CEA	0.314	1.369 (1.104-1.697)	0.004
CA19-9	0.278	1.321 (1.070-1.631)	0.010
CA125	0.896	2.450 (1.807-3.323)	0.000

SRC in 421 early gastric adenocarcinoma patients [11]. Zu et al. revealed the 5-year overall survival rate was significantly different between SRC and non-SRC (43.4% vs 87.1%, $P < 0.05$) [18]. The prognosis of SRC did not have significant difference compared with non-SRC in Heger's study [19]. Two independent retrospective studies including more than 3500 gastric adenocarcinoma patients respectively indicated a worse prognosis in SRC than in non-SRC [9, 16, 20]. So, in most studies, the clinical-pathological features of SRC included as followed: poor prognosis, younger patients, middle part of stomach, more lymph node metastasis and lower R0 resection rate.

Thus, unlike other researches, our study focused on the comparison between SRC and PGA patients after D2 radical gastrectomy to testify the

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prognostic significance of SRC histological type in gastric adenocarcinoma.

It is said the SRC patients were more found in younger mean age, our results showed the age distribution of SRC and PGA was in opposition. There were more younger patients (≤ 50) and less older patients (>60) in SRC compared to PGA. As for gender, the male patient of PGA was dominant, indicated SRC might relate to the level of estrogen. It is said the incidence of gastric adenocarcinoma in upper stomach increased yearly, more than half of the SRC patients were found in lower part of stomach in our study (56.7% vs PGA 45.5%), while the incidence of SRC in upper part was only half of PGA (17.5% vs 35.1%). The difference of N stage indicated the SRC might provide the preferential microenvironment for more lymph node metastasis. But the total T stage, macroscopic type and blood type had no difference between SRC and PGA, which might because D2 radical gastrectomy was performed on all candidates' patients.

Further, we explored the characteristic of tumor and pathology molecular markers in SRC. Among tumor biomarkers, more positive CA19-9 was found in SRC, which might because the intracytoplasmic mucins of SRC could be consider as a kind of undifferentiated cells or embryonic cells [21, 22]. However, less positive EGFR was observed in SRC might due to the lack of binding site on SRC cells [23, 24].

It is widely accepted the SRC patients had poor prognosis, our results indicated the 5-year survival rate of SRC are similar to that of PGA after D2 radical gastrectomy, even compared by T stages. But the two groups of patients had already showed the trend of significant difference, which might need longer follow-up period.

And the univariate analyses revealed 11 prognostic indicators (including SRC histological type); but the age, T and N stage, positive CEA, CA19-9, CA125 were confirmed to be the independent prognostic indicators by multivariate analysis, not including SRC histological type.

In conclusion, our study confirmed SRC patients had the similar prognosis as PGA patients after D2 radical gastrectomy, but the follow-up needed for a longer time. And the histological type of

SRC wasn't a positive prognostic factor in gastric adenocarcinoma patients underwent D2 radical gastrectomy.

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Disclosure of conflict of interest

None.

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