Association between fibroblast activation protein α expression and immune environment in gastric cancer

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Abstract. Tumor stroma serves an important role in cancer progression. However, the role of fibroblast activation protein α (FAP), a subpopulation marker of cancer-associated fibroblasts (CAFs), remains poorly understood, especially in gastric cancer. The present study investigated the role of FAP-positive CAFs in gastric cancer prognosis and immune environment. The retrospective study included patients with gastric cancer who underwent curative resection. The tumor-stroma ratio (TSR) and FAP expression status were evaluated using immunohistochemistry, after which their associations with recurrence-free survival (RFS) were investigated. In addition, immune cell infiltration was evaluated, including T cells, regulatory T cells (Tregs) and M2 macrophages, using immunohistochemistry, and its association with TSR or FAP status was analyzed. Among the 128 eligible patients, 29 (23%) had low TSR, whereas 39 (30%) were FAP-positive. No significant association was observed between TSR and FAP status (P=0.596). The low TSR group and FAP-positive group showed significantly worse survival than the high TSR group and FAP-negative group (log-rank P=0.004, P=0.011, respectively). Subsequently, a Cox multivariate analysis for RFS identified that FAP positivity was an independent factor for poor prognosis (P=0.009) but low TSR was not (P=0.088). FAP-positive patients had a higher tumor infiltrating $FOXP3^+$ Tregs/$CD3^+$ T-cell ratio (P<0.001) and $FOXP3^+$ Tregs/$CD8^+$ T-cell ratio (P=0.006), and higher levels of $CD163^+$ M2 macrophages (P=0.011) compared with FAP-negative patients, although such differences were not observed according to TSR status. In conclusion, the present study revealed that FAP status may be an independent prognostic factor in gastric cancer, potentially contributing to the formation of immune tolerance within the tumor microenvironment.

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Introduction

Gastric cancer is one of the most common causes of cancer death worldwide (1), with gastroenterological malignancies having the highest mortality rates in Japan (2). Despite the tremendous advances in diagnosis and therapy, the prognosis of gastric cancer patients still remains unsatisfactory (3-7). The large amount of stroma has been considered one of the factors contributing to the poor prognosis of gastric cancer. In recent years, the role of the tumor stroma in cancer progression has gained significant attention, with a considerable amount of data having been published (8-10). The tumor stroma consists of several components, among which cancer-associated fibroblasts (CAFs) constitute the majority. CAFs are activated through interactions with tumor cells. Once activated, they produce various molecules that regulate tumor growth (8). Prognostic studies using the tumor-stroma ratio (TSR), which evaluated the amount of CAFs in tumors, suggested that the TSR could be a vital prognostic factor in gastric cancer (11,12).

Studies have found that CAFs have four subpopulations (CAF-S1, S2, S3, S4). CAF-S1, a subpopulation identified based on fibroblast activation protein α (FAP) expression, has been reported to be involved in tumor growth and the tumor immune environment (13). FAP is a type II integral membrane protein that belongs to the serine protease family and is expressed on activated fibroblasts in cancer tissues and during wound healing processes in normal tissues. Studies using mouse models of pancreatic cancer have shown that FAP-positive CAFs were associated with poor therapeutic outcomes following treatment with immune checkpoint inhibitors (14). Other studies similarly support the immunosuppressive roles for FAP-positive CAFs in ovarian and breast cancers (13,15). In gastric cancer, FAP has been associated with tumor growth and distant metastasis (16). However, no studies have yet investigated the relationship between the immune environment and FAP expression status. Examining FAP, which is considered an important component among CAFs, would certainly help elucidate the mechanisms underlying gastric cancer tumor progression and the development of novel therapy. Therefore, the current study aimed to elucidate the role of FAP-positive

CAFs in the prognosis of patients with gastric cancer and their involvement in the immune environment.

Materials and methods

Patients and samples. This study included consecutive patients who satisfied the following inclusion criteria: i) Histologically confirmed primary gastric cancer; ii) diagnosed with macroscopic type 0, 1, 2, 3, or 5 gastric cancer; and iii) underwent curative surgical resection at the University of Osaka Hospital (Osaka, Japan) between January 2015 and December 2019. Patients who satisfied the following exclusion criteria were excluded: i) Diagnosed with clinical stage IV disease; ii) received neoadjuvant therapy; or iii) insufficient data. Patient with macroscopic type 4 gastric cancer were excluded because of their extremely poor prognosis and distinct epidemiological and pathological characteristics. Clinicopathological characteristics and staging were based on the Japanese Classification of Gastric Carcinoma, 14th edition (17). This study was approved by the Ethics Review Board of the University of Osaka Hospital (approval no. 24452). Written informed consent for the usage of their tumor specimens was obtained from all patients.

Immunohistochemical staining. Tumor tissue samples were obtained from surgically resected specimens. Specimens were fixed with 10% neutral buffered formalin for 24-72 h and embedded in paraffin. The resected specimens from the formalin-fixed paraffin-embedded blocks were cut into 4.0-μm-thick sections, deparaffinized with xylene, and then rehydrated with multistep descending concentrations of ethanol. The sections were autoclaved in citrate buffer at 115˚C for 20 min, immersed in 0.3% hydrogen peroxide to block endogenous peroxidase, and incubated in horse serum for 20 min to avoid nonspecific staining. The slides were incubated with specific primary antibodies (monoclonal primary antibody for FAP [ab207178; dilution, 1:250; Abcam, USA], FOXP3 [ab20034; dilution, 1:1,000; Abcam, USA], CD3 [M725401; dilution, 1:250; DAKO, USA], CD8 [M710301; dilution, 1:250; DAKO, USA], and CD163 [ab182422; dilution, 1:1,000; Abcam, USA] overnight at 4˚C), with ABC peroxidase (Vector Laboratories) for 20 min, with diaminobenzidine tetrahydrochloride for 2.5 min to visualize the reactions with FAP, with FOXP3 and CD163 for 1.5 min, and with CD3 and CD8 for 1.0 min. Human breast cancer was used as a positive control for FAP, and human tonsil tissue was used for CD3, CD8, CD163, and FOXP3 (Fig. S1). FOXP3 was used as a marker for regulatory T cells (Tregs), and CD163 was used as a marker for M2 macrophages.

Whole-slide images of the IHC slides were generated, and five FOXP3 hotspots fields at x100 magnification were carefully selected by visual inspection. Subsequently, IHC staining for CD3, CD8, and CD163 was performed on serial sections, and the same five corresponding fields as those selected for FOXP3 were selected. All positive cells in the fields were counted. Thus, all analyses were performed in the same five fields (Fig. S2). All scanning and counting were performed using software HALO™ (Indica Labs, Albuquerque, NM), an image analysis software designed for digital pathology applications. The detection thresholds in HALO were visually checked and individually adjusted to minimize nonspecific detection (FOXP3: 0.05; CD3: 0.025; CD8: 0.07; CD163: 0.025). Immunohistochemistry analysis was conducted while blinded to the clinical data.

Evaluation of the TSR and FAP status. The TSR was assessed in hematoxylin and eosin-stained slides at the invasive front of the tumor. Initially, areas with the highest amounts of tumor stroma were selected using low magnification (x5 objective). Thereafter, higher magnification (x10 objective) was used to evaluate the amount of tumor stroma in a selected field that had cancer cells present on the four sides of the microscopic field, as previously reported (11). The percentage of stroma in the selected field relative to the amount of cancer cells was estimated at 10% intervals. The TSR was calculated as follows: TSR=(tumor cell areas)/(microscopic field area) x100 (%). A TSR of <50 and ≥50% was regarded as low and high TSR, respectively, based on a previous study (11). Representative images for low and high TSRs are presented in Fig. 1A and B. Areas of necrosis, pre-existing lymphoid tissue, or any other normal structures were excluded from the microscopic field. If this was not possible, these irrelevant tissues (i.e., necrotic tumor or lymphoid stroma) were excluded when estimating the tumor stroma.

FAP expression status was evaluated in terms of positive area percentage and staining intensity, as previously reported (18). A single representative tumor area was selected for analysis to ensure consistency with the method used for TSR assessment. The positive area percentage was defined as follows: 0 points, <1%; 1 point, 1-24%; 2 points, 25-49%; 3 points, 50-74%; and 4 points, 75-100%. Staining intensity was defined as follows: 1 point, negative-weak; 2 points, moderate; 3 points, strong. The final score, which was estimated by multiplying both scores, was used to classify the patients into two groups: FAP-positive (>4) or FAP-negative (≤4). Representative immunohistochemical staining images of FAP-positive and FAP-negative cells are presented in Fig. 1C and D.

Statistical analysis. The relationships between clinicopathological characteristics and the TSR and FAP status of the tissue samples were analyzed using the χ^2 test for categorical variables. Recurrence-free survival (RFS) was defined as the interval from the date of surgery to the date of recurrence or death from any cause. Deaths from causes other than recurrence were treated as events. Survival rates were estimated using the Kaplan-Meier method and compared using the log-rank test. The association between recurrence patterns and FAP expression was analyzed using Fisher's exact test. To identify independent prognostic factors, a Cox multivariate analysis using a backward stepwise procedure was employed. The candidate variables included in the initial model were seven well-known clinicopathological prognostic factors (age, sex, histological type, tumor depth, lymph node metastasis, lymphatic invasion, and venous invasion), together with TSR and FAP expression. Variables were sequentially removed until all remaining variables satisfied the selection criterion of P<0.10. The association between TSR, FAP expression and tumor-infiltrating immune cells was analyzed using the Mann-Whitney U test. Multivariable analysis using

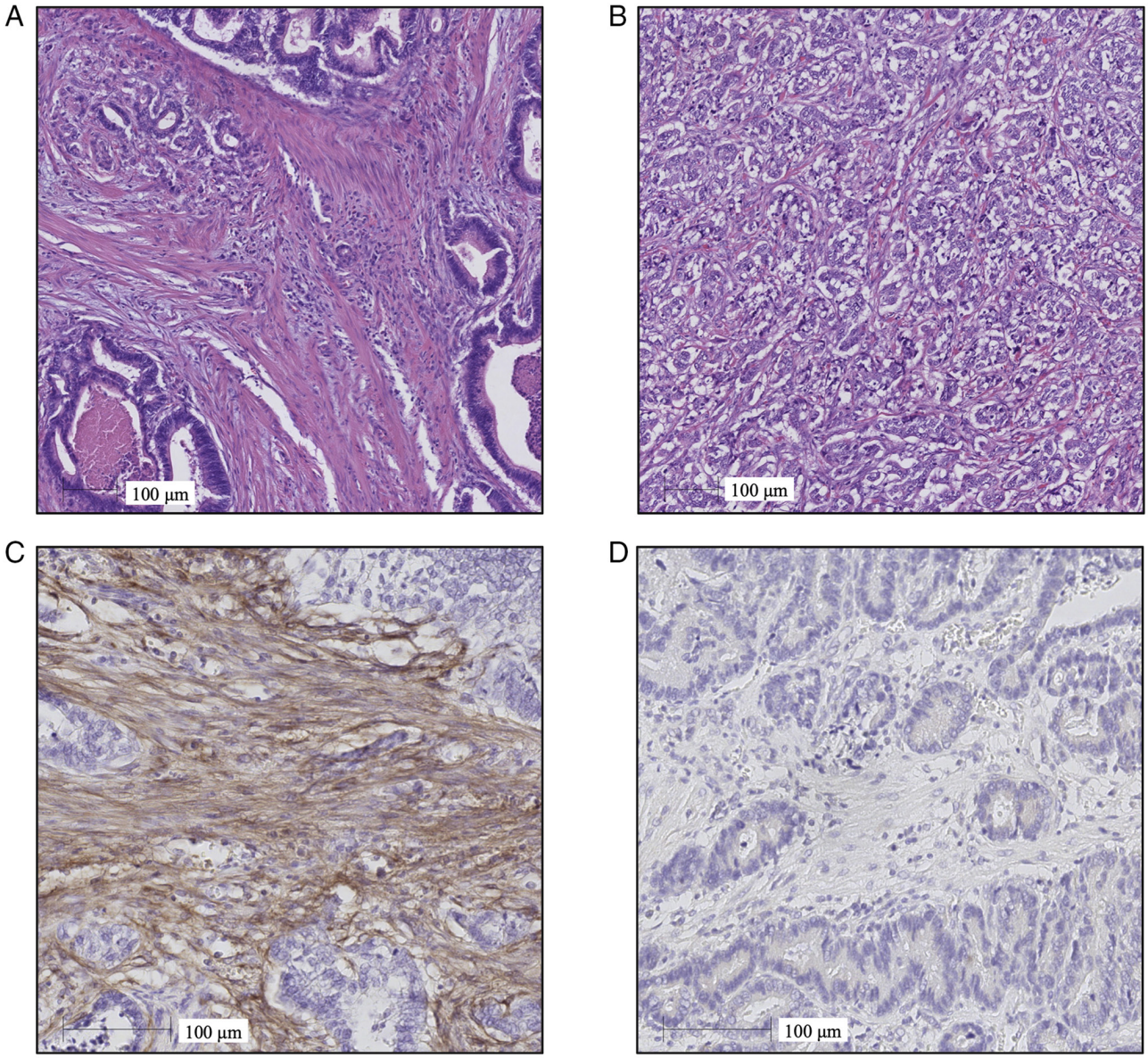

Figure 1. Representative immunohistochemical staining images for the TSR and FAP in the (A) low TSR, (B) high TSR, (C) FAP-positive, and (D) FAP-negative groups. FAP, fibroblast activation protein α; TSR, tumor-stroma ratio.

a generalized linear model with a γ distribution and log link was performed to estimate group differences in the levels of tumor-infiltrating immune cells, adjusting for covariates. For $CD163^+$ M2 macrophages, a natural log transformation was applied prior to modeling. All statistical analyses were performed using the JMP PRO software (JMP version 17.2.0; SAS Institute) and the SPSS software (version 29.0.2.0, IBM Corp.), with $P<0.05$ indicating a statistically significant difference.

Results

Patient characteristics and expression status. Among the 128 eligible patients, 29 (23%) had low TSR, whereas 39 (30%) were FAP-positive. The association between the TSR and FAP status and clinicopathological characteristics are detailed in Table I. Compared to the high TSR group, the low TSR group had a significantly increased frequency of lymph node metastasis (P=0.022) and venous invasion (P=0.010). Similarly, the FAP-positive group had a significantly higher frequency of lymph node metastasis (P=0.028) than did the FAP-negative group. Significant differences in pathological stage were observed according to both the TSR and FAP status. Regarding the pathological Lauren classification, the proportion of the intestinal type of histology was significantly higher in the FAP-positive group than in the FAP-negative group (P<0.001). No significant correlation was observed between the TSR and FAP status (P=0.596).

Survival according to the TSR and FAP status. Fig. 2 shows the Kaplan-Meier curves for RFS in 128 patients according to TSR or FAP status at the median follow up period of 60.0 months. There were 22 recurrences and 14 deaths from other causes without recurrence. The low TSR group and FAP-positive group showed significantly worse survival than did the high TSR group and FAP-negative group (log-rank

Table I. Association between TSR or FAP status and clinicopathological characteristics.

Characteristic	Low TSR (n=29)	High TSR (n=99)	P-value	FAP-positive (n=39)	FAP-negative (n=89)	P-value
Age, years			0.556			0.001
<70	12 (41%)	35 (35%)		6 (15%)	41 (46%)	
≥70	17 (59%)	64 (65%)		33 (85%)	48 (54%)	
Sex			0.694			0.677
Male	20 (69%)	72 (73%)		29 (74%)	63 (71%)	
Female	9 (31%)	27 (27%)		10 (26%)	26 (29%)	
Histological type			0.700			<0.001
Intestinal	21 (72%)	68 (69%)		36 (92%)	53 (60%)	
Diffuse	8 (28%)	31 (31%)		3 (8%)	36 (40%)	
pT status			0.634			0.233
2	11 (38%)	47 (48%)		15 (38%)	43 (48%)	
3	9 (31%)	28 (28%)		10 (26%)	27 (30%)	
4	9 (31%)	24 (24%)		14 (36%)	19 (21%)	
pN status			0.022			0.028
0	10 (33%)	58 (59%)		15 (38%)	53 (60%)	
1-3	19 (66%)	41 (41%)		24 (62%)	36 (40%)	
pStage			0.026			0.003
I	5 (17%)	31 (31%)		7 (18%)	29 (33%)	
II	10 (35%)	46 (47%)		13 (33%)	43 (48%)	
III	14 (48%)	22 (22%)		19 (49%)	17 (19%)	
Lymphatic invasion			0.108			0.149
Absent	3 (10%)	23 (23%)		5 (13%)	21 (24%)	
Present	26 (90%)	76 (77%)		34 (87%)	68 (76%)	
Venous invasion			0.010			0.887
Absent	7 (24%)	50 (51%)		17 (44%)	40 (45%)	
Present	22 (76%)	49 (49%)		22 (56%)	49 (55%)	
TSR			-			0.596
Low	-	-		10 (26%)	19 (21%)	
High	-	-		29 (74%)	70 (79%)	
FAP			0.596			
Positive	10 (34%)	29 (29%)		-	-	-
Negative	19 (66%)	70 (71%)		-	-	

pStage is according to the 14th Japanese Classification of Gastric Carcinoma (17). FAP, fibroblast activation protein α; pStage, pathological stage; TSR, tumor-stroma ratio.

P=0.004, P=0.011, respectively). Regarding the pattern of recurrence, FAP-positive patients tended to have a higher rate of liver metastasis than did FAP-negative patients (P=0.084) (Table II). Univariate analysis for RFS showed that pN1-3, lymphatic invasion, venous invasion, low TSR, and FAP positivity were significant poor prognostic factors (Table III). A Cox multivariate analysis using a backward stepwise procedure revealed that FAP positivity (P=0.009), venous invasion (P=0.036), and pN1-3 (P=0.023), but not low TSR (P=0.088), were independent indicators of poor RFS.

Relationship between the TSR and FAP status with tumor-infiltrating immune cells. We analyzed the association between the TSR and FAP status and tumor-infiltrating $FOXP3^+$ Tregs and $CD163^+$ M2 macrophages (Fig. 3). Notably, we found no significant differences in the tumor-infiltrating $FOXP3^+$ Treg/$CD3^+$ T-cell ratio (P=0.105), tumor-infiltrating $FOXP3^+$ Treg/$CD8^+$ T-cell ratio (P=0.093), and number of tumor-infiltrating $CD163^+$ M2 macrophages (P=0.690) between high and low TSR patients. In contrast, FAP-positive patients had a higher tumor-infiltrating $FOXP3^+$ Treg/$CD3^+$ T-cell ratio (P<0.001), tumor infiltrating $FOXP3^+$ Treg/$CD8^+$ T-cell ratio (P=0.006), and number of tumor-infiltrating $CD163^+$ M2 macrophages (P=0.011) than did FAP-negative patients. Even after adjustment for histological type (intestinal vs. diffuse) and pStage (I/II vs. III) using a generalized linear model with

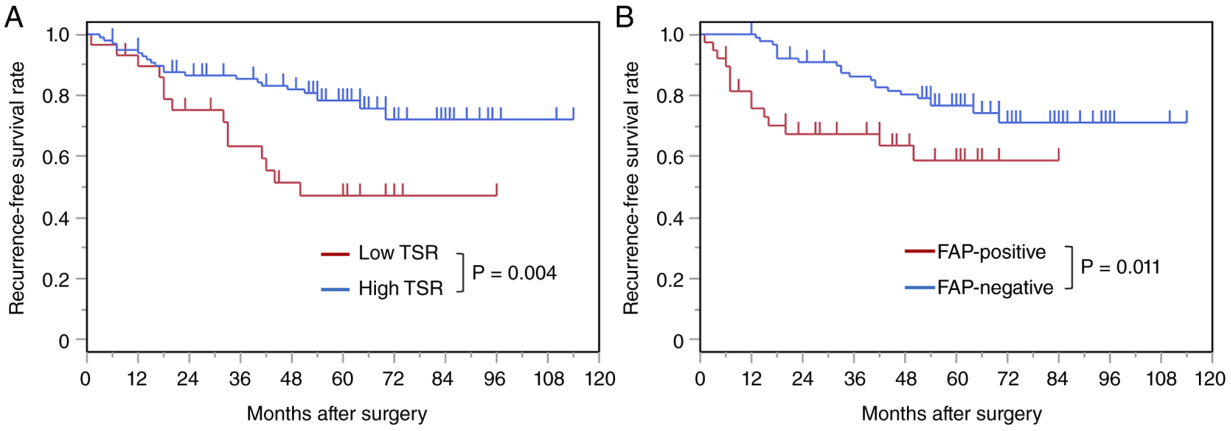

Figure 2. Kaplan-Meier recurrence-free survival curves for the 128 patients according to (A) TSR and (B) FAP status. FAP, fibroblast activation protein α; TSR, tumor-stroma ratio.

Table II. Associations between recurrence patterns and FAP status.

Recurrence pattern	FAP-positive (n=39)	FAP-negative (n=89)	P-value
Any recurrence	9 (23%)	13 (15%)	0.309
Peritoneum	3 (8%)	7 (8%)	1.000
Lymph nodes	3 (8%)	4 (4%)	0.435
Liver	3 (8%)	1 (1%)	0.084
Others	0 (0%)	1 (1%)	1.000

FAP, fibroblast activation protein α.

a γ distribution and log link, all of these levels of tumor-infiltrating immune cells were significantly higher in FAP-positive patients than in FAP-negative patients (Table SI).

Discussion

Both the TSR and FAP status have been considered indicators of the extent of the tumor stroma. However, our study demonstrated that only FAP status, but not TSR, was an independent prognostic factor in resectable gastric cancer. Furthermore, FAP status was correlated with the enrichment of tumor-infiltrating FOXP3$^+$ Tregs and tumor-infiltrating M2 macrophages, whereas the TSR showed no such association with these tumor-infiltrating immune cells. These findings suggest that FAP-positive gastric cancers have a higher proportion of infiltrating Tregs and M2 macrophages, which may promote immune tolerance and worsen prognosis. Although several studies have investigated the association between FAP status and prognosis, this study has been the first to investigate the involvement of immune cell infiltration in the correlation between FAP status and prognosis in gastric cancer.

Notably, our findings showed no significant correlation between the TSR and FAP status. Generally, the TSR evaluates the amount of CAFs in a tumor using only hematoxylin and eosin-stained slides (11). Additionally, FAP is one of many markers of CAFs, such as α-smooth muscle actin, platelet-derived growth factor receptor, and fibroblast-specific protein-1 that has often been used for evaluating the entire CAFs in tumors (19,20). Theoretically, a correlation between the TSR and FAP status should be expected. However, no such correlation had been observed in the current study, which may be attributed that TSR measures only the overall amount of CAFs, including FAP-negative populations, whereas FAP represents a marker of a single CAF subtype. FAP-positive CAF-S1 has been reported to be associated with angiogenesis and immune tolerance (13-15).

CAFs have been found to promote angiogenesis and induce epithelial-mesenchymal transition of tumor cells (21), which enhances the invasive and metastatic capabilities of the tumor (22). Given that low TSR tumors have high amounts of CAFs, these tumors are more likely to be influenced by the tumor microenvironment enhanced by CAFs. Moreover, studies have shown that FAP status also correlates with distant metastasis in gastric cancer (16). In the current study, FAP-positive cases tended to have a higher incidence of liver metastasis than did FAP-negative cases, although the difference was not significant. This may be attributed to the involvement of FAP in angiogenesis, as previously reported (16). Experiments on breast cancer cells have shown that FAP-positive CAFs promote tumor growth and enhance epithelial-mesenchymal transition (23). In the current study, gastric cancers with FAP-positive expression showed a higher proportion of FOXP3$^+$ Tregs and number of CD163$^+$ M2 macrophages. These findings suggest that FAP-expressing CAFs may induce immune tolerance, which could potentially worsen the prognosis of patients with gastric cancer. Previous reports have demonstrated a correlation between FAP expression and FOXP3$^+$ Tregs in ovarian and breast cancers (13,15). Meanwhile, *in vitro* experiments have shown that FAP-expressing CAFs secrete CXCL12, which induces FOXP3$^+$ Treg differentiation (13). Our study suggests the hypothesis that a similar mechanism occurs in gastric cancer. M2 macrophages, which play a role in immune regulation, anti-inflammatory responses, and tumor progression, have been associated with CAFs (24). Moreover, experiments using oral squamous cell carcinoma revealed that FAP-expressing CAFs secreted CXCL12 and recruited tumor-associated

Table III. Univariate and multivariate Cox analyses for recurrence-free survival.

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	P-value	HR (95% CI)	P-value
Age, ≥70 years	1.519 (0.747-3.087)	0.249		
Female	0.706 (0.322-1.551)	0.386		
Diffuse type	0.660 (0.310-1.406)	0.282		
pT3-4	1.847 (0.923-3.693)	0.083		
pN1-3	3.540 (1.736-7.221)	0.001	2.380 (1.126-5.032)	0.023
Lymphatic invasion (+)	3.417 (1.047-11.159)	0.042		
Venous invasion (+)	3.368 (1.532-7.407)	0.003	2.466 (1.060-5.739)	0.036
Low TSR	2.605 (1.329-5.107)	0.005	1.822 (0.916-3.627)	0.088
FAP-positive	2.330 (1.184-4.586)	0.014	2.522 (1.254-5.072)	0.009

CI, confidence interval; HR, hazard ratio; FAP, fibroblast activation protein α; TSR, tumor-stroma ratio.

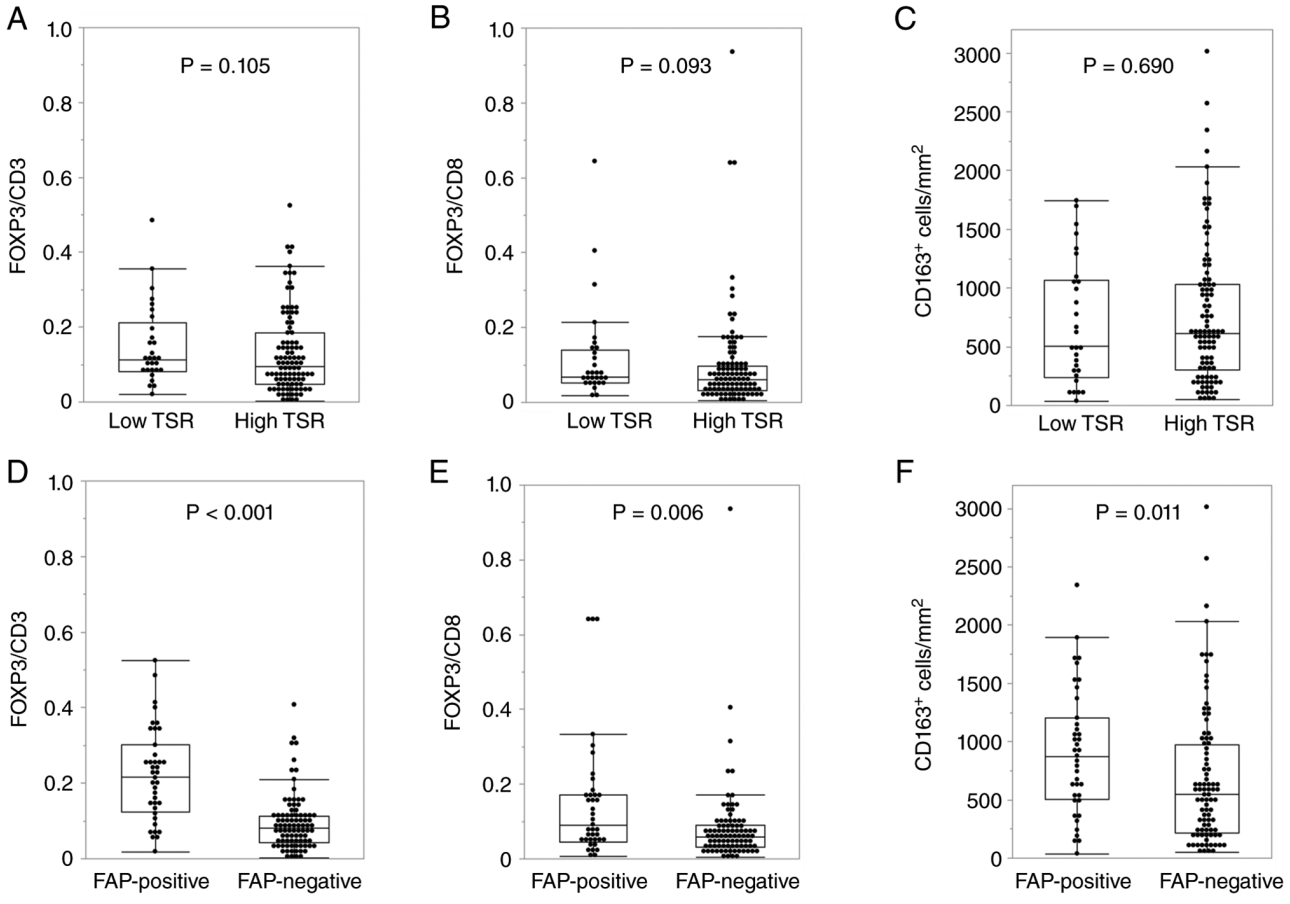

Figure 3. Association between TSR status and tumor infiltration: (A) FOXP3$^+$ Treg/CD3$^+$ T-cell ratio; (B) FOXP3$^+$ Treg/CD8$^+$ T-cell ratio; and (C) number of CD163$^+$ M2 macrophages. Association between FAP status and tumor infiltration: (D) FOXP3$^+$ Treg/CD3$^+$ T-cell ratio; (E) FOXP3$^+$ Treg/CD8$^+$ T-cell ratio; and (F) number of CD163$^+$ M2 macrophages. Box plots indicate the first, second (median), and third quartiles. Bars show the lowest data points within 1.5 times the interquartile range from the lower quartile boundary and the highest data points within 1.5 times the interquartile range from the upper quartile boundary. FAP, fibroblast activation protein α; Treg, regulatory T cell; TSR, tumor-stroma ratio.

macrophages into the tumor, thereby increasing the number of tumor-associated macrophages (25), which supports our findings.

In this study, a diffuse-type histology was less common in the FAP-positive group than in the FAP-negative group. However, no consistent findings regarding the relationship between FAP status and histological differentiation have been published. One study reported that the proportion of FAP-positive cases was 63.4% in well- or moderately differentiated adenocarcinoma and 59.0% in poorly differentiated adenocarcinoma (16). Another

study reported that the proportion of FAP-positive patients was 44.9% in intestinal-type gastric cancer and 34.1% in diffuse or mixed-type gastric cancer (18). Considering the small sample size of diffuse-type cases in the current study, subgroup analysis based on pathological type could not be performed in our determination of the association between FAP status and prognosis. Hence, further studies specifically focusing on diffuse-type cases are necessary.

This study has several limitations worth noting. First, the study was retrospective and based on a relatively small sample obtained via a cohort from a single center. By analyzing consecutive cases, we have minimized biases as much as possible. Second, this study excluded patients who underwent neoadjuvant therapy or were diagnosed with macroscopic type 4 gastric cancer. Given that neoadjuvant therapy cases exhibit necrosis and fibrosis in tumor samples, evaluating them through immunohistochemical staining was difficult. Additionally, studies have shown that macroscopic type 4 gastric cancer generally carries a poor prognosis and a different oncological background (26,27). In the present study, the relatively small number of cases with low TSR may be attributable to the exclusion of macroscopic type 4 gastric cancer. Investigation of TSR and FAP specifically in macroscopic type 4 gastric cancer is clinically important, and additional studies focusing exclusively on this subtype are warranted. Third, stepwise variable selection was used, which may introduce overfitting and instability in the selected variables. Therefore, the results should be interpreted with some caution. However, the observed associations were clinically plausible and internally consistent. Fourth, FAP expression status was analyzed in one spot of the tumor, which might introduce field selection bias. However, there is currently no standardized or universally accepted method for evaluating FAP expression using immunohistochemical staining, and this remains a subject of ongoing debate. Previous studies have also assessed FAP expression using a single-spot approach (28,29). In the present study, to maintain consistency with the evaluation method used for TSR, FAP expression was therefore assessed using a single representative spot. Considering the potential heterogeneity of FAP expression within tumors, evaluating FAP expression based on a single spot may not fully capture the spatial variability of its expression. We attempted to evaluate the entire immunohistochemical slides using HALO, but an accurate and reliable assessment across the whole slide could not be achieved. Furthermore, external validation was not performed, including validation of inter-assay variability in immunohistochemistry. Therefore, further investigation may be necessary to analyze FAP expression using immunohistochemical staining.

In conclusion, our findings demonstrated the impact of FAP expression status on prognosis, highlighting its relationship with immune cells as one of the contributing factors. Future studies are therefore warranted to further elucidate the role of FAP expression in gastric cancer, which may facilitate the development of novel therapeutic strategies and improve clinical outcomes for patients with gastric cancer.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

KK, YK and TH designed the overall experiments. KK and SN had unrestricted access to all data and performed the data analysis. KK, YK and TH performed statistical analyses and drafted the manuscript. KK, YK, TT, TS, KaY, KM, KoY, KT, TM and KN collected clinical specimens and associated patient data. HE and YD contributed to data acquisition and interpretation, and critically revised the manuscript for important intellectual content. KK and SN confirm the authenticity of all the raw data. All authors read and approved the final draft of the manuscript and take full responsibility for its content.

Ethics approval and consent to participate

The present study was approved by the Ethics Review Board of the University of Osaka Hospital (approval no. 24452). Written informed consent for the usage of their tumor specimens was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Globalcancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Higashi T and Kurokawa Y. Incidence, mortality, survival, and treatment statistics of cancers in digestive organs-Japanese cancer statistics 2024. Ann Gastroenterol Surg 8: 958-965, 2024.
3. Kurokawa Y, Doki Y, Mizusawa J, Terashima M, Katai H, Yoshikawa T, Kimura Y, Takiguchi S, Nishida Y, Fukushima N, *et al*: Bursectomy versus omentectomy alone for resectable gastric cancer (JCOG1001): A phase 3, open-label, randomised controlled trial. Lancet Gastroenterol Hepatol 3: 460-468, 2018.
4. Kurokawa Y, Doki Y, Mizusawa J, Yoshikawa T, Yamada T, Kimura Y, Takiguchi S, Nishida Y, Fukushima N, Cho H, *et al*: Five-year follow-up of a randomized clinical trial comparing bursectomy and omentectomy alone for resectable gastric cancer (JCOG1001). Br J Surg 110: 50-56, 2022.
5. Yanagimoto Y, Kurokawa Y, and Doki Y. Essential updates 2021/2022: Perioperative and surgical treatments for gastric and esophagogastric junction cancer. Ann Gastroenterol Surg 7: 698-708, 2023.
6. Kurokawa Y, Kawase T, Takeno A, Furukawa H, Yoshioka R, Saito T, Takahashi T, Shimokawa T, Eguchi H and Doki Y: Phase 2 trial of neoadjuvant docetaxel, oxaliplatin, and S-1 for clinical stage III gastric or esophagogastric junction adenocarcinoma. Ann Gastroenterol Surg 7: 247-254, 2022.

7. Kurokawa Y, Matsuyama J, Nishikawa K, Takeno A, Kimura Y, Fujitani K, Kawabata R, Makari Y, Terazawa T, Kawakami H, *et al*: Docetaxel plus S-1 versus cisplatin plus S-1 in unresectable gastric cancer without measurable lesions: A randomized phase II trial (HERBIS-3). Gastric Cancer 24: 428-434, 2021.
8. Xu M, Zhang T, Xia R, Wei Y and Wei X: Targeting the tumor stroma for cancer therapy. Mol Cancer 21: 208, 2022.
9. Dong W, Jia L and YiMing T: Tumor-stroma ratio predicts prognosis and PD-L1 expression in hepatocellular carcinoma. BMC Cancer 23: 434, 2023.
10. Luz S, Richard RP, Michel K, Anne C and Hwajeong L: Tumor stroma ratio and its significance in locally advanced colorectal cancer. Curr Oncol 29: 3232-3241, 2022.
11. Kim EY, Abdul-Ghafar J, Chong Y and Yim K: Calculated tumor-associated neutrophils are associated with the tumor-stroma ratio and predict a poor prognosis in advanced gastric cancer. Biomedicines 10: 708, 2022.
12. Peng C, Liu J, Yang G and Li Y: The tumor-stromal ratio as a strong prognosticator for advanced gastric cancer patients: Proposal of a new TSNM staging system. J Gastroenterol 53: 606-617, 2018.
13. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, Cardon M, Sirven P, Magagna I, Fuhrmann L, Bernard C, *et al*: Fibroblast heterogeneity and immunosuppressive environment in human breast cancer. Cancer Cell 33: 463-479.e10, 2018.
14. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, *et al*: Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci USA 110: 20212-20217, 2013.
15. Givel AM, Kieffer Y, Scholer-Dahirel A, Sirven P, Cardon M, Pelon F, Magagna I, Gentric G, Costa A, Bonneau C, *et al*: miR200-regulated CXCL12β promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. Nat Commun 9: 1056, 2018.
16. Gao LM, Wang F, Zheng Y, Fu ZZ, Zheng L and Chen LL: Roles of fibroblast activation protein and hepatocyte growth factor expressions in angiogenesis and metastasis of gastric cancer. Pathol Oncol Res 25: 369-376, 2019.
17. Japanese Gastric Cancer Association: Japanese classification of gastric carcinoma:3rd English edition. Gastric Cancer 14: 101-112, 2011.
18. Tong Y, Zhao Z, Zhang J, Wang W and Zhu Y: High expressions of CD10, FAP and GPR77 in CAFs are associated with chemoresistance and worse prognosis in gastric cancer. Front Oncol 12: 984817, 2022.
19. Orimo A and Weinberg RA: Heterogeneity of stromal fibroblasts in tumors. Cancer Biol Ther 6: 618-619, 2007.
20. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA and Fearon DT: Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science 330: 827-830, 2010.
21. Kitadai Y: Cancer-stromal cell interaction and tumor angiogenesis in gastric cancer. Cancer Microenviron 3: 109-116, 2010.
22. Marsh T, Pietras K and McAllister SS: Fibroblasts as architects of cancer pathogenesis. Biochim Biophys Acta 1832: 1070-1078, 2013.
23. Pelon F, Bourachot B, Kieffer Y, Magagna I, Mermet-Meillon F, Bonnet I, Costa A, Givel AM, Attieh Y, Barbazan J, *et al*: Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms. Nat Commun 11: 404, 2020.
24. Cassetta L and Pollard JW: Targeting macrophages: Therapeutic approaches in cancer. Nat Rev Drug Discov 17: 887-904, 2018.
25. Li X, Bu W, Meng L, Liu X, Wang S, Jiang L, Ren M, Fan Y and Sun H: CXCL12/CXCR4 pathway orchestrates CSC-like properties by CAF recruited T tumor associated macrophage in OSCC. Exp Cell Res 378: 131-138, 2019.
26. Hashimoto T, Nakayama I, Ohashi M, Mizusawa J, Kawachi H, Kita R, Fukuda H, Kurokawa Y, Boku N, Yoshikawa T and Terashima M: Randomized phase II study comparing neoadjuvant 5-fluorouracil/oxaliplatin/docetaxel versus docetaxel/oxaliplatin/S-1 for patients with type 4 or large type 3 gastric cancer. Future Oncol 19: 2147-2155, 2023.
27. Iwasaki Y, Terashima M, Mizusawa J, Katayama H, Nakamura K, Katai H, Yoshikawa T, Ito S, Kaji M, Kimura Y, *et al*: Gastrectomy with or without neoadjuvant S-1 plus cisplatin for type 4 or large type 3 gastric cancer (JCOG0501): An open-label, phase 3, randomized controlled trial. Gastric Cancer 24: 492-502, 2021.
28. Zou B, Liu X, Zhang B, Gong Y, Cai C, Li P, Chen J, Xing S, Chen J, Peng S, *et al*: The expression of FAP in hepatocellular carcinoma cells is induced by hypoxia and correlates with poor clinical outcomes. J Cancer 9: 3278-3286, 2018.
29. Zhang Q, Wang C and Cliby WA: Cancer-associated stroma significantly contributes to the mesenchymal subtype signature of serous ovarian cancer. Gynecol Oncol 152: 368-374, 2019.