Poorly cohesive gastric cancer with increased epithelial-mesenchymal transition is associated with a poor prognosis

NOBUHIRO NAKAZAWA[1], MAKOTO SOHDA[1], MUNENORI IDE[2], YUKI SHIMODA[2], AKIHIKO SANO[1], MAKOTO SAKAI[1], TETSUNARI OYAMA[2], KEN SHIRABE[1] and HIROSHI SAEKI[1]

[1]Department of General Surgical Science, Graduate School of Medicine, Gunma University, Maebashi, Gunma 371-8511, Japan; [2]Department of Diagnostic Pathology, Graduate School of Medicine, Gunma University, Maebashi, Gunma 371-8511, Japan

Received December 19, 2023; Accepted May 10, 2024

DOI: 10.3892/ol.2024.14554

Abstract. The present study examined the surgical outcome and prognosis of patients with poorly cohesive carcinoma (PCC), and characterized the molecular pathological factors, epithelial-mesenchymal transition (EMT) and interstitial signals of the disease. A total of 281 patients who underwent gastric cancer (GC) surgery between April 2015 and August 2020 were included. Furthermore, tissue samples from another 197 patients with GC who underwent surgery between 1999 and 2003 were assessed using a tissue microarray. Preoperatively treated cases and endoscopic submucosal dissection cases were excluded, and multiple blocks containing the invasion region were collected for tissue microarray. For tissue microarray analysis, the clinicopathological factors of protein wnt3a (wnt3a), leucine-rich repeat-containing G-protein coupled receptor 5, transforming growth factor-β-induced, phosphorylated serine/threonine-protein kinase mTOR and E-cadherin expression were collected as EMT markers. The results of the surgical case evaluation and tissue microarray indicated that PCC was more common in younger patients and women, as the ratio of women to men was higher in the PCC group compared with that in the non-PCC group. However, none of the results revealed that the prognosis was worse in all patients with PCC compared with the non-PCC group. Furthermore, in the tissue microarray study, PCC samples exhibited significantly decreased expression of the cell adhesion molecule E-cadherin, suggesting enhanced EMT, which activates wnt3a signaling. PCC with increased EMT was significantly associated with a poor prognosis.

Correspondence to: Dr Makoto Sohda, Department of General Surgical Science, Graduate School of Medicine, Gunma University, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan
E-mail: msohda@gunma-u.ac.jp

Key words: gastric cancer, poorly cohesive carcinoma, epithelial-mesenchymal transition

Introduction

Although the number of gastric cancer (GC) cases is gradually decreasing, GC is the third leading cause of cancer death worldwide (1,2). Overall, >40% of patients who undergo radical resection for GC experience recurrence within 2 years of surgery (3). The treatment of GC has gradually advanced with the development of minimally invasive surgical treatments, such as robotic technology (4), and anticancer drugs, and the introduction of immune checkpoint inhibitors; however, treatment outcomes are not yet optimal. The incidence of intestinal-type GC in the West has decreased; however, the proportion of diffuse-type GC according to the Lauren classification has increased (5-7). Furthermore, the incidence of poorly cohesive carcinoma (PCC), including cases comprising signet ring cell (SRC) histology and non-solid cases [not otherwise specified (NOS)] according to the World Health Organization (WHO) classification, has increased (6,8). Therefore, the interest in PCC has increased worldwide. The fifth edition of the WHO classifies PCC into SRC and NOS (8). In clinical practice, PCC is considered to comprise a combination of SRC and NOS tumors (9). According to the Japanese classification of GC, PCC corresponds to poorly differentiated non-solid-type adenocarcinoma (por2) and SRC carcinoma cases (10). In the intestinal type, frequent genetic abnormalities include loss of heterozygosity and mutations in tumor protein p53 (11), as well as APC regulator of WNT signaling pathway (12). By contrast, the importance of the cadherin 1 (CDH1) gene has been recognized in the diffuse type due to the high frequency of cases with decreased E-cadherin expression and hypermethylation of its promoter (13). However, treatment outcomes and clinicopathological characteristics of PCC in Japan have not been adequately studied.

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells acquire mesenchymal properties, and it is involved in the metastasis, invasion and proliferation of cancer cells (14). *Helicobacter pylori* infection is considered to have a significant effect on the gastric microenvironment by inducing several inflammatory responses via the infiltration of macrophages, neutrophils, regulatory T cells and natural killer cells, which are associated with promoting EMT (15).

Transforming growth factor β (TGF-β) in gastric cancer is a representative signal of the promotion of EMT (16). PCC and its microenvironmental changes, including EMT, have not been adequately assessed. Therefore, it is important to examine the characteristics of PCC by focusing on intra-tumor-infiltrating CD8-positive T cells, protein wnt3a (wnt3a) and phosphorylated serine/threonine-protein kinase mTOR (p-mTOR) signaling, cancer stemness markers [leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5)] contributing to drug resistance, EMT markers (E-cadherin) and TGF-β signaling.

Therefore, the present novel study examined the surgical outcome of PCC and its prognosis, and further characterized the molecular pathological factors of PCC, EMT and interstitial signals.

Patients and methods

Patients. The present study included 281 patients who underwent surgery, including total, proximal and distal resection, for GC between April 2015 and August 2020 at Gunma University Hospital (Maebashi, Japan). Furthermore, samples from 197 patients with GC who underwent surgery, including total, proximal and distal resection, between 1999 and 2003 were evaluated using a tissue microarray (TMA). Preoperatively treated cases and endoscopic submucosal dissection cases were excluded, and multiple blocks containing the invasion region were collected for TMA. PCC was defined as cases in which the main component of the cancer was poorly differentiated non-solid-type adenocarcinoma (por2) and SRC carcinoma. For surgical case analysis, clinicopathological factors, including age, sex, body mass index (BMI), tumor size, tumor depth, presence of lymph node metastasis, number of lymph node metastases, peritoneal washing cytology, presence of distant metastasis, stage, surgical method (such as open and laparoscopic surgery), lymph node dissection, resection method, operative time and blood loss, were collected. Additionally, for TMA analysis, clinicopathological factors, including age, sex, tumor size, depth of tumor, presence of lymph node metastasis, number of lymph node metastases and stage, were collected. In terms of TMA analysis, existing immunohistochemistry staining data was used to analyze PCC characteristics (17-21). Wnt3a (representative of Wnt signaling), LGR5 (a cancer stemness marker), TGF-β-induced (TGFBI) (a representative of downstream genes of TGF-β signaling), p-mTOR signaling, and E-cadherin as an EMT marker were assessed. This study was approved by the Institutional Review Board (IRB) of Gunma University (Gunma, Japan; approval no. HS2022-153) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. As this was a retrospective study, the requirement to obtain informed consent was waived by the IRB of Gunma University. An opt-out method was used to obtain consent from the participants. The data obtained were all collected from medical records or existing immunohistochemistry staining. Both the application for the waiver of informed consent and the document of the opt-out consent were posted on the hospital's website for viewing.

Immunohistochemistry. All paraffin-embedded specimens were cut into 4-μm thick sections and mounted on glass slides. Sections were deparaffinized with xylene, hydrated and incubated with 0.3% hydrogen peroxide for 30 min at room temperature to block endogenous peroxidase activity. Non-specific binding sites were blocked by incubation with Protein Block Serum-Free (Dako; Agilent Technologies, Inc.) for 30 min at room temperature. The primary antibodies were diluted with REAL Antibody Diluent (Dako; Agilent Technologies, Inc.) and incubated overnight at 4˚C. Wnt3a polyclonal antibodies (1:200 dilution; cat. no. bs-1700R; BIOSS), LGR5 antibodies (1:200 dilution; cat. no. ab75850; Abcam), TGFBI antibodies (1:200 dilution; anti-TGFBI/BIGH3 antibody; cat. no. 10188-1-AP; Proteintech Group, Inc.), p-mTOR antibodies (1:80 dilution; anti-rabbit monoclonal antibody; cat. no. #2976; Cell Signaling Technology, Inc.) and E-cadherin antibodies (1:500 dilution; HECD-1; mouse monoclonal; cat. no. M108; Takara Bio, Inc.) were used as the primary antibodies. For Wnt3a, the citric acid method was adopted as the antigen activation method, and specimens were immersed in hot water at 98˚C for 30 min using sodium citrate buffer (LSI Medience Corporation), followed by immersion in hot water at 75˚C for 10 min. For TGFBI, antigen retrieval was performed using ImmunoSaver (Nisshin EM, Co., Ltd.) at 98˚C for 45 min. Antigen activation of LGR5 was performed by heating citrate buffer (pH 6.0) in an autoclave for 5 min. To enable E-cadherin activation, the sections were boiled in 10 mM citrate buffer (pH 6.0) at 98˚C for 30 min. Antigen retrieval of p-mTOR was not conducted. The secondary antibody from the Histofine Simple Stain MAX-PO (Multi) Kit (cat. no. 414152F; Nichirei Biosciences, Inc.) was used, which was incubated for 30 min at room temperature. Staining with 3,3-diaminobenzidine tetrahydrochloride was performed in a 0.02% solution in 50 mM ammonium acetate-citrate buffer (pH 6.0) containing 0.005% hydrogen peroxide. Sections were stained with hematoxylin at room temperature for 1 min, placed on a cover glass and observed under a light microscope. The evaluation methods were as previously described (17-21). Wnt3a and TGFBI were evaluated using intensity scores. No staining and weak staining were classified as low expression, and moderate staining and strong staining as high expression. These evaluations were conducted manually as described previously (17,19). For LGR5, the percentage of stained cells was classified into four categories (0, negative; 1, 1-25% positive; 2, 25-50% positive; and 3, >50% positive) and staining intensity into four categories (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining). Percentage and intensity scores were added (0-6) and ranks of 3 or higher were classified as high expression (18). p-mTOR was evaluated using the following intensity scores: 0, no tumor cells are stained, only cytoplasm; 1, 0-10% of tumor cells show weak to moderate staining; 2, >10% of tumor cells show moderate staining or 1-10% of tumor cells show strong staining; and 3, >10% of tumor cells show strong staining. Scores 2 and 3 were classified as positive (20). E-cadherin positive cases were defined as those in which at least 50% of the cancer cells had moderate or higher staining intensity (21).

Statistical analysis. Normally distributed data are presented as the mean ± standard deviation and were analyzed using an unpaired t-test, and non-normally distributed data are presented

Table I. Association between PCC and clinicopathological factors in surgical cases.

Factor	PCC (n=77)	Non-PCC (n=204)	P-value
Age, years	66±11	71±9	<0.0001[a]
Sex			0.0015[a]
Male	42	152	
Female	35	52	
BMI, kg/m^2	22.08±0.26	21.70±0.43	0.448
Tumor size, mm	45.0 (27.3-76.5)	41.0 (25.0-61.8)	0.265[a]
Depth			0.048[a]
M,SM,MP	39	128	
SS,SE,SI	38	73	
Lymph node metastasis			0.443
Absent	42	121	
Present	35	82	
Number of lymph node metastases	0 (0-4.8)	0 (0-3.0)	0.303[a]
Cy			0.191
0	72	198	
1	5	6	
Distant metastasis			>0.999
Absent	74	194	
Present	3	8	
Stage			0.098
I/II	50	153	
III/IV	27	51	
Surgical method			0.906
Open	27	70	
Laparoscopic	50	134	
Lymph node dissection			0.402
D0,D1,D1+	38	111	
D2,D3	39	91	
Resection method			0.229
Total gastrectomy	27	61	
Proximal gastrectomy	5	23	
Distal gastrectomy	43	119	
Other	2	1	
Operation time, min	299±9	301±5	0.849
Blood loss, ml	76 (18-230)	103 (16-253)	0.510

[a]P<0.05. Data were undetermined in 3 cases for depth, 1 case for lymph node metastasis, and 2 cases each for distant metastasis and lymph node resection. PCC, poorly cohesive carcinoma; BMI, body mass index; M, mucosa; SM, submucosa; MP, muscularis propria; SS, subserosa; SE, serosa; SI, serosal invasion; Cy, cytology.

as the median (interquartile range) and were analyzed using the Mann-Whitney U test. The χ^2 test was used for categorical data, with the exception of distant metastasis and resection method data, which were analyzed using Fisher's exact test. Overall survival (OS) and progression-free survival (PFS) from the date of surgery were determined and plotted using the Kaplan-Meier method. The log-rank test was employed for comparison. P<0.05 was used to indicate a statistically significant difference. All statistical analyses were performed using the JMP Pro software (version 15.0; SAS Institute, Inc.).

Results

Association between PCC and clinicopathological factors and prognosis in surgical cases. The associations between PCC and clinicopathological factors are shown in Table I. PCC occurred in 77 (27.4%) patients, with a higher proportion of younger (P<0.0001) and female (P=0.002) patients compared with the non-PCC group. There was no difference in BMI; however, the tumor was significantly deeper (P=0.048) in patients with PCC than in those without. There was no significant difference

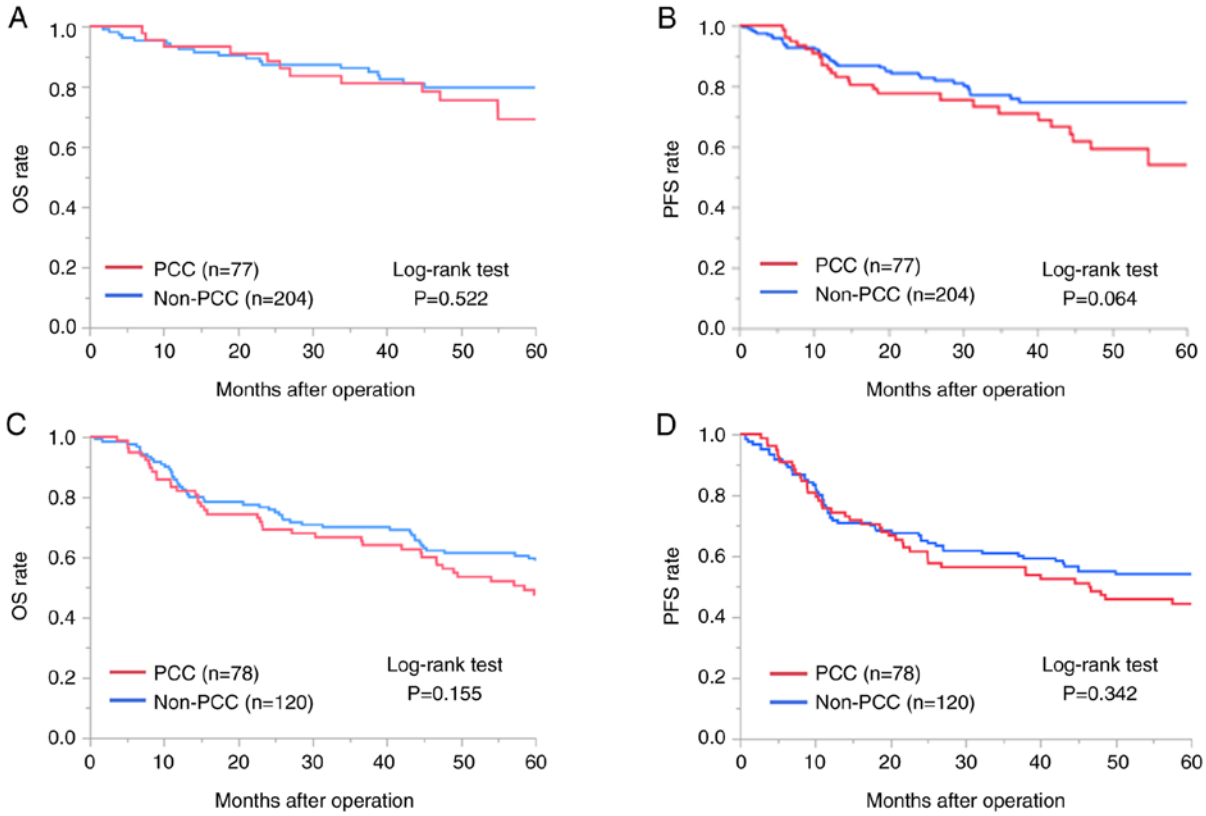

Figure 1. Kaplan-Meier analysis of 5-year OS and PFS of PCC. (A) OS and (B) PFS of surgical cases. (C) OS and (D) PFS of cases evaluated using a tissue microarray. OS, overall survival; PFS, progression-free survival; PCC, poorly cohesive carcinoma.

in the presence of lymph node metastasis. No significant associations were observed for cytology, distant metastasis and stage. Additionally, no significant associations were found for surgical methods, such as open and laparoscopic surgery, lymph node dissection, resection method, operative time and blood loss. There was no significant difference in prognosis for PCC in terms of OS (P=0.522; Fig. 1A) and PFS (P=0.064; Fig. 1B).

Association between PCC and clinicopathological factors and prognosis in TMA cases. The associations between PCC and clinicopathological factors are shown in Table II. In the present TMA study, 78 cases of PCC were included (39.4% of the total). The patients with PCC were younger (P=0.0015), more often women (P=0.045) and had larger tumors (P=0.0199) compared with the patients without PCC. Although there were no associations with tumor depth and lymph node metastasis, the results of this study indicated that stage III-IV cases were significantly more common in patients with PCC (P=0.0041). The representative immunohistochemistry staining images of low and high expression levels of Wnt3a, LGR5, E-cadherin, p-mTOR and TGFBI are presented in Fig. 2. Examination of the expression of the various proteins revealed that wnt3a expression was significantly upregulated in PCC (P=0.0078), whereas E-cadherin (P=0.022) was significantly downregulated. No significant associations were observed with the expression of LGR5, TGFBI and p-mTOR. Furthermore, there was no significant difference in prognosis for PCC in terms of OS (P=0.155; Fig. 1C) and PFS (P=0.342; Fig. 1D).

Expression of E-cadherin and TGFBI associated with EMT and OS in PCC TMA cases. As the immunohistochemistry results revealed that PCC cells exhibited increased EMT, as determined based on E-cadherin levels (Table II), a focus was placed on the expression of E-cadherin as an EMT marker. Furthermore, TGFBI is a representative downstream gene of TGF-β signaling that is known to be associated with EMT (22). Therefore, the significance of E-cadherin and TGFBI expression levels in PCC was investigated with regard to survival (Fig. 3). Among patients with PCC, low E-cadherin expression (P=0.049; Fig. 3A) and high TGFBI expression (P<0.0001; Fig. 3B), which are involved in EMT, were associated with a significantly poorer prognosis. Furthermore, during the evaluation of PFS, low expression of E-cadherin indicated a non-significant tendency for a poor prognosis (P=0.076), and high expression of TGFBI was significantly associated with a poor prognosis (P<0.0001) (Fig. S1). However, there was no association between E-cadherin expression level (P=0.771; Fig. 3C) or TGFBI expression level (P=0.843; Fig. 3D) and a poor prognosis in non-PCC cases.

Discussion

The present study evaluated surgical cases using a TMA and revealed that PCC was more common in younger patients and women, as the ratio of women to men was higher in the PCC group compared with that in the non-PCC group. However, none of the results showed that the prognosis was worse for patients with PCC. Furthermore, in the TMA study, PCC was associated with both decreased expression of EMT markers,

Table II. Association between PCC and clinicopathological factors in tissue microarray cases.

Factor	PCC (n=78)	Non-PCC (n=120)	P-value
Age, years	62±12	67±10	0.0015[a]
Sex			0.045[a]
Male	48	90	
Female	30	30	
Tumor size, mm	62.5 (41.5-108.3)	54.5 (40.0-71.5)	0.0199[a]
Depth			0.111
M,SM,MP	15	35	
SS,SE,SI	63	85	
Lymph node metastasis			0.798
Absent	24	39	
Present	54	81	
Stage			0.0041[a]
I/II	28	68	
III/IV	50	52	
Wnt3a			0.0078[a]
Low expression	23	58	
High expression	55	62	
LGR5			0.855
Low expression	22	35	
High expression	56	84	
E-cadherin			0.022[a]
Low expression	48	54	
High expression	30	66	
TGFBI			0.786
Low expression	47	74	
High expression	31	45	
p-mTOR			0.770
Low expression	35	52	
High expression	42	68	

[a]P<0.05. Data were undetermined in 1 case each for LGR5, TGFBI and p-mTOR. PCC, poorly cohesive carcinoma; M, mucosa; SM, submucosa; MP, muscularis propria; SS, subserosa; SE, serosa; SI, serosal invasion; Cy, cytology; Wnt3a, protein wnt3a; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; p-mTOR, phosphorylated serine/threonine-protein kinase mTOR; TGFBI, transforming growth factor-β-induced.

such as E-cadherin, and the activation of wnt3a signaling. In the TMA study, patients with elevated EMT in the PCC group had a significantly poorer prognosis compared with patients without PCC.

The prognosis for PCC varies from study to study and remains controversial (23,24). In the present study of 478 cases, a poor prognosis of PCC compared with non-PCC was not observed. The proportion of SRC in PCC is inversely associated with tumor invasiveness and has been reported to be an independent predictor of survival (25). We hypothesized that the proportion of SRC may contribute to these controversial results.

Previous studies have indicated that PCC with increased EMT has a poorer prognosis (26,27). The present study focused on E-cadherin and TGF-β signaling as indicators of EMT. PCC patients with low E-cadherin or high TGFBI expression had a significantly poorer prognosis, indicating that E-cadherin and TGFBI may be prognostic factors for PCC. The wnt/β-catenin pathway, TGF-β signaling, hypoxia, neurogenic locus notch homolog protein 3 signaling and matrix metalloproteinases are known to induce EMT (28). EMT contributes to resistance to chemotherapy and immune checkpoint inhibitors (29). A previous study reported that PCC was associated with low PD-L1 expression (30). Furthermore, we previously reported that high TGFBI expression contributes to EMT and treatment resistance in nivolumab-treated patients with lung cancer (19). Hence, the possibility of immunotherapy resistance has also been considered in PCC, and further development of chemotherapy treatment selection strategies is mandatory.

The present study evaluated both surgical cases and TMA results, and these results indicated that PCC was more common in younger patients and women. Koseki *et al* (31) also reported

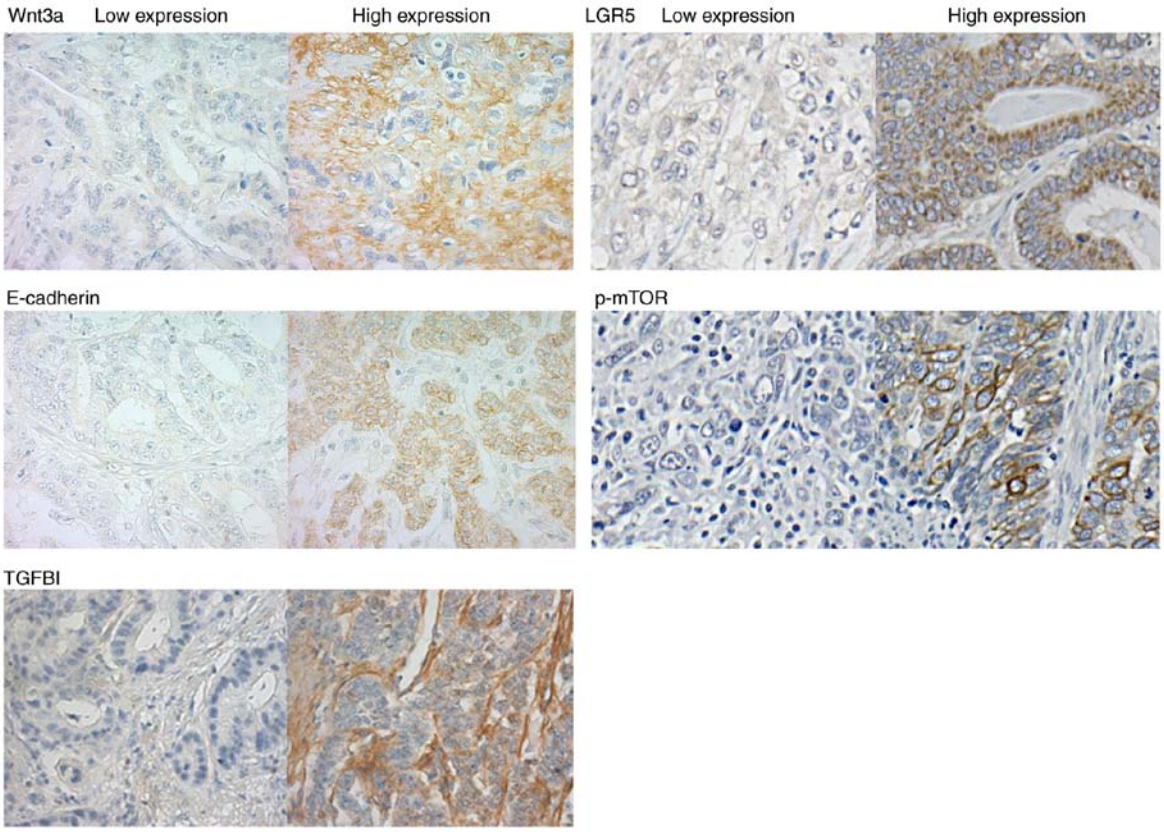

Figure 2. Representative immunohistochemistry. Results of immunohistochemistry staining of representative low and high expression levels of Wnt3a, LGR5, E-cadherin, p-mTOR and TGFBI (x400 magnification). Wnt3a, protein wnt3a; LGR5, leucine-rich repeat-containing G-protein coupled receptor 5; p-mTOR, phosphorylated serine/threonine-protein kinase mTOR; TGFBI, transforming growth factor-β-induced.

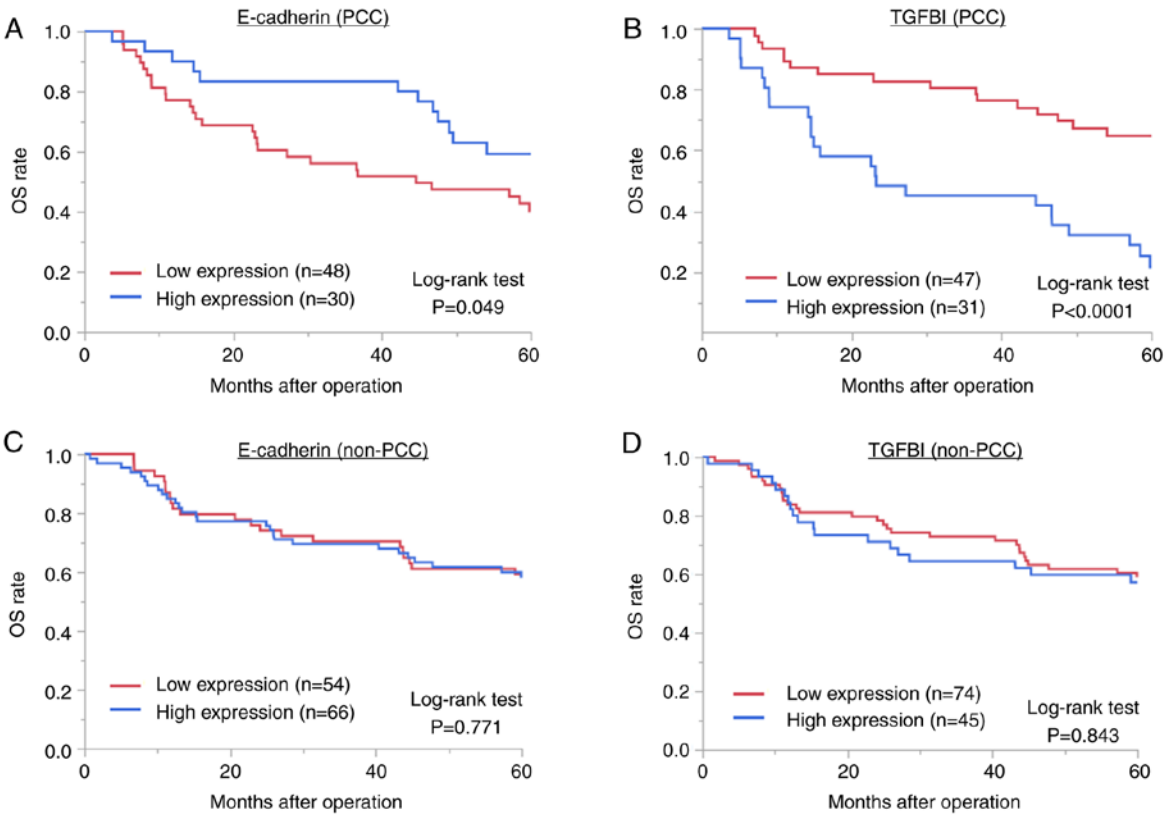

Figure 3. Kaplan-Meier analysis of 5-year OS of PCC for different EMT markers. OS according to (A) E-cadherin and (B) TGFBI expression levels in PCC cases. OS according to (C) E-cadherin and (D) TGFBI expression levels in non-PCC cases. OS, overall survival; PFS, progression-free survival; PCC, poorly cohesive carcinoma; EMT, epithelial-mesenchymal transition; TGFBI, transforming growth factor-β-induced.

that PCC was significantly more common in younger women. Furthermore, the study suggested that mutations in CDH1 and ras homolog family member A (RHOA) are more frequent in PCC. GC is classified as Epstein-Barr virus-CpG island methylator phenotype, hypermutated (microsatellite instability), genomically stable (GC) or chromosomal instability based on its pathological features (32), while PCC is classified as a GC and has frequent mutations in CDH1 and RHOA.

The present study showed that TGFBI expression was associated with prognosis in PCC (high expression was associated with poor OS), but that there was no significant difference in terms of prognosis between PCC and non-PCC patients with different expression levels of TGFBI. Mizoi *et al* (33) reported that TGF-β signaling is upregulated in the GC stroma, and we previously reported (34) that TGFBI, a representative downstream gene of TGF-β signaling, is secreted by cancer-associated fibroblasts in the cancer stroma and that suppressing TGFBI inhibits cancer cell invasion. The reason for the poor prognosis in the PCC group with high TGFBI expression may be due to the fact that PCC is rich in cancer stroma and contains many cancer-associated fibroblasts, which mediated the activation of TGF-β signaling.

The present study had several limitations. First, this was a single-center, retrospective study. Therefore, large-scale, multicenter prospective studies are required for a detailed analysis of the pathological characteristics and prognosis of PCC. Second, this study focused on immunohistochemical staining and did not include cellular experiments.

In conclusion, PCC was more common in younger patients and women. Furthermore, PCC was associated with the absence of cell adhesion molecules and the activation of wnt signaling. In the present study, there was no clear association between PCC and prognosis. However, PCC with increased EMT was associated with a significantly poorer prognosis than PCC without EMT.

Acknowledgements

Not applicable.

Funding

This study was supported by a Japan Society for the Promotion of Science KAKENHI grant (no. 23K15513).

Availability of data and materials

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

Authors' contributions

NN was responsible for study conception and design. Acquisition of data was performed by NN, MI and YS. NN, MSo, AS, MSa, TO, KS and HS analyzed and interpreted the data. Writing, review and/or revision of the manuscript was completed by NN, MSo, KS and HS. TO, KS and HS supervised the study. NN and MSo confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Gunma University (Gunma, Japan; approval no. HS2022-153). As this was a retrospective study, the requirement to obtain informed consent was waived by the Institutional Review Board of Gunma University. An opt-out method was used to obtain the participant's consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Marubashi S, Takahashi A, Kakeji Y, Hasegawa H, Ueno H, Eguchi S, Endo I, Goi T, Saiura A, Sasaki A, *et al*: Surgical outcomes in gastroenterological surgery in Japan: Report of the national clinical database 2011-2019. Ann Gastroenterol Surg 5: 639-658, 2021.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
3. Liu D, Lu M, Li J, Yang Z, Feng Q, Zhou M, Zhang Z and Shen L: The patterns and timing of recurrence after curative resection for gastric cancer in China. World J Surg Oncol 14: 305, 2016.
4. Kikuchi K, Suda K, Shibasaki S, Tanaka T and Uyama I: Challenges in improving the minimal invasiveness of the surgical treatment for gastric cancer using robotic technology. Ann Gastroenterol Surg 5: 604-613, 2021.
5. Marrelli D, Pedrazzani C, Morgagni P, de Manzoni G, Pacelli F, Coniglio A, Marchet A, Saragoni L, Giacopuzzi S and Roviello F; Italian Research Group for Gastric Cancer: Changing clinical and pathological features of gastric cancer over time. Br J Surg 98: 1273-1283, 2011.
6. Henson DE, Dittus C, Younes M, Nguyen H and Albores-Saavedra J: Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: Increase in the signet ring cell type. Arch Pathol Lab Med 128: 765-770, 2004.
7. Laurén PA and Nevalainen TJ: Epidemiology of intestinal and diffuse types of gastric carcinoma. A time-trend study in Finland with comparison between studies from high- and low-risk areas. Cancer 71: 2926-2933, 1993.
8. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F and Cree IA; WHO Classification of Tumours Editorial Board: The 2019 WHO classification of tumours of the digestive system. Histopathology 76: 182-188, 2020.
9. Mariette C, Carneiro F, Grabsch HI, van der Post RS, Allum W and de Manzoni G; European Chapter of International Gastric Cancer Association: Consensus on the pathological definition and classification of poorly cohesive gastric carcinoma. Gastric Cancer 22: 1-9, 2019.
10. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma. 15th edition. Tokyo: Kanehara Shuppan, 2017.
11. Joypaul BV, Newman EL, Hopwood D, Grant A, Qureshi S, Lane DP and Cuschieriet A: Expression of p53 protein in normal, dysplastic, and malignant gastric mucosa: An immunohistochemical study. J Pathol 170: 279-283, 1993.
12. Horii A, Nakatsuru S, Miyoshi Y, Ichii S, Nagase H, Ando H, Yanagisawa A, Tsuchiya E, Kato Y and Nakamuraet Y: Frequent somatic mutations of the APC gene in human pancreatic cancer. Cancer Res 52: 6696-6698, 1992.
13. Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, Kong D, Smolinski KN, Wilson KT, James SP, *et al*: E-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. J Natl Cancer Inst 92: 569-573, 2000.

14. Ribatti D: Epithelial-mesenchymal transition in morphogenesis, cancer progression and angiogenesis. Exp Cell Res 353: 1-5, 2017.
15. Ma HY, Liu XZ and Liang CM: Inflammatory microenvironment contributes to epithelial-mesenchymal transition in gastric cancer. World J Gastroenterol 22: 6619-6628, 2016.
16. Zhang X, Zhang P, Shao M, Zang X, Zhang J, Mao F, Qian H and Xu W: SALL4 activates TGF-β/SMAD signaling pathway to induce EMT and promote gastric cancer metastasis. Cancer Manag Res 10: 4459-4470, 2018.
17. Nakazawa N, Sohda M, Yokobori T, Gombodorj N, Sano A, Sakai M, Oyama T, Kuwano H, Shirabe K and Saeki H: Cytoplasmic localization of connexin 26 suppresses transition of β-catenin into the nucleus in intestinal- and mix-type gastric cancer. Ann Gastroenterol Surg 6: 505-514, 2022.
18. Nakazawa N, Sohda M, Ide M, Shimoda Y, Tateno K, Watanabe T, Sano A, Sakai M, Yokobori T, Ogawa H, *et al*: PROX1 was associated with LGR5 and wnt signaling and contributed to poor prognosis in gastric cancer. Oncology 100: 569-575, 2022.
19. Nakazawa N, Yokobori T, Kaira K, Turtoi A, Baatar S, Gombodorj N, Handa T, Tsukagoshi M, Ubukata Y, Kimura A, *et al*: High stromal TGFBI in lung cancer and intratumoral CD8-positive T cells were associated with poor prognosis and therapeutic resistance to immune checkpoint inhibitors. Ann Surg Oncol 27: 933-942, 2020.
20. Nakazawa N, Sohda M, Ide M, Shimoda Y, Ubukata Y, Kuriyama K, Hara K, Sano A, Sakai M, Yokobori T, *et al*: High L-type amino acid Transporter 1 levels are associated with chemotherapeutic resistance in gastric cancer patients. Oncology 99: 732-739, 2021.
21. Nakazawa N, Yokobori T, Sohda M, Hosoi N, Watanabe T, Shimoda Y, Ide M, Sano A, Sakai M, Erkhem-Ochir B, *et al*: Significance of lipopolysaccharides in gastric cancer and their potential as a biomarker for nivolumab sensitivity. Int J Mol Sci 24: 11790, 2023.
22. Liu Y, Xue M, Du S, Feng W, Zhang K, Zhang L, Liu H, Jia G, Wu L, Hu X, *et al*: Competitive endogenous RNA is an intrinsic component of EMT regulatory circuits and modulates EMT. Nat Commun 10: 1637, 2019.
23. Nakamura K, Eto K, Iwagami S, Ogawa K, Sawayama H, Ishimoto T, Iwatsuki M, Baba Y, Miyamoto Y, Yoshida N and Baba H: Clinicopathological characteristics and prognosis of poorly cohesive cell subtype of gastric cancer. Int J Clin Oncol 27: 512-519, 2022.
24. Sarriugarte Lasarte A, García Alberdi E, Martínez Indart L, Gutiérrez Grijalba O, Álvarez Abad I, Guerra Lerma M, Calle Baraja M and Colina Alonso A: From Lauren's diffuse gastric cancer to WHO's poorly cohesive carcinoma. Clinicopathological and prognostic characteristics. Rev Esp Enferm Dig 113: 324-331, 2021.
25. Roviello F, Marano L, Ambrosio MR, Resca L, D'Ignazio A, Petrelli F, Petrioli R, Costantini M, Polom K, Macchiarelli R, *et al*: Signet ring cell percentage in poorly cohesive gastric cancer patients: A potential novel predictor of survival. Eur J Surg Oncol 48: 561-569, 2022.
26. Bencivenga M, Simbolo M, Ciaparrone C, Vicentini C, Torroni L, Piredda ML, Sacco M, Alloggio M, Castelli C, Tomezzoli A, *et al*: Poorly cohesive gastric cancers showing the transcriptomic hallmarks of epithelial-mesenchymal transition behave aggressively. Ann Surg 276: 822-829, 2022.
27. Bencivenga M, Treppiedi E, Dal Cero MD, Torroni L, Verlato G, Iglesias M, Renaud F, Tomezzoli A, Castelli C, Piessen G, *et al*: The amount of signet ring cells is significantly associated with tumour stage and survival in gastric poorly cohesive tumours. J Surg Oncol 121: 1084-1089, 2020.
28. Polyak K and Weinberg RA: Transitions between epithelial and mesenchymal states: Acquisition of malignant and stem cell traits. Nat Rev Cancer 9: 265-273, 2009.
29. Fischer KR, Durrans A, Lee S, Sheng J, Li F, Wong STC, Choi H, Rayes TE, Ryu S, Troeger J, *et al*: Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. Nature 527: 472-476, 2015.
30. Kim H, Heo YJ, Cho YA, Kang SY, Ahn S and Kim KM: Tumor immune microenvironment is influenced by frameshift mutations and tumor mutational burden in gastric cancer. Clin Transl Oncol 24: 556-567, 2022.
31. Koseki Y, Hatakeyama K, Terashima M, Nagashima T, Urakami K, Ohshima K, Aizawa D, Sugino T, Furukawa K, Fujiya K, *et al*: Molecular profile of poorly cohesive gastric carcinoma with special reference to survival. Gastric Cancer 26: 553-564, 2023.
32. Cancer Genome Atlas Research Network: Comprehensive molecular characterization of gastric adenocarcinoma. Nature 513: 202-209, 2014.
33. Mizoi T, Ohtani H, Miyazono K, Miyazawa M, Matsuno S and Nagura H: Immunoelectron microscopic localization of transforming growth factor beta 1 and latent transforming growth factor beta 1 binding protein in human gastrointestinal carcinomas: Qualitative difference between cancer cells and stromal cells. Cancer Res 53: 183-190, 1993.
34. Suzuki M, Yokobori T, Gombodorj N, Yashiro M, Turtoi A, Handa T, Ogata K, Oyama T, Shirabe K and Kuwano H: High stromal transforming growth factor β-induced expression is a novel marker of progression and poor prognosis in gastric cancer. J Surg Oncol 118: 966-974, 2018.