Molecular profile of poorly cohesive gastric carcinoma with special reference to survival

日本大学大学院医学研究科博士課程
外科系消化器外科学専攻

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修了年（2024）

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Gastric Cancer
https://doi.org/10.1007/s10120-023-01390-5

ORIGINAL ARTICLE

Molecular profile of poorly cohesive gastric carcinoma with special reference to survival

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Received: 20 December 2022 / Accepted: 1 April 2023

Abstract

Background Patients with poorly cohesive gastric carcinoma (PCC) are known to have poor survival. However, detailed molecular biology of PCC has not been elucidated, except for mutations in *CDH1* and *RHOA*. Additionally, the molecular profiles of signet-ring cell carcinoma (SRC) have not been fully investigated. We aimed to investigate the association between molecular profiles and survival in PCC and PCC subtypes.

Methods The present study included 455 patients with gastric adenocarcinoma underwent radical gastrectomy. Whole-exome sequencing and gene expression profiling were conducted. Patients were classified according to the WHO classification as PCC or non-PCC, with PCC being further classified into SRC, combined, and PCC not-otherwise-specified (NOS). Clinicopathological factors and survival were compared with molecular profiles.

Results Of the patients, 159 were classified with PCC, while 296 were classified with non-PCC. Among PCC, 44 were classified with SRC, 64 with combined, and 51 with PCC-NOS. Mutations in *CDH1* and *RHOA* were remarkably more frequent in PCC than in non-PCC. PCC had worse overall survival (OS) and disease-specific survival (DSS) compared to non-PCC. For PCC, the SRC group had good OS and DSS, whereas PCC-NOS classification with *CDH1* mutations was associated with extremely poor survival. In the PCC-NOS and combined groups, patients with mutations in the extracellular domain 1 of *CDH1* had poor survival.

Conclusions Our findings suggest that PCC has poorer survival than non-PCC. Accumulation of *CDH1* and *RHOA* mutations are unique profiles in PCC. Among PCC, *CDH1* mutations may play a crucial role in the survival of non-SRC PCC.

Keywords Gastric cancer · Poorly cohesive · *CDH1* mutation · Extracellular domain 1

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Published online: 10 April 2023

Introduction

Gastric cancer is the fifth most common cancer worldwide and is the fourth leading cause of cancer deaths [1]. Due to the decreased prevalence of *Helicobacter pylori* infections in more developed countries, the incidence of the intestinal type of gastric cancer, as per the Lauren classification [2], has reduced. However, the proportion of the diffuse type has increased in tandem [3].

According to the Japanese Classification of Gastric Carcinoma [4, 5], the histological type of gastric cancer is classified into common and special types. The common types are papillary adenocarcinoma (pap), tubular adenocarcinoma of well (tub1) or moderate (tub2) differentiation, poorly differentiated adenocarcinomas of the solid (por1) or non-solid (por2) type, signet-ring cell carcinoma (sig), and mucinous adenocarcinoma (muc). The Nakamura classification system groups pap, tub1, and tub2 as differentiated types and por1, por2, sig, and muc are classified as undifferentiated. In the WHO classification, PCC includes por2 and sig, whereas non-PCC corresponds to pap, tub, and por1. The Japanese classification classifies a tumor with mixed different histological types according to their quantitative predominance, with the predominant type being categorized as the histological subtype. In the WHO classification, gastric cancer is classified into tubular adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma, and poorly cohesive carcinoma (PCC) (signet-ring cell phenotype (SRC) and other cell types) [6]. In the Japanese Classification of Gastric Carcinoma, PCC includes non-solid, poorly differentiated adenocarcinomas and signet-ring cell carcinoma (SRC). According to the Lauren classification, most PCCs belong to the diffuse type. These types have a high incidence of peritoneal metastasis at diagnosis and a poor prognosis [7–9]. While molecular profiling studies have characterized diffuse types or PCCs using *CDH1* [10, 11] and *RHOA* mutations [12], to our knowledge, no study has demonstrated the impact in a large cohort or using a comprehensive histological classification system.

Although a higher proportion of SRC has been linked to better outcomes in PCCs [13–16], the differences in molecular profiles associated with the SRC proportion remain largely unexplored.

Advances in next-generation sequencing have facilitated molecular biological research, resulting in the development of new classifications for gastric cancer proposed by programs such as The Cancer Genome Atlas (TCGA) [17] and the Asian Cancer Research Group [18]. Since 2014, our hospital has employed whole-exome sequencing (WES) and comprehensive gene expression profiling (GEP) analysis [19, 20] as a high-tech omics-based patient evaluation (HOPE), and the genetic characteristics of each tumor have been reported. We have classified gastric adenocarcinoma into four molecular subtypes based on their genetic profiles: hypermutators (HMTs) with a high tumor mutational burden (TMB), tumors with a high T-cell-inflamed expression signature (TCI), tumors with low epithelial–mesenchymal transition (EMT) (EMT-low), and tumors with high EMT (EMT-high). Furthermore, survival outcomes for HMTs and TCIs were found to be superior to those of other subtypes [21].

Despite these findings, detailed molecular biological characteristics associated with each histological subtype have yet to be investigated. In this study, we used WES and GEP data obtained through the HOPE project to delineate the molecular profiles of PCC and its subtypes.

Methods

Study design and participants

Between January 2014 and March 2019, a total of 600 patients who underwent radical resection for advanced gastric cancer and had sufficient tissue for genetic analysis were considered for this study. Patients with special types of gastric carcinoma, such as gastric carcinoma with lymphoid stroma and mucinous histology according to the Japanese Classification of gastric carcinoma, or remnant gastric cancer, were excluded. Additionally, patients without available WES or gene expression data, patients receiving preoperative chemotherapy, and patients undergoing R2 surgery were also excluded. As a result, a total of 455 patients were included in the analysis. Clinicopathological data were collected from electronic medical records. The American Joint Committee on Cancer (AJCC) TNM classification, 8th edition [22], was used to classify clinicopathological factors, whereas the Japanese Classification of Gastric Carcinoma was used for residual tumors [4]. Whenever possible, the surgical technique, adequacy of postoperative adjuvant chemotherapy, and postoperative follow-up were performed in accordance with the Japanese Gastric Cancer Treatment Guidelines [23].

Pathological diagnosis

All pathological diagnoses were made by pathologists (D.A. and T.S.). Initially, PCC and other non-PCC were classified based on the WHO classification. PCC were further classified into two subtypes according to the WHO classification system: the signet-ring cell phenotype (SRC) and not-otherwise-specified (PCC-NOS). We then subclassified PCC-NOS into PCC-NOS/SRC (combined) and PCC-NOS based on the component ratios of signet-ring cells, following

the consensus of the European Chapter of the International Gastric Cancer Association [24]. Therefore, we subclassified PCC into three groups based on the proportion of signet-ring cells: SRC (> 90% signet-ring cells), combined (< 90% but > 10% signet-ring cells), and PCC-NOS (< 10% signet-ring cells). Pathological diagnosis was performed by two pathologists (D.A., T.S.) (Supplemental Fig. 1). Figure 1 shows representative histopathological images of these three groups.

Clinical samples

A total of ≥ 0.1 g of gastric cancer and adjacent non-cancerous tissue were dissected from surgically resected specimens. To ensure sufficient tumor tissue was included, the collection site was verified by both a pathologist and a surgeon at the time of collection. The collected specimens were immediately stored in liquid nitrogen [20].

Data for the analysis of somatic alterations

DNA extraction and mutational analysis were performed as previously described [20]. Briefly, we extracted somatic mutations by analyzing differential mutations between cancer tissues and peripheral blood cells, as well as between single nucleotide substitutions (SNVs) and insertion–deletions (indels). Mutations identified in protein-encoding exon regions and splice sites were analyzed. Along with WES (mean read depth of 130), 409 genes (including cancer-related genes) were analyzed by the comprehensive cancer panel (CCP) at a deeper read depth (mean read depth of 1169). The cancer-related genes were defined in a previous publication [20]. Briefly, the gene set included oncogenes and tumor suppressor genes and was curated in-house based on multiple databases.

Gene expression signature analysis

RNA extraction and gene expression analysis were conducted according to a previous report [20]. In the GEP analysis, we used microarrays to analyze tumor tissue and adjacent non-cancerous tissue as controls. The WES and GEP data of all samples used for our analysis were registered in the National Bioscience Database Center Human Database as 'Controlled-Access Data' (Research ID, hum0127.v1; https://humandbs.biosciencedbc.jp/en/).

Statistical analysis

Continuous variables were analyzed using Kruskal–Wallis or Wilcoxon rank sum tests, and Fisher's exact test or the χ^2 test was used for categorized variables. Overall survival

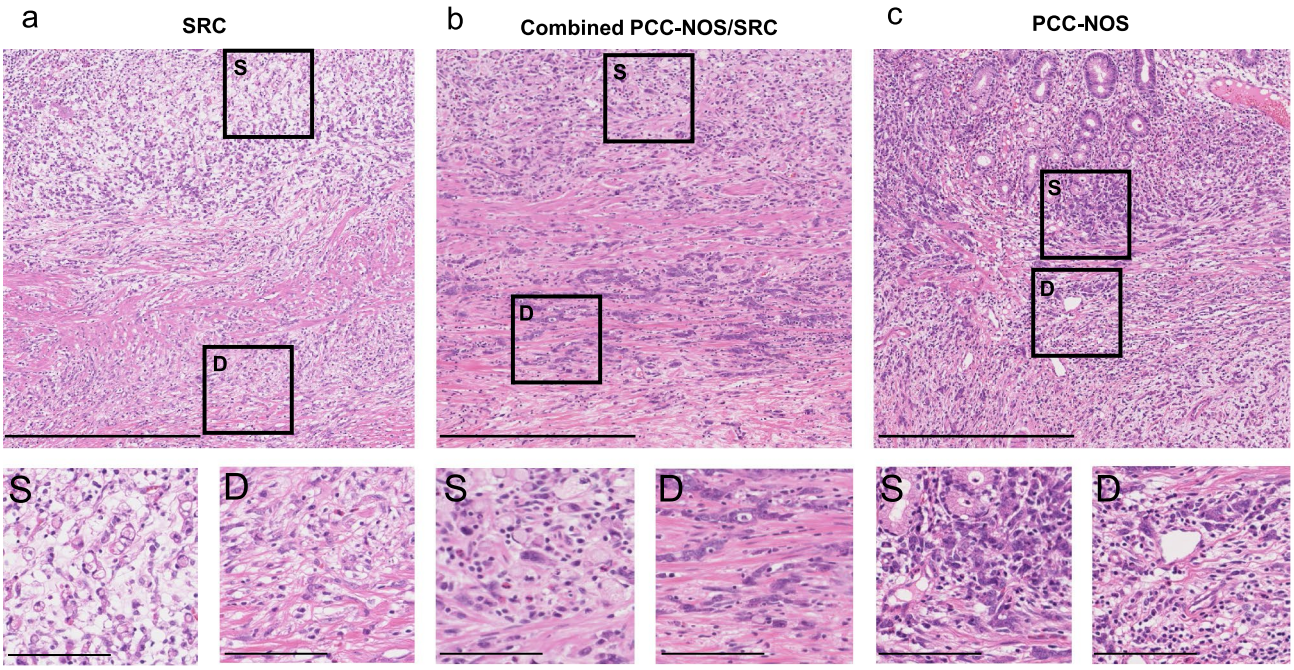

Fig. 1 Hematoxylin and eosin staining. Representative histopathological images of PCC classified into three histological categories. SRC (> 90% of signet-ring cells) (**a**), combined (< 90% but > 10% of signet-ring cells) (**b**), and PCC-NOS (< 10% of signet-ring cells) (**c**). Three images of the low magnification surface layer and the deeper layer are shown, respectively. S in black squares in low magnification indicates the superficial layer, and D indicates the deep layer in high magnification. The black frame in the low magnification is enlarged and shown in high magnification. The low magnification scale is 500 μm, and the high magnification scale is 100 μm

(OS) time was defined as the time from surgery to death due to any cause and censored on the date of last contact for surviving patients. Disease-specific survival (DSS) time was defined as the time from surgery to death due to gastric cancer and censored on the date of death due to other causes or last contact for surviving patients. The OS and DSS were calculated using the Kaplan–Meier method, and the log-rank test was used to determine their significance among the subgroups. The Benjamini–Hochberg correction (q value) was carried out to control the false discovery rate, with two-sided q values < 0.05 considered significant. Clustering using gene expression data was carried out by the ward D2 method after Z-score transformation. All statistical analyses were performed using JMP version 14.3.0 software (SAS Institute, Cary, NC, USA).

Results

The clinicopathological features of the patients are shown in Table 1. Of the total 455 patients, 159 were diagnosed with pheochromocytoma (PCC), while 296 were diagnosed with non-PCC. The PCC group had a significantly higher proportion of women who were significantly younger. Moreover, it had a higher incidence of tumor location in the M region than that in the non-PCC group. Additionally, patients with

Table 1 Clinicopathological features between PCC and non-PCC

Characteristics	PCC ($n = 159$)	Non-PCC ($n = 296$)	p value
Gender			0.004
Male	101 (63.5%)	227 (76.7%)	
Female	58 (36.5%)	69 (23.3%)	
Age (years)	69 (62−76)	72 (66−78)	< 0.001
Location			0.004
E	0 (0%)	7 (2.4%)	
U	32 (20.1%)	92 (31.1%)	
M	68 (42.8%)	89 (30.0%)	
L	59 (37.1%)	108 (36.5%)	
Pathological T classification[a]			< 0.001
T1a	3 (1.9%)	7 (2.4%)	
T1b	7 (4.4%)	31 (10.5%)	
T2	24 (15.1%)	69 (23.3%)	
T3	34 (21.4%)	90 (30.4%)	
T4a	82 (51.6%)	88 (29.7%)	
T4b	9 (5.7%)	11 (3.7%)	
Pathological N classification[a]			< 0.001
N0	35 (22.0%)	97 (32.8%)	
N1	22 (13.8%)	73 (24.7%)	
N2	37 (23.3%)	70 (23.6%)	
N3a	27 (17.0%)	37 (12.5%)	
N3b	38 (23.9%)	19 (6.4%)	
Pathological stage[a]			< 0.001
I	17 (10.7%)	66 (22.3%)	
II	38 (23.9%)	100 (33.8%)	
III	62 (39.0%)	100 (33.8%)	
IV	42 (26.4%)	30 (10.1%)	
Residual tumor[b]			< 0.001
*R*0	116 (73.0%)	268 (90.5%)	
*R*1	43 (27.0%)	28 (9.5%)	
Recurrence	58 (36.5%)	72 (24.3%)	0.009

Data are n (%) or median (interquartile range)

PCC poorly cohesive carcinoma

[a]The eighth cancer staging manual of the American Joint Committee on Cancer (AJCC)

[b]Japanese classification of gastric carcinoma, 3rd English edition

PCC had more advanced T and N classifications and a higher number of stage IV cases compared to non-PCC patients. The R1 resection and recurrence rates were also significantly higher in the PCC group than in the non-PCC group.

Figure 2 shows the mutation profiles of cancer-related genes. The estimated tumor purity and TMB were significantly lower in PCC than in non-PCC (Supplemental Fig. 2a, b). Supplemental Fig. 2c shows the percentage of TCGA classifications in the PCC and non-PCC groups. The proportion of percentage of genomically stable (GS) was high in PCC, whereas the proportion of percentage of chromosomal instability (CIN) was high in non-PCC. The association between the HOPE classification and histopathological type is also shown in Supplementary Fig. 2d. There was no difference in proportion of hypermutator/ T-cell. However, the proportion of EMT-high tumors was significantly higher in PCC than in non-PCC ($p = 1.21 \times 10^{-6}$). The mutation accumulation rates for the cancer-related genes *CDH1* and *RHOA* were significantly higher in PCC than in non-PCC. Conversely, the rate of accumulation of mutations in

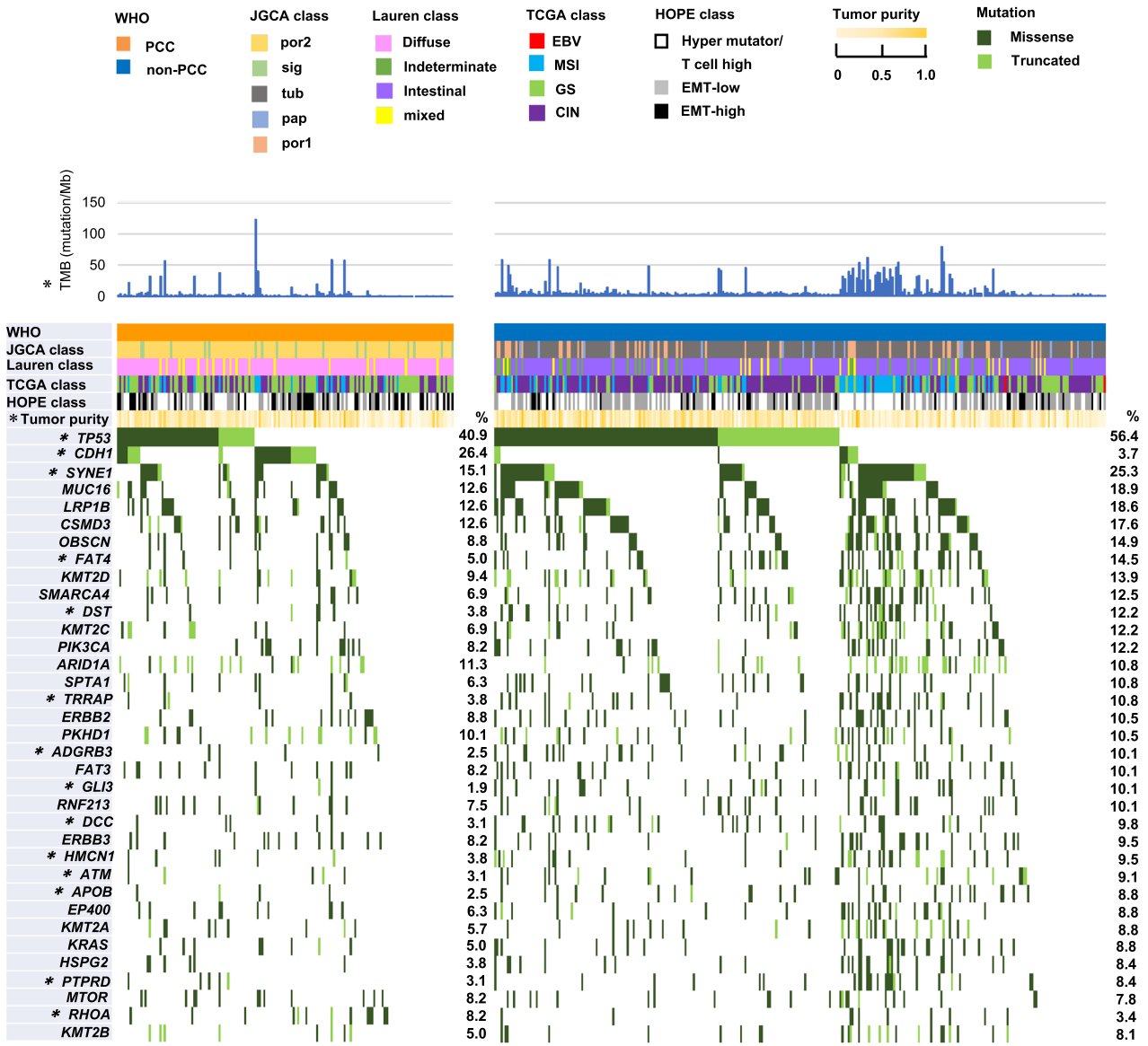

Fig. 2 Mutational landscape of PCC and non-PCC tumors of cancer-related genes. Mutated genes were classified as missense and truncated by selecting nonsynonymous mutations. Those with 8% or more mutations in either group were selected. TMB and tumor purity were estimated from the WES. We combine WES and CCP to see the rate of mutation. * indicates items and genes with significant differences ($p < 0.05$) between the two groups. *WHO* world health organization; *JGCA* Japanese gastric cancer association; *PCC* poorly cohesive carcinoma; *TMB* tumor mutation burden; *WES* whole exome sequence; *CCP* comprehensive cancer panel; *EBV* Epstein–Barr virus; *MSI* microsatellite instability; *GS* genomically stable; *CIN* chromosomal instability; *EMT* epithelial–mesenchymal transition

TP53, SYNE1, FAT4, DST, TRRAP, ADGRB3, GLI3, DCC, HMCN1, ATM, APOB, and *PTPRD* was significantly higher in non-PCC.

In terms of comprehensive GEP analysis, our cohort did not reveal any characteristic gene clusters for PCC. Therefore, we conducted an expression analysis to verify whether our PCC cohort showed concordance with previously reported characteristics of PCCs (Supplemental Fig. 3). The analysis demonstrated that the gene clusters reported as characteristic of PCCs [25] were significantly upregulated in our PCC cohort. There was no significant difference in *CLDN18* expression between PCC and non-PCC. Although *CDH1* expression was significantly lower in PCC than in non-PCC, *CDH1* expression in subgroup analysis showed no significant difference in survival (Supplementary Fig. 4a–c).

Survival analysis revealed that patients with PCC had worse OS ($p = 0.002$) and DSS ($p < 0.001$) (Supplemental Fig. 5) compared to those with non-PCC.

The PCC group was subclassified into three groups: 44 patients as SRC, 64 as combined PCC-NOS/SRC, and 51 as PCC-NOS. Table 2 presents the clinicopathological characteristics of these groups. The age in the SRC group was significantly lower, and the tumor location was predominantly in the M region. The combined and PCC-NOS groups had a relatively high proportion of stage IV cancers, and consequently, the R1 resection rate and recurrence rate were also higher in these groups.

The mutational profiles of these three groups were also investigated (Fig. 3). No significant difference was observed in estimated tumor purity among the three groups

Table 2 Clinicopathological features classified into three histological groups of PCC

	SRC ($n = 44$)	Combined ($n = 64$)	PCC-NOS ($n = 51$)	p value
Gender				0.622
Male	30 (68.2%)	38 (59.3%)	33 (64.7%)	
Female	14 (31.8%)	26 (40.7%)	18 (35.3%)	
Age (years)	65.0 (61–73)	67.5 (64–75)	72.0 (66–79)	0.026
Location				0.105
U	11 (25.0%)	11 (17.2%)	10 (19.6%)	
M	23 (52.3%)	22 (34.4%)	23 (45.1%)	
L	10 (22.7%)	31 (48.4%)	18 (35.3%)	
Pathological T classification[a]				0.224
T1a	0 (0%)	3 (4.7%)	0 (0%)	
T1b	4 (9.1%)	1 (1.6%)	2 (3.9%)	
T2	5 (11.4%)	12 (18.8%)	7 (13.7%)	
T3	11 (25.0%)	13 (20.3%)	10 (19.6%)	
T4a	23 (52.2%)	29 (45.2%)	30 (58.9%)	
T4b	1 (2.3%)	6 (9.4%)	2 (3.9%)	
Pathological N classification[a]				0.537
N0	9 (20.5%)	14 (21.9%)	12 (23.5%)	
N1	5 (11.4%)	9 (14.0%)	8 (15.7%)	
N2	12 (27.2%)	12 (18.8%)	13 (25.5%)	
N3a	7 (15.9%)	16 (25.0%)	4 (7.8%)	
N3b	11 (25.0%)	13 (20.3%)	14 (27.5%)	
Pathological stage[a]				0.409
I	5 (11.4%)	9 (14.0%)	3 (5.9%)	
II	11 (25.0%)	12 (18.8%)	15 (29.4%)	
III	20 (45.4%)	22 (34.4%)	20 (39.2%)	
IV	8 (18.2%)	21 (32.8%)	13 (25.5%)	
Residual tumor[b]				0.094
R0	37 (84.1%)	42 (65.6%)	37 (72.5%)	
R1	7 (15.9%)	22 (34.4%)	14 (27.5%)	
Recurrence	12 (27.3%)	27 (42.2%)	19 (37.3%)	0.289

Data are *n* (%) or median (interquartile range)

SRC signet-ring cell; *NOS* not otherwise specified

[a]The eighth cancer staging manual of the American Joint Committee on Cancer (AJCC)

[b]Japanese classification of gastric carcinoma, 3rd English edition

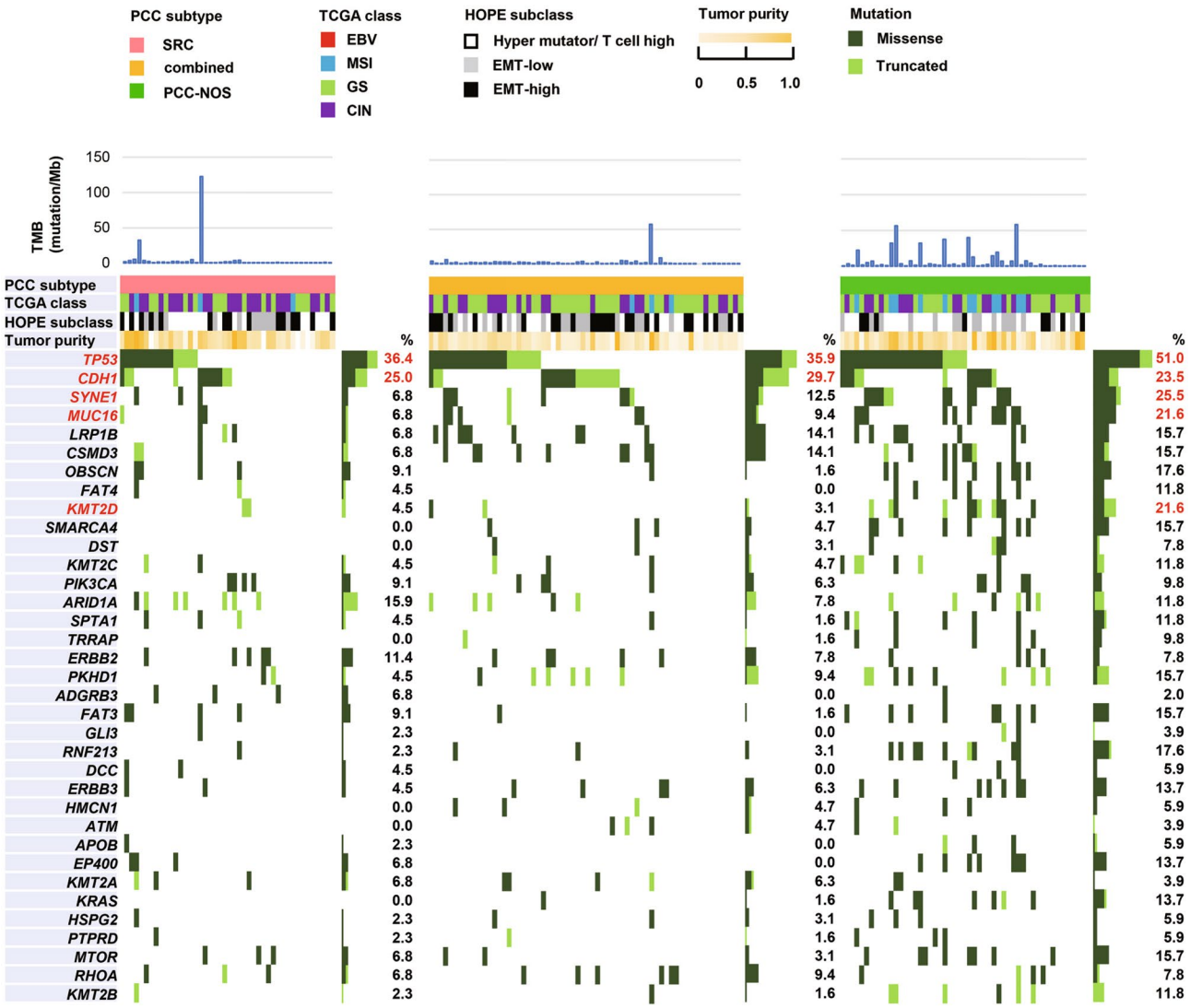

Fig. 3 Mutation profiles of cancer-related genes in the three histopathological classifications of PCC. The gene order conforms to Fig. 2. Red letters indicate that at least one of the PCC subtypes has a mutation accumulation rate of 20% or more. The number of mutations and the rate of accumulation of mutations in each gene are shown in bars and %. *NOS*, not otherwise specified

(Supplemental Fig. 6a). However, TMB was significantly higher in the PCC-NOS group than in the SRC and combined groups (Supplemental Fig. 6b). Supplemental Fig. 6c depicts the percentage of the TCGA classifications in the PCC subgroups. The PCC-NOS group had a high percentage of microsatellite instability (MSI), whereas the combined group had a high percentage of GS tumors. The accumulation of mutations in cancer-related genes differed among the subgroups, with *SYNE1, MUC16*, and *KMT2D* mutations more frequently observed in PCC-NOS gastric cancers.

Survival analysis revealed that although no significant difference was observed when comparing the three groups, the SRC group had a tendency to exhibit better survival than the combined or PCC-NOS groups (Supplemental Fig. 7a and b). Survival in the combined and PCC-NOS groups was comparable. Therefore, by combining the combined and PCC-NOS groups a comparing with SRC group, both OS ($p = 0.029$) and DSS ($p = 0.027$) were significantly higher in the SRC group (Figs. 4a and 4b).

PCC-NOS cancers exhibit a high proportion of MSI-high (MSI-H) (Supplemental Fig. 6c), which is generally associated with better survival outcomes. However, patients with PCC-NOS cancers demonstrated poor survival outcomes. To investigate further, we performed an additional survival analysis by comparing DSS (Supplemental Fig. 8a–c) and OS (Supplemental Fig. 8d–f) between MSI-H and microsatellite stable (MSS) in each subgroup. The MSI-H group tended to have better survival in SRC cancers, but this trend was not observed in PCC-NOS and combined cancers. We also evaluated

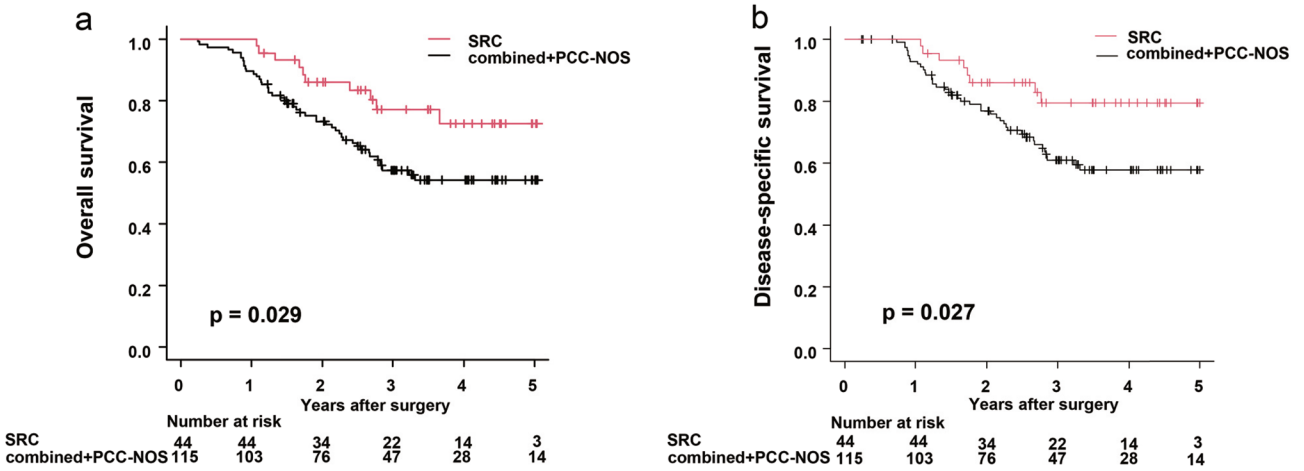

Fig. 4 Comparison of survival between the SRC group and the combined + PCC-NOS groups. OS (**a**) and DSS (**b**) are shown. The Kaplan–Meier survival curve shows the SRC group as a red line and the combined + PCC-NOS group as a black line

clinicopathological features in different PCC subgroups according to MSI status (Supplemental Table 1). MSI-H patients with PCC-NOS cancers were slightly more likely to be female and had more U regions than those with SRC or combined cancers. However, for MSI-H patients with PCC-NOS cancers, the proportion of stage III and IV cancers was smaller than that in the SRC group, and the proportion of lymph node metastasis was comparable to that in the SRC group. There were no specific factors that affected survival.

In the relationship between PCC subgroups and HOPE classification, the proportion of EMT-high was lower in the PCC-NOS group than that in the other groups, although the difference did not reach statistical significance ($p = 0.051$) (Supplemental Fig. 6d).

We selected the top five cancer-related genes, *TP53, CDH1, SYNE1, MUC16*, and *KMT2D*, with mutations in at least one of the top 20% of the PCC subgroups, and investigated their associations with survival. We found that patients with PCC-NOS cancer with *CDH1* mutations had significantly worse DSS ($p < 0.001$) (Supplemental Fig. 9a–c) and OS ($p = 0.002$) (Supplemental Fig. 9d–f). Supplemental Table 2 shows the clinicopathological features for each subgroup according to the presence or absence of *CDH1* mutations. There was no significant difference in pathological T classification, N classification, or stage. However, the group with PCC-NOS cancers with the *CDH1* mutation tended to have higher proportions of stage IV cancers, CY1, and R1 resections. Regarding the recurrence, nine patients with PCC-NOS and *CDH1* mutations had recurrence. Recurrence in the peritoneum was also found to be higher in this group. Similar analyses were performed for the remaining four cancer-related genes, but no significant differences were found in OS and DSS (data not shown).

To investigate the relationship between *CDH1* mutation characteristics and survival in PCC subgroups, a Lollipop plot was used to compare the site and type of *CDH1* mutation for each subgroup (Fig. 5). Mutations were scattered from extracellular domain 1 (EC1 domain) to EC4 domain in SRC and combined cancers. However, *CDH1* mutations were concentrated in the EC1 domain in PCC-NOS cancers ($p = 0.075$). Moreover, missense mutations were significantly more frequent in the EC1 domain than in the other domains ($p = 0.015$) in PCC. Almost all missense mutations of *CDH1* in PCC were variants of unknown significance except for one likely-pathogenic variant in the combined tumor. However, no relationship between mutation type and survival was observed. We further investigated the relationship between the site of mutation and DSS. We found that patients with mutations in the EC1 domain frequently died of gastric cancer, especially in the PCC-NOS and combined groups ($p = 0.038$). DSS was assessed in terms of the presence or absence of mutations in the EC1 domain (Supplemental Fig. 10). Patients with mutations in the EC1 domain had significantly worse DSS in the PCC-NOS ($p = 0.003$) and combined groups ($p = 0.015$). Multivariate analysis of DSS in patients with PCC-NOS and combined cancers revealed that *CDH1* mutations were not selected as an independent prognostic predictor, whereas *CDH1* mutations in the EC1 domain were ($p = 0.046$) (Supplemental Tables 3a, b).

Discussion

In this study, we aimed to investigate the disparities in clinicopathological features, survival outcomes, and mutational profiles between PCC and non-PCC. In our cohort, we observed that PCC patients had worse survival outcomes,

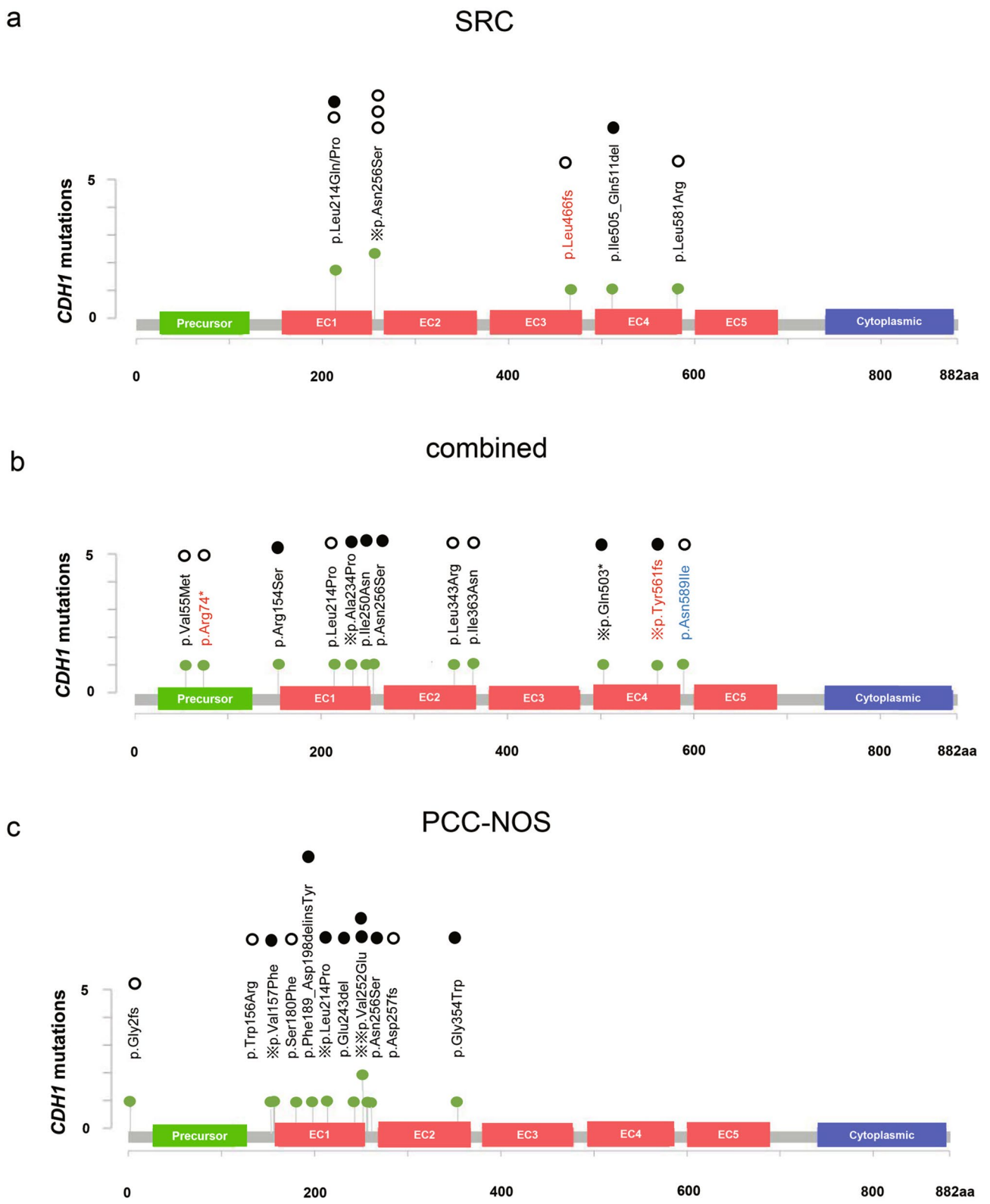

Fig. 5 Lollipop plot of mutation sites on *CDH1* between subgroups of PCC. a indicates SRC, b indicates combined, and c indicates PCC-NOS tumors. NM_004360/NP_004351 was used as the *CDH1* isoform. Filled circles indicate patients with DSS events. Open circles indicate patients without DSS events. * indicates CY1 patients. The figure is color-coded with reference to ClinVar for the evaluation of each mutations. Red letters indicate pathogenic variant. Blue letters indicate likely-pathogenic variant. Black letters indicate variant of unknown significance

corroborating previous findings [9, 16]. Additionally, in gene mutation analysis, we identified a higher accumulation of *CDH1* and *RHOA* mutations in PCCs than in non-PCCs. Conversely, a greater accumulation of *TRRAP, ADGRB3, GLI3, DCC, ATM, APOB*, and *PTPRD* mutations was observed in non-PCCs than that observed in PCCs. These genes are not selected at the top of the mutation frequency in conventional gene mutation analysis in gastric adenocarcinoma [17], and hence, they may serve as genetic mutations characterizing non-PCC.

In addition, PCC was subclassified into three groups according to the consensus of the European Chapter of the International Gastric Cancer Association [24]: SRC, PCC-NOS, and combined PCC-NOS/SRC. Analyzing mutations of cancer-related genes among these three subgroups revealed that *SYNE1*, *MUC16*, and *KMT2D* mutations were more frequent in the PCC-NOS group. To the best of our knowledge, only two studies have previously reported the molecular profile of SRC. Kwon et al. [13] reported no SRC-specific gene mutations, which coincides with our results. In contrast, Wang et al. [26] identified mutations in eight genes, including *CDKN2A*, *POLQ*, *SETBP1*, *SOX9*, *TNFAIP3*, *ZFHX3*, *CREBBP*, and *MAP2K4*, in SRC. However, these genes were not among the most mutated in our SRC group. This inconsistency may be ascribed to the differences in the patient population, as Wang et al. targeted patients with peritoneal dissemination. In terms of survival, the SRC group exhibited significantly better OS and DSS than those of the other two subgroups. Furthermore, PCC-NOS cancers with *CDH1* mutations exhibited poor survival. Specifically, there was a suggested association between mutations in the EC1 domain and survival.

PCCs generally have a low tumor cell content in resected specimens. Our cohort similarly had significantly lower estimated tumor purity when comparing PCCs with non-PCCs. In WES, low tumor purity results in a small number of mutations, making mutation identification challenging [27]. To address this issue, we combined WES with a targeted panel of 409 key genes associated with gastric cancer (with an average read depth of 1169), referred to as the CCP test. Therefore, we believe that mutations in cancer-related genes could be identified in PCCs and non-PCCs without being affected by tumor purity.

When we classified our tumors according to the TCGA, the GS type accounted for approximately 60% of PCCs and was significantly more prevalent than the other subtypes. Moreover, GS-type cancer is known to be characterized by *CDH1* and *RHOA* mutations. Accordingly, *CDH1* and *RHOA* mutations were significantly accumulated in our PCCs.

PCCs exhibited poorer DSS and OS than non-PCCs. It has been reported that many patients with PCC have progressive disease at the time of diagnosis, which was also true in our cohort. In the PCC group, many patients had T4a or deeper tumors, were pathologically stage III or IV, and underwent R1 surgery. According to the TCGA classification, patients with GS-type cancer have poor survival outcomes [28]. The findings of this study of poor survival in patients with PCC, including many with GS-type cancer, support previous research.

In this study, we conducted a subgroup analysis of patients with PCCs and compared their survival outcomes. We found that the SRC group had significantly better survival than those in the PCC-NOS or combined groups. Clinicopathologically, patients in the PCC-NOS and combined groups tended to undergo R1 resection more frequently, and PCC-NOS patients were older. When we compared the PCC subclassification with the TCGA classification, we found that most patients were classified as GS or CIN types, regardless of subgroup. However, approximately 20% of PCC-NOS cancers were classified as the MSI type, which is generally associated with a good prognosis [29]. Surprisingly, survival in PCC-NOS cancers, which contained the most MSI, was poor. When comparing the survival between MSI-H and MSS within each subgroup, SRC cancers showed a trend towards better survival in the MSI-H type, whereas no remarkable difference in survival was observed between PCC-NOS and combined cancers. This finding indicates that tumors containing non-solid adenocarcinomas may be linked to poor survival outcomes. Additionally, we compared patient demographics between MSI-H and MSS for each subgroup and found that MSI-H PCC-NOS cancer was slightly more prevalent in females and in the U region. However, we observed no differences in other clinicopathological factors.

To further investigate the cause of the poor survival in PCC-NOS tumors, we analyzed the clinicopathological features of each subgroup according to the presence or absence of *CDH1* mutations. We found that PCC-NOS tumors with *CDH1* mutations had extremely poor survival. CY1 was more common in PCC-NOS tumors with *CDH1* mutations, and the proportion of stage IV tumors was high, which may explain the high rate of recurrence, especially in the peritoneum, in patients with PCC-NOS cancer. We also considered whether *CDH1* mutations may be associated with tumor aggressiveness. It has been reported that *CDH1* expression may have an impact on survival [30]. Therefore, we further investigated the role of *CDH1* expression on survival in our cohort. Contrary to the previous report, *CDH1* expression did not exhibit significant differences in survival rates among PCC subgroups.

However, this trend was not evident in the combined groups, prompting us to investigate the link between *CDH1* mutations and survival to understand the role of *CDH1* in this aspect. Our analysis showed that in tumors with non-solid components, patients with mutations in the

EC1 domain of *CDH1* exhibited a high incidence of DSS. Mutations in EC1 were also identified as an independent prognostic factor in multivariate analysis. It is possible that mutations in EC1 contribute to the poor survival observed in tumors with non-solid components.

EC1 is the outermost domain at the N-terminus of the extracellular domain of *CDH1*, and it plays a pertinent role in cell-to-cell binding [31]. Mutations in this region are thought to weaken intercellular adhesion and affect the progression and metastasis of cancer cells [32, 33]. In other words, mutations in EC1 can cause cell-to-cell adhesion to become more easily detached, allowing cancer cells on the serosal surface to be released into the peritoneal cavity. This process may be associated with the detection of free cancer cells and recurrence in the peritoneum. Hence, mutations in the EC1 domain of *CDH1* in PCC-NOS and combined PCC-NOS/SRC cancers may be indicators of tumor malignancy.

This research has some limitations. Firstly, it is a study of a Japanese cohort, and as the rate of mutation varies by race [34], the differences among races were not taken into account. Although racial differences in the accumulation of mutations have been reported, the mutational characteristics of PCC subgroups have not been characterized to date. Therefore, the results of this study, which focused on *CDH1* mutations, may be extrapolated to different races. Further research is needed in this field to gain a more comprehensive understanding. Additionally, recent evidence indicates that *CLDN18.2* is a promising target for treating gastric cancer. It has been reported that high *CLDN18* expression is associated with the diffuse type of gastric cancer [11, 35]. Moreover, the *CLDN18-ARHGAP26* gene fusion, which is frequently observed in diffuse-type tumors, has been linked to the prognosis of gastric cancer and the presence of the *RHOA* mutation [17, 35, 36]. However, the use of our fusion gene panel based on next-generation sequencing does not allow the detection of the *CLDN18-ARHGAP26* fusion [37]. Therefore, it is imperative to develop new methods to detect novel fusion genes in PCC and explore their potential clinical implications. Furthermore, as microdissection was not performed in this study, there is a possibility that the characteristics of mutations at the cellular level of SRC- and PCC-NOS tumors are not clearly represented. Nevertheless, since there is a clear difference in the characteristics of mutations between these tumors, we believe that the present results are interpretable. Moreover, dividing PCC into subgroups reduces the number of instances in our study, which is undeniably underpowered for statistical analysis. Despite these limitations, we believe that this study provides useful information, as there have been no reports of genetic analysis in PCC on this scale.

Conclusions

In conclusion, PCC exhibit more *CDH1* and *RHOA* mutations and have a worse survival rate than non-PCC. Among PCCs, SRC cancers demonstrated superior survival rates. We suggest that mutations in the *CDH1* functional domain may be associated with survival in patients with gastric cancers that include a non-solid component.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10120-023-01390-5.

Acknowledgements We would like to thank all of the patients and their families who participated in Project HOPE. We also would like to thank Y. Shimoda, Su. Ohnami, T. Tanabe, F. Kamada for performing next-generation or Sanger sequencing, and A. Naruoka, Sh. Ohnami, K. Maruyama, and T. Mochizuki for data acquisition.

Author contributions YK, KH, YO, KY, and MT contributed to the conception and design of the study. KY chaired the project. DA and TS evaluated pathological specimens. KH, KU, KO, YA, KF, KF, YT, and EB contributed to acquisition of data. YK, KH, and TN contributed to the analysis and interpretation of data. YK, KH, TN, KU, KO, and YA drafted the paper. MT, YO, KF, KF, YT, EB, DA, TS, and KY revised the paper. All authors approved the final version of the manuscript.

Funding Not applicable.

Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest MT has received honorarium from Taiho Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., Bristol Myers Squib Japan K.K., Yakult Honsha Co., Ltd, Takeda Pharmaceutical Co., Ltd, Eli Lilly Japan K.K., Pfizer Japan Inc., Daiichi Sankyo Ltd., Johnson and Johnson K.K., Medtronic Japan Co., Ltd., Intuitive Japan Inc., and Olympus Co., Ltd. EB has received honorarium from EIZO Corporation, TERUMO CORPORATION, and EIZAI.

Ethical approval All procedures were conducted in accordance with the ethical standards of the corresponding committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. The institutional review board at Shizuoka Cancer Center approved all aspects of this study (authorization number 25-33). Informed consent was obtained from all patients.

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論文題名：poorly cohesive 胃癌の生存と関連した分子プロファイル

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Abstract

背景 胃 Poorly cohesive carcinoma（PCC）の患者は生存率が低い。CDH1 と RHOA の変異以外の PCC の分子生物学的な詳細な検討は行われていない。さらに、印環細胞癌（SRC）の分子プロファイルも十分には解明されていない。我々は、PCC および PCC のサブグループの分子プロファイルと生存との関連を明らかにすることを目的とした。

方法 本研究は、根治的胃切除を受けた胃腺癌患者 455 名を対象とし、全エクソームシークエンシングおよび遺伝子発現プロファイリングを実施した。WHO 分類に従い、PCC と non-PCC に分類し、PCC はさらに SRC、combined、PCC not-otherwise-specified（NOS）に分類した。分子プロファイルと臨床病理学的因子と生存を比較した。

結果 159 人が PCC、296 人が non-PCC であった。PCC のうち、44 人が SRC、64 人が combined、51 人が PCC-NOS であった。CDH1 と RHOA の変異は、PCC において有意に多かった。PCC は、non-PCC よりも全生存率（OS）と疾患特異的生存率（DSS）が不良だった。PCC のサブグループ解析では、SRC 群が良好な OS と DSS を示した一方、CDH1 変異を有する PCC-NOS 群は非常に生存が不良だった。PCC-NOS および combined 群では、

CDH1 の細胞外ドメイン 1 の変異を有する患者は、特に生存が不良だった。

結論 PCC は、non-PCC よりも生存が不良だった。CDH1 と RHOA の変異蓄積は、PCC に特徴的なプロファイルだった。PCC の中で、CDH1 変異が non-SRC の生存に重要な役割を果たす可能性が示唆された。

背景

胃癌は世界で第 5 位の有病率、4 位の死亡率の癌腫である[1]。ピロリ菌の除菌により、Lauren 分類[2]の intestinal type は減少しているが diffuse type の割合は増えている[3]。日本の胃癌取扱い規約[4, 5]の por2,sig は WHO 分類の poorly cohesive carcinoma（PCC）に分類される[6]。PCC の多くは Lauren 分類の diffuse type に含まれる。Diffuse type や PCC は診断時に腹膜播種を有していることが多く、予後不良と考えられている[7–9]。Diffuse や PCC は分子プロファイルとしては、CDH1[10, 11]や RHOA の変異[12]を有していることが知られているが、十分なコホートでの普遍的な病理分類と生存との関係は不明である。また signet ring cell の含有割合で生存と関連することが報告[13–16]されているが、分子プロファイルとの関連は明らかではない。

The Cancer Genome Atlas (TCGA)[17]や Asian Cancer Research Group[18]により、次世代シーケンサーを用いた解析により胃癌が分子生物学的に分類されてきた。我々も、当院で行っている project HOPE（High-tech Omics-based Patient Evaluation）というマルチオミクス解析を行う研究により、whole exome sequence (WES) や gene expression profiling (GEP) により、得られた結果を報告してきた[19,20]。胃癌においては、発現と変異データを用いてクラスタリングを行い、Hypermutator、T cell inflamed、EMT high、low により分類し、生存との関連を報告した[21]。

しかし、胃癌の病理組織分類による分子生物学的な特徴はいまだ不明で、PCC と PCC の

サブグループの分子生物学的な特徴を明らかにすることを目的に、project HOPE のデータを用いて、WES や GEP の解析を行った。

方法

Study design and participants

2014 年から 2019 年に根治胃切除が施行された 600 人のうち、病理で特殊型、GCLS、mucinous type、残胃癌を除外し、WES や GEP のデータが不十分なもの、R2 手術となった患者を除外した。455 人が、本研究の解析対象となった。

臨床病理学的因子は TNM 分類[22]、腫瘍遺残については胃癌取扱い規約[4]を基にして分類した。術後のフォローアップは胃癌治療ガイドラインに従った[23]。

Pathological diagnosis

病理分類は、2 人の病理医によって分類された。まず WHO 分類に従い PCC とその他の non-PCC に分類した。PCC は SRC とその他（PCC-NOS）に分類された。PCC-NOS は国際胃癌学会欧州支部の SRC 含有率による分類の提唱[24]に従い、さらに PCC-NOS/SRC と PCC-NOS に分類した。すなわち PCC は 3 群に分類し、sig が 90%以上の SRC、sig が 10－90%の PCC-NOS/SRC、10%以下の PCC-NOS に分類した。

Clinical samples

腫瘍とその近傍の非腫瘍組織を切除検体から採取した。採取箇所は、外科医と病理医で決定した。採取した組織は、液体窒素に入れ凍結した[20]。

Data for the analysis of somatic alterations

がん組織と末梢血液細胞の間の変異の差異、および単一塩基置換（SNV）と挿入-欠失

（indels）の中での差異変異によって体細胞変異を抽出し、タンパク質をコードするエクソン領域とスプライスサイトで検出された変異を解析した。WES（平均リード深度 130）に加えて、がんパネル（comprehensive cancer panel; CCP）によって 409 個の遺伝子（がん関連遺伝子を含む）がより深いリード深度（平均リード深度 1169）で分析された。がんに関連するがん遺伝子やがん抑制遺伝子などの遺伝子セットは、複数のデータベースに基づいて当該研究所によってキュレーションされた。

Gene expression signature analysis

RNA 抽出と遺伝子発現解析は、以前の文献 20 に従って実施した。GEP 解析では、マイクロアレイを使用して腫瘍組織を解析し、腫瘍に隣接した非腫瘍組織を対照として使用した。我々の解析に使用されたすべてのサンプルの WES および GEP データは、「コントロールされたアクセスデータ」として National Bioscience Database Center Human Database に登録された。CDH1 発現は、腫瘍組織と非腫瘍組織の発現変化量を用いて、発現変化量の中央値で二分し、高発現（high）と低発現（low）に分類した。

Statistical analysis

連続変数については Kruskal-Wallis 検定または Wilcoxon の順位和検定を、カテゴリー変数については Fisher の正確確率検定または $\chi 2$ 検定を使用した。全生存期間（OS）は、手術から死亡までの時間と定義し、生存患者の最終確認日はセンサーとして扱った。疾患特異的生存期間（DSS）は、手術から胃癌による死亡までの時間と定義し、他の原因による死亡

または生存患者の最終確認日はセンサーとして扱った。OS および DSS は Kaplan–Meier 法を用いて計算した。サブグループ間の OS および DSS の有意性を決定するために、ログランク検定を使用した。偽陽性率を調整するために、Benjamini-Hochberg 補正（q 値）を行い、両側の q 値が<0.05 であれば有意と考えた。遺伝子発現データを用いたクラスタリングは、Z スコア変換後の ward D2 法によって行われた。すべての統計解析は、ソフトウェア JMP バージョン 14.3.0 を使用して実施した。

結果

患者の臨床病理学的特徴を Table1 に示す。PCC 群は 159 人で、non-PCC 群は 296 人だった。PCC 群は、有意に女性の割合が高く、有意に若年であり、腫瘍の占居部位も non-PCC 群と比較し M 領域に多かった。PCC 群は、non-PCC 群に比べて、T および N 因子が進行しており、その結果ステージ IV の症例が多くなっていた。R1 切除率および再発率も PCC 群では non-PCC 群よりも有意に高かった。

Figure2 に、がん関連遺伝子の変異プロファイルを示す。推定腫瘍含有量と Tumor mutation burden（TMB）は、PCC 群は non-PCC 群よりも有意に低かった（Supplemental Fig. 2a, 2b)。Supplemental Fig. 2c は、PCC 群と non-PCC 群の TCGA 分類の割合を示している。ゲノム安定型（GS）の割合は、PCC で高く、一方で染色体不安定性（CIN）の割合は non-PCC で高かった。当科で報告した HOPE 分類との関連を Supplemental Fig. 2d に示す。Hyper mutator/ T cell high の割合に違いは認められなかったが、EMT-high の割合は PCC 群で non-PCC 群よりも有意に高かった（$p = 1.21 \times 10^{-6}$)。CDH1 と RHOA の変異蓄積率は、PCC 群で non-PCC 群よりも有意に高かった。一方、TP53、SYNE1、FAT4、DST、TRRAP、ADGRB3、GLI3、DCC、HMCN1、ATM、APOB、および PTPRD の変異蓄積率は、non-PCC 群で有意に高かった。

GEP 解析によると、PCC に特徴的な遺伝子クラスターは観察されなかった。そのため、PCC に関する既報に一致するか否かを確認するために、発現解析を行った（Supplemental

Fig. 3)。この解析では、以前に PCC に特徴的であると報告されていた遺伝子クラスター[25]が、我々の PCC コホートでも有意に上昇していることが示された。また CLDN18 の発現には PCC と non-PCC の間に差はなかった。CDH1 の発現は PCC で有意に低かったが、サブグループ解析では、CDH1 の発現による生存率に有意な差は認めなかった(Supplemental Fig. 4a–4c)。

PCC 群は non-PCC 群と比較し、OS ($p = 0.002$) および DSS ($p < 0.001$) において生存率が不良であることが示された（Supplemental Fig. 5)。

PCC のサブグループは、44 例が SRC、64 例が PCC-NOS/SRC、51 例が PCC-NOS に分類された。Table 2 に、3 群の臨床病理学的特徴を示す。SRC 群の年齢は有意に低く、腫瘍の占居部位が M 領域である割合が高かった。また、combined 群および PCC-NOS 群では、ステージ IV の割合が比較的高く、それに応じて R1 切除率および再発率も高くなる傾向があった。

これら 3 群の変異プロファイルを調べた（Fig. 3)。3 群間で推定腫瘍含有量に有意差は認めなかった（Supplemental Fig. 6a)。TMB は、PCC-NOS 群が SRC 群および combined 群よりも有意に高かった（Supplemental Fig. 6b)。Supplemental Fig. 6c に、PCC サブグループの TCGA 分類の割合を示す。マイクロサテライト不安定性（MSI）の割合が PCC-NOS 群で高く、一方で GS 型の割合が combined 群で高かった。がん関連遺伝子の変異蓄積を比較すると、SYNE1、MUC16、KMT2D の変異は、PCC-NOS 群でより多い傾向があった。

生存に関して、3 群間を比較した結果、有意差は見られなかったが、SRC 群は combined 群または PCC-NOS 群よりも生存率が良好な傾向であった（Supplemental Fig. 7a, 7b)。Combined 群と PCC-NOS 群の生存率は類似していたため、combined 群と PCC-NOS 群を結合し SRC 群と比較すると、SRC 群の OS (p = 0.029）と DSS (p = 0.027）は、いずれも有意に良好だった（Fig. 4a, 4b)。Stage 別での生存曲線を補足 Fig. 1 に示す。Stage 別で比較した場合、SRC が生存がよい傾向にあるが、有意差はなかった。

生存に関して、PCC-NOS 群は MSI-H の割合が高かった（Supplemental Fig. 7c)。MSI-H は一般的に生存に有利と考えられている。それにもかかわらず、PCC-NOS 群の生存率は低いことがわかった。そのため、各サブグループ内で MSI-H と microsatellite stable (MSS) を比較し、DSS (Supplemental Fig. 8a–8c）および OS (Supplemental Fig. 8d–8f）を追加で解析した。SRC 群では MSI-H 群の生存率が改善される傾向が見られたが、PCC-NOS と combined 群ではこの傾向は見られなかった。PCC のサブグループにおける MSI と MSS の比較を、臨床病理学的に評価した（Supplemental Table 1)。MSI-H 患者は女性の割合がやや高く、PCC-NOS 群では U 領域がより多い傾向があり、SRC および combined よりも少ないが、PCC-NOS の MSI-H 患者では、ステージ III および IV の癌の割合が SRC 群よりも低く、リンパ節転移の割合は SRC 群と同様だった。生存に影響を与える特定の要因はなかった。

PCC のサブグループと HOPE 分類の関係について、PCC-NOS 群は、他の群と比べて

EMT-high の割合が低かったが、この差は統計的に有意ではなかった（p = 0.051)(Supplemental Fig. 6d)。

少なくとも1つのPCCサブグループで、上位20%の変異を有するTP53、CDH1、SYNE1、MUC16、およびKMT2Dの上位5種のがん関連遺伝子を選択し、それらと生存との関連を調べた。CDH1変異を有するPCC-NOS群では、DSS（p < 0.001)(Supplemental Fig. 9a–9c）およびOS（p = 0.002)(Supplemental Fig. 9d–9f）が有意に不良であることがわかった。Supplemental Table 2に、CDH1変異の有無による各サブグループの臨床病理学的特徴を示す。T因子、N因子、ステージ分類に有意差はなかったが、CDH1変異を有するPCC-NOS群は、ステージIV、CY1、およびR1手術の割合が高い傾向だった。再発形式に関しては、CDH1変異を有するPCC-NOSの9人に再発を認め、このうち8人は腹膜再発だった。腹膜での再発率は他の群より高かった。残りの 4 つの上位の変異を認めたがん関連遺伝子についても同様の分析が行われたが、OS および DSS に有意差は無かった（データは示されていない)。

PCC の各サブグループにおける CDH1 の変異の特徴と生存率の関係を調べるため、Lollipop プロットを用いてサブグループごとに CDH1 の変異箇所と種類を比較した（Fig. 5)。SRC および combined では、変異は細胞外（Extra cellular）ドメイン1(EC1ドメイン）から EC4 ドメインまで散在していたが、PCC-NOS では EC1 ドメインに集中していた（p = 0.075)。また、PCC における CDH1 の変異の種類に関しては、ミスセンス変異が EC1 ド

メインで有意に頻度が高いことが分かった（p = 0.015)。PCC における CDH1 のほとんどのミスセンス変異は、臨床的意義不明のバリアント（VUS）であったが、combined 群においては 2 つの pathogenic variant と 1 つの likely pathogenic variant が見つかった。SRC においても 1 つの pathogenic variant が同定された。これらの 4 人の生存と CDH1 変異を有するその他の 38 人の生存を調べた（補足 Fig. 2)。病的変異のある患者の生存は不良とは言えなかった。変異の種類と生存の関係については、有意な関連は認められなかった。次に、変異の箇所と DSS の関係を調べた。EC1 ドメインでの変異を有する患者は、特に PCC-NOS 群および combined 群において、胃癌で死亡することが多いことが分かった（p = 0.038)。CDH1 の EC1 ドメインでの変異の有無に基づいて、DSS を評価した（Supplemental Fig. 10)。EC1 ドメインでの変異のある患者は、PCC-NOS（p = 0.003）および combined（p = 0.015）において有意に DSS が不良だった。PCC-NOS および combined における DSS の多変量解析では、CDH1 変異は独立した予後因子として選択されなかったが、CDH1 の EC1 変異は選択された（p = 0.046)(Supplemental Fig. 3a, 3b)。CDH1 変異の 42 人の臨床病理学的背景を補足 Table1 に示す。CDH1 変異の PCC-NOS/SRC と PCC-NOS は StageIV や R1 切除割合が多い傾向であったが有意差はなかった。また、EC1 変異と臨床病理学的因子との関連を補足 Table2 に示す。EC1 変異と CY は関連なし（p=0.730)、pStage は関連なし（p=0.965)、再発は傾向あるも有意差なし（p=0.062)、腫瘍遺残も関連なし（p=.0499）であった。

考察

この研究では、PCC と non-PCC の臨床病理学的因子、生存率、遺伝子変異プロファイルの違いを初めて調べた。我々のコホートでは、以前報告されたように PCC の生存率が低く[9, 16]、遺伝子変異解析では CDH1 と RHOA の変異が non-PCC よりも PCC で高い蓄積割合を認めた。一方、TRRAP、ADGRB3、GLI3、DCC、ATM、APOB、PTPRD の変異は、non-PCC で PCC よりも高い蓄積割合を認め、これらの遺伝子は胃癌の従来的な遺伝子変異解析では上位に選択されない遺伝子変異で、non-PCC を特徴づける遺伝子変異と考えられた[17]。

さらに、PCC は国際胃癌学会欧州支部の提唱に基づいて SRC、PCC-NOS、および combined PCC-NOS/SRC の 3 つのサブグループに分類した[24]。これらの 3 つのサブグループ間でがん関連遺伝子の変異を比較した結果、PCC-NOS 群では SYNE1、MUC16、KMT2D の変異蓄積率が高くなる傾向があったことが示された。胃癌においては SYNE1 や MUC16 に関して生物学的意義は不明。しかし、SYNE1、MUC16 は TMB 高値の腫瘍において、変異が多いといわれている遺伝子[補足文献 1]で、TMB 高値の PCC-NOS 群において、変異蓄積割合が高かったと考えられる。

私たちの知る限り、SRC の分子プロファイルに関する研究は 2 つしか報告されていない。Kwon ら[13]によると、SRC 固有の遺伝子変異は報告されていないため、私たちの結果と一致していた。一方、Wang ら[26]は SRC で CDKN2A、POLQ、SETBP1、SOX9、TNFAIP3、

ZFHX3、CREBBP、MAP2K4 の 8 つの遺伝子変異が報告されたが、これらの遺伝子は我々の SRC 群で最も変異が高い遺伝子の中には含まれていなかった。この違いは、Wang らが腹膜播種を有する患者に限定したため、患者背景の違いによるものと考えられる。生存に関しては、SRC 群が他の 2 つのサブグループよりも有意に優れた OS および DSS を示した。CDH1 変異を有する PCC-NOS の生存率が低いことが示され、特に EC1 ドメインの変異と生存率の関連が示唆された。

PCC は、一般的に手術標本の中に腫瘍細胞が少ない傾向がある。我々のコホートでも、PCC と non-PCC を比較すると推定腫瘍含有量が有意に低くなっていた。WES では、腫瘍含有量が低い場合、変異数が少なくなり、変異の同定が困難になる可能性がある[27]。この問題を回避するために、胃癌に関連する 409 の遺伝子を含む CCP（平均読み込み深度 1169）を WES と組み合わせて解析した。従って、この解析では腫瘍含有量の影響を受けることなく、がん関連遺伝子の変異が PCC と non-PCC で同定できたと考えられる。

TCGA 分類は胃癌の分子生物学的分類としてよく知られている。TCGA に基づいて腫瘍を分類したところ、GS 型が PCC の約 60%を占め、他のタイプよりも有意に多いことがわかった。さらに GS 型が CDH1 および RHOA の変異で特徴づけられていると報告されており、我々の PCC でも CDH1 および RHOA の変異が有意に蓄積されていることがわかった。

PCC は、non-PCC に比べて DSS と OS が不良だった。多くの PCC 患者は、診断時に進行していると報告されている。我々のコホートでも同様で、PCC 群の多くの患者は、T4a 以

上であり、病理学的には III 期または IV 期であり、R1 手術の割合が多かった。TCGA 分類では、GS 型の癌の患者の生存率が低いと報告されている[28]。本研究の結果は、GS 型の癌を含む多くの PCC 患者の生存率が低いことを示しており、これは以前の研究の結果を支持している。

PCC のサブグループ解析では、SRC 群は PCC-NOS 群または combined 群よりも有意に良好な生存率を示した。臨床病理学的に、PCC-NOS 群は高齢であり、combined と PCC-NOS はより多くの R1 切除を受けていた。PCC のサブグループ分類を TCGA 分類と比較すると、サブグループに関係なく、ほとんどの患者が GS 型または CIN 型に分類されたが、約 20%の PCC-NOS 群が MSI 型と分類された。MSI は一般的に良好な予後と関連していると考えられている[29]が、最も MSI を含む PCC-NOS 群の生存率は低かった。各サブグループにおいて MSI-H と MSS の生存率を比較すると、SRC 群は MSI-H 型において生存率が良好である傾向があったが、PCC-NOS と combined の間には生存率の差は無かった。この結果は、non-solid 成分を含む腫瘍が生存率の低下に寄与する可能性があることを示唆している。

MSI-H と MSS の各サブグループ間で患者の臨床病理学的因子を比較すると、MSI-H を有する PCC-NOS は女性や U 領域でやや多く見られたが、その他の臨床病理学的要因には差が無かった。術後補助化学療法と MSI に関して補足 Fig. 3 に示す。StageIIB 以上で術後補助化学療法の有無により、生存曲線を示す。StageIII, IV では、術後補助化学療法施行群

の生存が有意に良好だった。MSI-H と MSS との比較を補足 Fig. 4 に示す。MSS では有意に化学療法施行群の生存が良好だった。一方、MSI-H では化学療法施行群と非施行群で生存に差を認めなかった。

PCC-NOS の生存が不良となる原因をさらに調べた。その結果、CDH1 遺伝子変異がある PCC-NOS 群の生存率が極端に悪いことがわかった。CDH1 変異の有無により各サブグループの臨床病理学的特徴を分析した結果、CY1 は CDH1 遺伝子変異を有する PCC-NOS 群でより多く、ステージ IV の割合が高かったことが明らかになった。おそらくそのため、PCC-NOS 群の患者では、特に腹膜再発が高率に観察された。CDH1 変異が腫瘍の悪性度に関連する可能性があるかどうかを検討した。CDH1 発現が生存に影響を与える可能性があることが報告されている[30]。そこで、我々はコホート内で CDH1 発現が生存率に及ぼす影響を調べた。以前の報告とは異なり、CDH1 発現は PCC サブグループ間の生存率に有意な差を示さなかった。

しかし、この傾向は combined 群では観察されなかったため、CDH1 変異部位と生存率との関係をさらに調べ、CDH1 の役割を明らかにすることとした。その結果、non-solid の成分を有する腫瘍において、CDH1 の EC1 ドメインに変異がある患者では、DSS が不良となることがわかった。EC1 の変異は、多変量解析で独立予後予測因子として選択された。EC1 の変異は、non-solid 成分を有する腫瘍における生存率が不良となる原因の一つである可能性が示唆された。

CDH1 の EC1 は、CDH1 の細胞外ドメインの N 末端にある最外層ドメインであり、細胞間結合に重要な役割を果たしている[31]。この領域における変異は、細胞間接着を弱め、がん細胞の進行と転移に影響を与えると考えられている[32, 33]。つまり EC1 に変異がある場合、細胞間接着がより簡単に解離し、腹膜表面に露出したがん細胞が腹腔内に放出されやすくなる。これが、フリー癌細胞の検出や腹膜内再発に関連すると推測される。したがって、PCC-NOS および PCC-NOS/SRC の腫瘍における CDH1 の EC1 領域の変異は、腫瘍の悪性度の指標である可能性がある。

PCC-NOS/SRC では、CDH1 変異の有無では、生存に差はなかったが、EC1 変異の有無で生存に差が出た（Supple Fig. 9,10)。また PCC-NOS の CDH1 変異の多くは EC1 変異だった（83.3%)。SRC における EC1 変異は生存に影響を与えない。PCC-NOS/SRC+PCC-NOS において、CDH1 変異は、独立予後予測因子ではなかった（Supple Table 3a)。一方、EC1 変異は独立予後予測因子だった（Supple Table 3b)。CDH1 の変異だけでは、生存には影響を与えないが、CDH1 における EC1 変異が、生存に強く影響するという結果が得られた。PCC-NOS/SRC と PCC-NOS の違いは PCC-NOS において EC1 の変異が多かった。形態学的に sig の含有割合が下がるにつれて、CDH1 の EC1 変異の割合が増加し、生存にも影響を与える可能性があるということは結果から言うことが可能。por2 が形態学的に多く存在した場合に、細胞の接着性が低下し、癌の進展に関連している可能性がある。EC1 変異が PCC の生存に影響を与える可能性があるが、形態学的な関連や、詳細なメカニズムは

不明で、今後タンパク発現を解析し、遺伝子変異とタンパク発現の癌の進展に関するメカニズムを追求する。

この研究にはいくつかの制限がある。まず、日本の集団を対象にした研究で人種によって突然変異の割合は異なるが[34]、人種間の違いは考慮されていない。変異の特性が PCC のサブグループについては今まで特定されていなかったが、本研究は CDH1 の変異に焦点を当てており、この結果は異なる人種にも適用できる可能性がある。また、最近、CLDN18.2 が胃癌の治療対象となることが示された。CLDN18 は diffuse type に高く見られると報告されている[11, 35]。さらに、diffuse type に豊富に含まれる CLDN18-ARHGAP26 遺伝子融合は、胃癌の生存率や RHOA 変異と関連があることが報告されている[17, 35, 36]。残念ながら、我々の次世代シーケンシングを用いた融合遺伝子パネルでは CLDN18-ARHGAP26 は検出できない[37]。そのため PCC において今後、新たな融合遺伝子の検査が必要で臨床的意義も含め、検査方法を開発することが重要と考えられる。また、本研究ではマイクロ切片検査は行われておらず、SRC および PCC-NOS 群の細胞レベルでの変異の特性が必ずしも明確に表現されているわけではない。本研究の組織抽出は、診断に影響がない箇所（辺縁部）を、病理医と外科医で決定している。PCC と診断した部位と、遺伝子解析の部位は完全には一致しない。ホルマリン固定パラフィン包埋（Formalin-fixed paraffin-embedded（FFPE））にて microdissection を行い、浸潤部を遺伝子解析した訳ではないので、胃癌の heterogeneity を鑑みると limitation になると考える。しかし、これらの腫瘍の変異

の特性に明確な差があることから、本研究の結果の解釈には重大な影響はないと考えられる。さらに、PCC をサブグループに分類することにより、統計分析において十分なパワーが得られていないことは否定できない。しかし、この規模での PCC における遺伝子解析の報告がなかったことから、本研究は有用な情報を提供するものと考えられる。

結論

PCC は、non-PCC に比べて CDH1 および RHOA の変異が多く、生存率が低いことが示された。PCC の中でも SRC 群の生存率が優れていた。CDH1 の機能領域の変異は、non-solid 成分を有する胃癌患者の生存と関連している可能性があることが示唆された。

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自身の役割

PCC159 人の病理スライドを見直し、病理医と分類

切除標本から腫瘍部、非腫瘍部を抽出（全例ではない）

Supplemental Table 1 Clinicopathological features of PCC subgroup according to MSI-H or MSS

	SRC (n=51)				combined (n=64)				PCC-NOS (n=51)				P value
	MSI-H (n=3)		MSS (n=41)		MSI-H (n=3)		MSS (n=61)		MSI-H (n=11)		MSS (n=40)		
Gender													0.774
Male	3	(100%)	27	(65.9%)	2	(66.7%)	36	(59.0%)	6	(54.5%)	27	(67.5%)	
Female	0	(0%)	14	(34.1%)	1	(33.3%)	25	(41.0%)	5	(45.5%)	13	(32.5%)	
Age, years (Range)	74 (73–83)		65 (31–86)		70 (62–76)		67 (23–84)		78 (71–82)		71 (64-77)		
Location													0.726
U	0	(0%)	11	(26.8%)	0	(0%)	11	(18.0%)	1	(9.1%)	9	(22.5%)	
M, L	3	(100%)	30	(73.2%)	3	(100%)	50	(82.0%)	10	(90.9%)	31	(67.5%)	
Pathological T classification[a]													0.355
T1, T2	2	(66.7%)	7	(17.1%)	0	(0%)	15	(24.6%)	1	(9.1%)	8	(20.0%)	
T3, T4	1	(33.3%)	34	(82.9%)	3	(100%)	46	(75.4%)	10	(90.9%)	32	(80.0%)	
Pathological N classification[a]													0.936
N0	1	(33.3%)	8	(19.5%)	0	(0%)	14	(23.0%)	3	(27.3%)	9	(22.5%)	
N positive	2	(66.7%)	33	(80.5%)	3	(100%)	47	(77.0%)	8	(72.7%)	31	(67.5%)	
Pathological stage[a]													0.231
I, II	0	(0%)	28	(68.3%)	0	(0%)	21	(34.4%)	3	(27.3%)	15	(37.5%)	
III, IV	3	(100%)	13	(31.7%)	3	(100%)	40	(65.6%)	8	(72.7%)	25	(62.5%)	
Residual tumor[b]													0.194
R0	3	(100%)	34	(82.9%)	2	(66.7%)	40	(65.6%)	10	(90.9%)	27	(67.5%)	
R1	0	(0%)	7	(17.1%)	1	(33.3%)	21	(34.4%)	1	(9.1%)	13	(32.5%)	
Recurrence	0	(0%)	12	(29.3%)	2	(66.7%)	25	(41.0%)	4	(36.4%)	15	(37.5%)	0.542

Data are *n* (%) or median (interquartile range)

[a] The eighth cancer staging manual of the American Joint Committee on Cancer (AJCC)

[b] Japanese classification of gastric carcinoma, 3rd English edition

MSI-H, microsatellite instability high; MSS, microsatellite stability

Supplemental Table 2 Clinicopathological features of PCC subgroup according to *CDH1* mutation

	SRC (n=44)				combined (n=64)				PCC-NOS (n=51)				P value
	MT (n=11)		WT (n=33)		MT (n=19)		WT (n=45)		MT (n=12)		WT (n=39)		
Gender													0.926
Male	7	(63.6%)	23	(69.7%)	11	(57.9%)	27	(60.0%)	7	(58.3%)	26	(66.7%)	
Female	4	(36.4%)	10	(30.3%)	8	(42.1%)	18	(40.0%)	5	(41.7%)	13	(33.3%)	
Age, years (IQR)	65.0	(61.5–73.0)	65.0	(59.5–71.8)	62.0	(53.5–67.0)	70.0	(63.0–76.0)	72.5	(70.0–78.3)	71.0	(65.5–79.0)	0.022
Location													0.098
U	4	(36.4%)	7	(21.2%)	2	(10.5%)	9	(20.0%)	3	(25.0%)	7	(17.9%)	
M	7	(63.6%)	16	(48.5%)	8	(42.1%)	14	(31.1%)	5	(41.7%)	18	(46.2%)	
L	0	(0%)	10	(30.3%)	9	(47.4%)	22	(48.9%)	4	(33.3%)	14	(35.9%)	
Pathological T classification[a]													0.261
T1a	0	(0%)	0	(0%)	1	(5.3%)	2	(4.4%)	0	(0%)	0	(0%)	
T1b	0	(0%)	4	(12.1%)	0	(0%)	1	(2.2%)	0	(0%)	2	(5.1%)	
T2	0	(0%)	5	(15.2%)	3	(15.7%)	9	(20.0%)	0	(0%)	7	(17.9%)	
T3	4	(36.4%)	7	(21.2%)	4	(21.1%)	9	(20.0%)	2	(16.7%)	8	(20.5%)	

T4a	7	(63.6%)	16	(48.5%)	9	(47.4%)	20	(44.5%)	10	(83.3%)	20	(51.3%)	
T4b	0	(0%)	1	(3.0%)	2	(10.5%)	4	(8.9%)	0	(0%)	2	(5.1%)	
Pathological													0.723
N													
classification[a]													
N0	3	(27.3%)	6	(18.2%)	6	(31.6%)	8	(17.8%)	2	(16.7%)	10	(25.6%)	
N1	2	(18.2%)	3	(9.0%)	2	(10.5%)	7	(15.6%)	1	(8.3%)	7	(17.9%)	
N2	3	(27.3%)	9	(27.3%)	2	(10.5%)	10	(22.2%)	2	(16.7%)	11	(28.3%)	
N3a	1	(9.0%)	6	(18.2%)	4	(21.1%)	12	(26.6%)	2	(16.7%)	2	(5.1%)	
N3b	2	(18.2%)	9	(27.3%)	5	(26.3%)	8	(17.8%)	5	(41.6%)	9	(23.1%)	
CY													
Positive	2	(18.2%)	5	(15.2%)	4	(21.1%)	17	(37.8%)	5	(41.7%)	8	(20.5%)	0.168
Negative	9	(81.8%)	28	(74.8%)	15	(78.9%)	28	(62.2%)	7	(58.3%)	31	(79.5%)	
Pathological													0.206
stage[a]													
I	0	(0%)	5	(15.2%)	3	(15.8%)	6	(13.3%)	0	(0%)	3	(7.7%)	
II	4	(36.4%)	7	(21.2%)	3	(15.8%)	9	(20.0%)	1	(8.3%)	14	(35.9%)	
III	5	(45.4%)	15	(45.4%)	9	(47.3%)	13	(28.9%)	6	(50.0%)	14	(35.9%)	

IV	2	(18.2%)	6	(18.2%)	4	(21.1%)	17	(37.8%)	5	(41.7%)	8	(20.5%)	
Residual tumors[b]													0.056
R0	9	(81.8%)	28	(84.8%)	15	(78.9%)	27	(60.0%)	6	(50.0%)	31	(79.5%)	
R1	2	(18.2%)	5	(15.2%)	4	(21.1%)	18	(40.0%)	6	(50.0%)	8	(20.5%)	
Recurrence	4	(36.4%)	8	(24.2%)	7	(36.8%)	20	(44.4%)	9	(75.0%)	10	(25.6%)	0.024
Recurrence site													
Peritoneum	4	(36.4%)	5	(15.2%)	5	(26.3%)	15	(33.3%)	8	(66.7%)	7	(17.9%)	
Lymph node	0	(0%)	3	(9.1%)	2	(10.5%)	2	(4.4%)	1	(8.3%)	1	(2.6%)	
Hematogenous	0	(0%)	1	(3.0%)	2	(10.5%)	4	(8.9%)	0	(0%)	2	(5.1%)	
Local	0	(0%)	0	(0%)	0	(0%)	2	(4.4%)	0	(0%)	1	(2.6%)	
Others	0	(0%)	0	(0%)	0	(0%)	0	(0%)	0	(0%)	0	(0%)	
Die of gastric cancer	3	(27.3%)	5	(15.2%)	6	(31.6%)	17	(37.8%)	8	(66.7%)	10	(25.6%)	0.030

Data are *n* (%) or median (interquartile range)

[a] The eighth cancer staging manual of the American Joint Committee on Cancer (AJCC)

[b] Japanese classification of gastric carcinoma, 3rd English edition

WT, wild type; MT, mutated

Supplemental Table 3 Univariate and multivariate analyses of factors associated with disease-specific survival of non-solid dominant and mixed patients using Cox proportional hazards models

3a

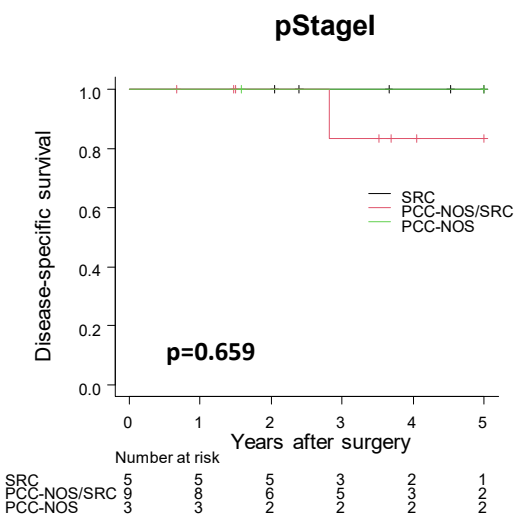
	Univariate			Multivariate		
	HR	**95%C.I.**	**P value**	**HR**	**95%C.I.**	**P value**
Age (≥ 75 vs. < 75)	1.329	0.696–2.538	0.388			
Gender (Male vs. Female)	0.522	0.283–0.964	0.038	0.500	0.269–0.930	0.029
BMI (≥ 25 vs. < 25)	0.492	0.176–1.382	0.178			
Tumor location (U vs. M/L)	3.045	1.557–5.955	0.001	2.697	1.364–5.331	0.004
Pathological stage (III, IV vs. I, II)	8.735	2.693–28.33	<0.001	8.480	2.604–27.62	<0.001
Adjuvant chemotherapy (Yes vs. No)	0.983	0.516–1.876	0.959			
***CDH1* mutation (Yes vs. No)**	1.864	0.975–3.566	0.060			

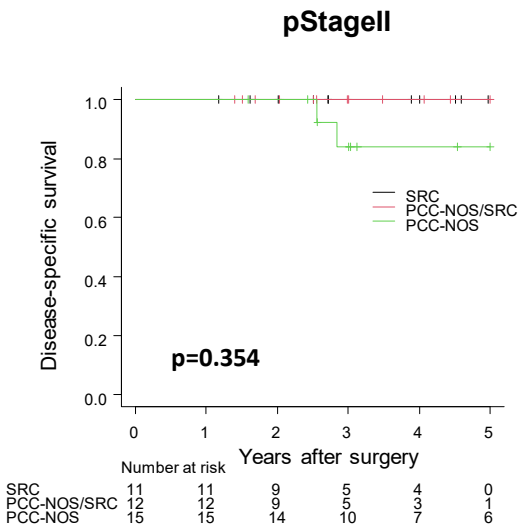
3b

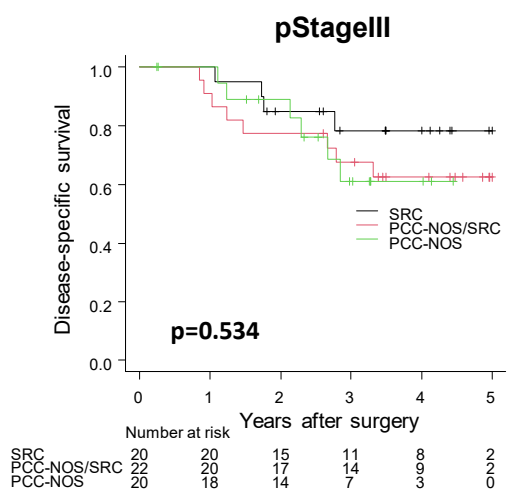
	Univariate			Multivariate		
	HR	**95%C.I.**	**P value**	**HR**	**95%C.I.**	**P value**
Age (≥ 75 vs. < 75)	1.329	0.696–2.538	0.388			
Gender (Male vs Female)	0.522	0.283–0.964	0.038	0.511	0.275–0.950	0.034
BMI (≥ 25 vs. < 25)	0.492	0.176–1.382	0.178			
Tumor location (U vs. M/L)	3.045	1.557–5.955	0.001	2.213	1.086–4.513	0.029
Pathological stage (III, IV vs. I, II)	8.735	2.693–28.33	<0.001	8.028	2.453–26.27	<0.001
Adjuvant chemotherapy (Yes vs. No)	0.983	0.516–1.876	0.959			
EC1 domain of *CDH1* mutation (Yes vs. No)	3.267	1.594–6.697	0.001	2.118	1.011–4.436	0.046

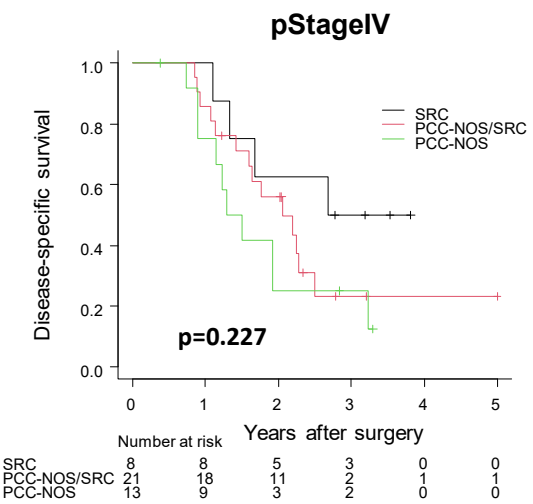
HR, hazard ratio; CI, confidence interval

補足Fig.1

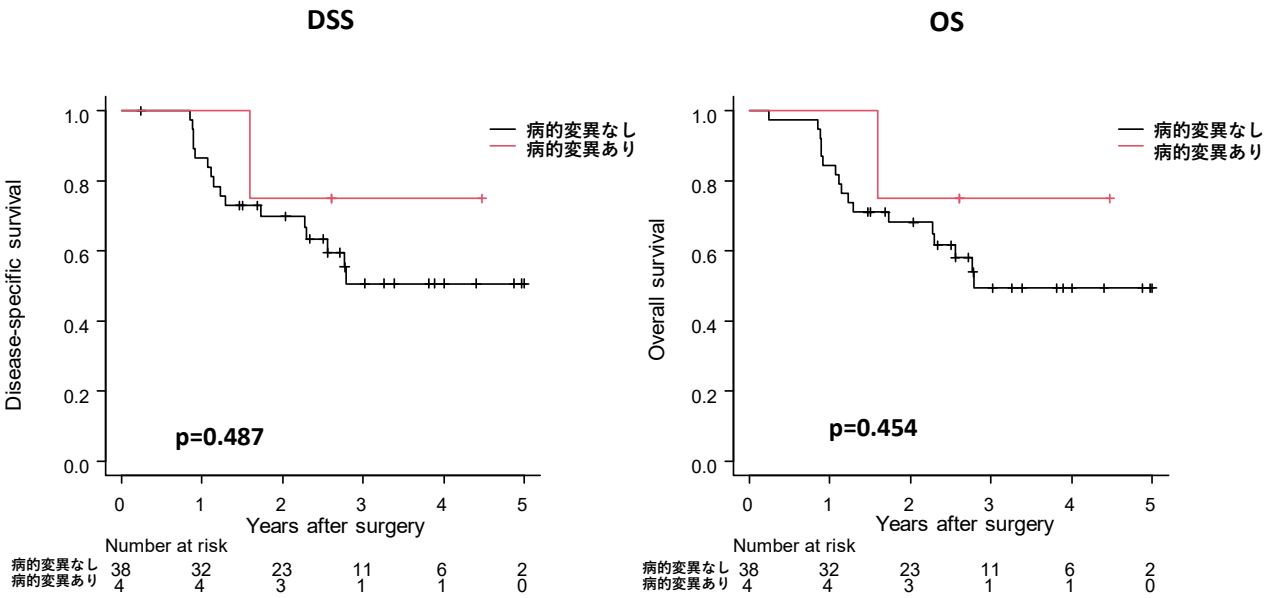

pStageI
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
SRC
PCC-NOS/SRC
PCC-NOS
p=0.659
0
1
2
3
4
5
Years after surgery
Number at risk
SRC 5 5 5 3 2 1
PCC-NOS/SRC 9 8 6 5 3 2
PCC-NOS 3 3 2 2 2 2


pStageII
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
SRC
PCC-NOS/SRC
PCC-NOS
p=0.354
0
1
2
3
4
5
Years after surgery
Number at risk
SRC 11 11 9 5 4 0
PCC-NOS/SRC 12 12 9 5 3 1
PCC-NOS 15 15 14 10 7 6

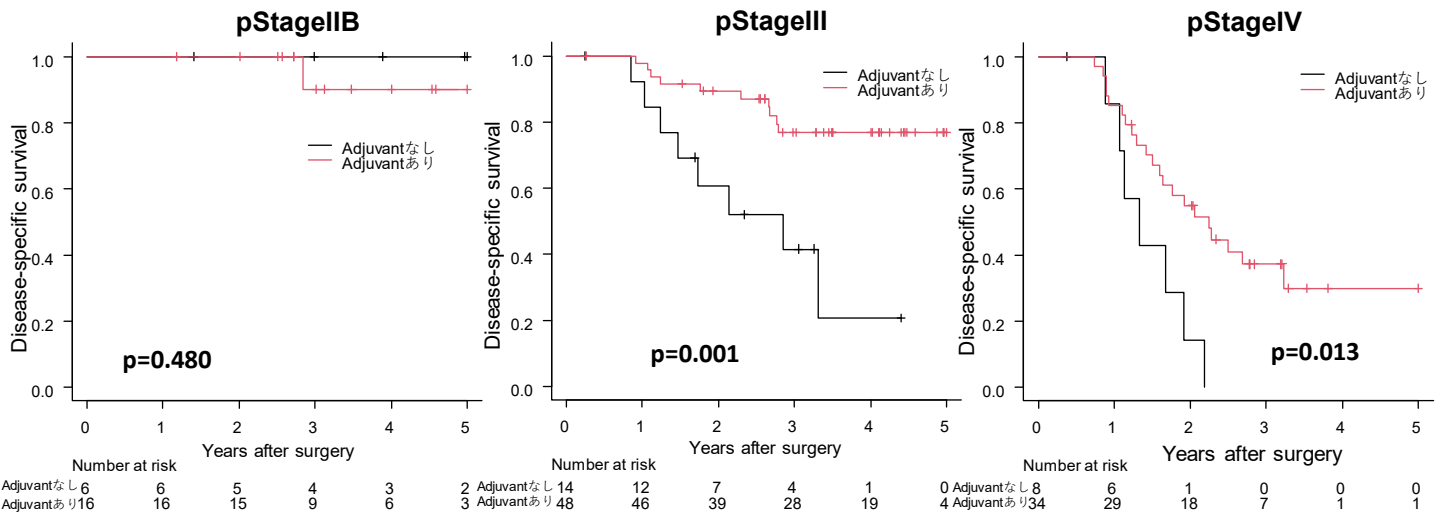

pStageIII
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
SRC
PCC-NOS/SRC
PCC-NOS
p=0.534
0
1
2
3
4
5
Years after surgery
Number at risk
SRC 20 20 15 11 8 2
PCC-NOS/SRC 22 20 17 14 9 2
PCC-NOS 20 18 14 7 3 0


pStageIV
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
SRC
PCC-NOS/SRC
PCC-NOS
p=0.227
0
1
2
3
4
5
Years after surgery
Number at risk
SRC 8 8 5 3 0 0
PCC-NOS/SRC 21 18 11 2 1 1
PCC-NOS 13 9 3 2 0 0

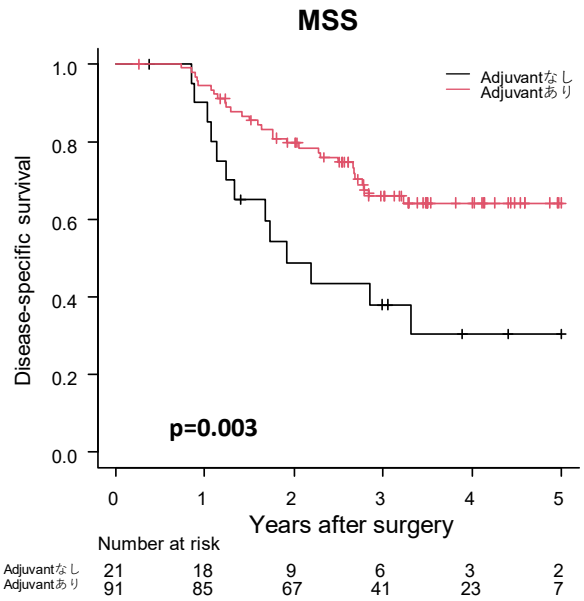
補足Fig.2

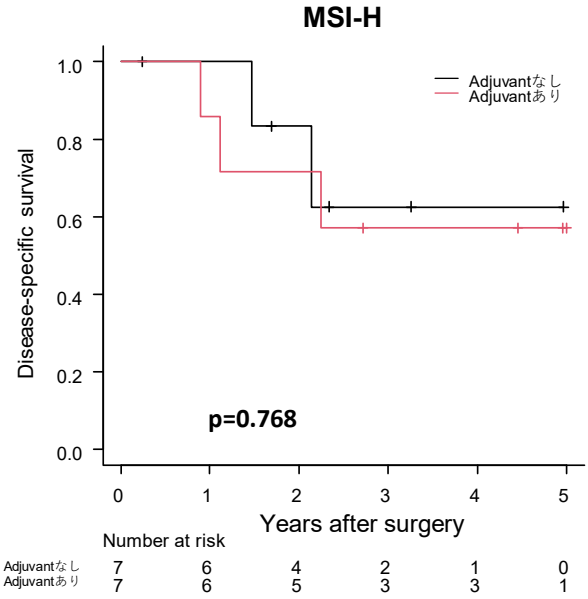

DSS
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
病的変異なし
病的変異あり
p=0.487
0
1
2
3
4
5
Years after surgery
Number at risk
病的変異なし 38 32 23 11 6 2
病的変異あり 4 4 3 1 1 0
OS
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
病的変異なし
病的変異あり
p=0.454
0
1
2
3
4
5
Years after surgery
Number at risk
病的変異なし 38 32 23 11 6 2
病的変異あり 4 4 3 1 1 0

補足Fig.3


pStageIIB
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Adjuvantなし
Adjuvantあり
p=0.480
0
1
2
3
4
5
Years after surgery
Number at risk
Adjuvantなし 6 6 5 4 3 2
Adjuvantあり 16 16 15 9 6 3
pStageIII
Disease-specific survival
Adjuvantなし
Adjuvantあり
p=0.001
Years after surgery
Number at risk
Adjuvantなし 14 12 7 4 1 0
Adjuvantあり 48 46 39 28 19 4
pStageIV
Disease-specific survival
Adjuvantなし
Adjuvantあり
p=0.013
Years after surgery
Number at risk
Adjuvantなし 8 6 1 0 0 0
Adjuvantあり 34 29 18 7 1 1

補足Fig.4


MSS
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Adjuvantなし
Adjuvantあり
p=0.003
0
1
2
3
4
5
Years after surgery
Number at risk
Adjuvantなし 21 18 9 6 3 2
Adjuvantあり 91 85 67 41 23 7


MSI-H
Disease-specific survival
1.0
0.8
0.6
0.4
0.2
0.0
Adjuvantなし
Adjuvantあり
p=0.768
0
1
2
3
4
5
Years after surgery
Number at risk
Adjuvantなし 7 6 4 2 1 0
Adjuvantあり 7 6 5 3 3 1

補足Table1　CDH1変異42人の臨床病理学的因子

	SRC (n=11)	PCC-NOS/SRC (n=19)	PCC-NOS (n=12)	p値
年齢 (範囲)	65 (49–85)	62 (23–81)	72.5 (31–84)	0.040
性別				1.000
男性	7 [63.6]	11 [57.9]	7 [58.3]	
女性	4 [36.4]	8 [42.1]	5 [41.7]	
占居部位				0.051
U	4 [36.4]	2 [10.5]	3 [25.0]	
M	7 [63.6]	8 [42.1]	5 [41.7]	
L	0 [0]	9 [47.4]	4 [33.3]	
pT				0.387
T1a	0 [0]	1 [5.2]	0 [0]	
T1b	0 [0]	0 [0]	0 [0]	
T2	0 [0]	3 [15.8]	0 [0]	
T3	4 [36.4]	4 [21.1]	2 [16.7]	
T4a	7 [63.6]	9 [47.4]	10 [83.3]	
T4b	0 [0]	2 [10.5]	0 [0]	
pN				0.879
N0	3 [27.3]	6 [31.6]	2 [16.7]	
N1	2 [18.2]	2 [10.5]	1 [8.3]	
N2	3 [27.3]	2 [10.5]	2 [16.7]	
N3a	1 [9.0]	4 [21.1]	2 [16.7]	
N3b	2 [18.2]	5 [26.3]	5 [41.6]	
pStage				0.403
I	0 [0]	3 [15.8]	0 [0]	
II	4 [36.4]	3 [15.8]	1 [8.3]	
III	5 [45.5]	9 [47.4]	6 [50.0]	
IV	2 [18.2]	4 [21.1]	5 [41.7]	
腫瘍遺残				0.175
R0	9 [81.8]	15 [78.9]	6 [50.0]	
R1	2 [18.2]	4 [21.1]	6 [50.0]	
CY				0.440
CY0	9 [81.8]	15 [78.9]	7 [58.3]	
CY1	2 [18.2]	4 [21.1]	5 [41.7]	

補足Table2
CDH1変異42人のEC1変異と臨床病理学的因子との相関

	EC1変異あり (n=20)		EC1変異なし (n=22)		p値
pStage					0.965
I	1	[5.0]	2	[9.1]	
II	4	[20.0]	4	[18.2]	
III	9	[45.0]	11	[50.0]	
IV	6	[30.0]	5	[22.7]	
CY					0.730
CY0	14	[70.0]	17	[77.3]	
CY1	6	[30.0]	5	[27.3]	
腫瘍遺残					0.499
R0	13	[65.0]	17	[77.3]	
R1	7	[35.0]	5	[27.3]	
再発					0.062
あり	13	[65.0]	7	[31.8]	
なし	7	[35.0]	15	[68.2]	