Potential neuroendocrine differentiation in poorly differentiated colorectal adenocarcinoma: A hidden trait?

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Abstract. Neuroendocrine carcinoma (NEC) of the colon and rectum is a rare malignancy with a poor prognosis that is characterized by distinct clinical and histopathological features that differ significantly from those of more prevalent adenocarcinomas. Poorly differentiated colorectal adenocarcinoma (PDC) is also rare and carries a poor prognosis. Considering the morphological similarities between these two rare, poorly differentiated cancers of the colon and rectum, it is plausible that certain cases of colorectal cancer (CRC) diagnosed as PDC may contain NEC as well. In the present study, cases of CRC that were diagnosed as PDC at our institution were investigated, searching for patients who exhibited NEC characteristics based on the expression of neuroendocrine markers (NEMs), including chromogranin A, synaptophysin and insulinoma-associated 1 (INSM1), and the loss of retinoblastoma 1 (Rb). Of 816 total CRC cases, 74 cases (9.1%) were identified as PDC. These were further divided into 13 (17.5%) cases that were positive for NEMs and others. Of these 13 cases, the expression rates for chromogranin A and synaptophysin were 69.2% each, while that of INSM1 was 100%. Upon re-examination of the 13 PDC cases, two cases were morphologically identified as NEC, including one large- and one small-cell NEC. A total of two cases showed loss of Rb in their PDC lesions. NEM positivity was considered an independent prognostic factor in the 74 PDC cases. Among these cases, some may exhibit characteristics of NEC. Unraveling the molecular mechanisms using CRC that harbors both PDC and NEC will be a task for future research.

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Introduction

Neuroendocrine carcinoma (NEC) of the colon and rectum is rare. The reported incidence of NEC in these regions ranges from <0.6% to as high as 5% (1,2). NEC is characterized as an epithelial cancer that is distinguished by the expression of neuroendocrine markers (NEMs), such as chromogranin A (CgA), synaptophysin (Syn), and insulinoma-associated 1 (INSM1) (3). The 2019 World Health Organization (WHO) update on colorectal cancer (CRC) classification emphasized that NEC of the colon and rectum is distinctly classified as a high-grade, poorly differentiated NEC, which is distinct from low-grade grade 3 neuroendocrine tumors (NETs) (4). NEC of the colon and rectum has been reported to have a poor prognosis (1). On the other hand, the most common histological type of CRC is adenocarcinoma, which accounts for ~90% of cases (5). However, the majority of these are low-grade cases of well-differentiated adenocarcinoma (WDC) and moderately differentiated adenocarcinoma (MDC). Poorly differentiated adenocarcinoma (PDC), corresponding to the high grade of NEC, is also a rare histological type of CRC, with an incidence rate of 3.3-18% in Japan (6,7).

Considering the morphological similarities between these two rare, poorly differentiated cancers of the colon and rectum (i.e., NEC and PDC), it is plausible that certain colorectal adenocarcinomas with poor prognoses that contain PDC components may have morphological or biomarker-related similarities to NEC. The retinoblastoma 1 (Rb) protein is a tumor suppressor that is frequently dysfunctional across numerous cancer types. Loss of Rb, which is detected in approximately half of pancreatic NECs, is considered a hallmark of NEC. To elucidate the clinicopathological features and clinical outcomes of colorectal NECs, as well as enhance the current understanding of this disease, cases of CRC diagnosed as PDC at our institution were investigated and those that exhibited NEC-like characteristics, such as the expression of certain NEMs and the loss of Rb, were analyzed.

Materials and methods

Patients and clinical data collection. Between January 2009 and December 2019, a total of 816 patients underwent CRC

resection surgery at the Department of Gastroenterological Surgery of Yokohama City University Hospital (Yokohama, Japan). Cases of CRC that exhibited PDC, either wholly or in part, were selected based on pathological diagnoses that were confirmed by two independent pathologists. This study retrospectively analyzed clinical data from a total of 74 diagnosed PDC cases. The reviewed data included variables, such as age at diagnosis, sex, histology, lymph node metastasis, clinical stage and curability. Tumor locations were classified into right-sided colon (cecum, ascending colon, transverse colon and appendix) and left-sided colon (descending colon, sigmoid colon, rectum and anus). All patients underwent clinical evaluation at the hospital and received appropriate management. Follow-up information was secured for all 74 cases. The present study was approved by the Ethical Review Board of Yokohama City University (Yokohama, Japan; approval no.: B200700086).

Immunohistochemistry (IHC) staining. Tumor tissues from the 74 PDC cases were formalin-fixed and paraffin-embedded. The resultant paraffin blocks were sectioned to a thickness of 4 μm for IHC staining. The sections were stained with antibodies against CgA (1:400 dilution; cat. no. ab15160; Abcam), Syn (1:200 dilution; cat. no. ab14692; Abcam), INSM1 (1:400 dilution; clone A-8; cat. no. sc-271408; Santa Cruz Biotechnology, Inc.), Rb (1:800 dilution; cat. no. ab181616; Abcam) and Ki-67 (1:50 dilution; clone MIB-1; cat. no. m7240; Dako; Agilent Technologies, Inc.). All sections were incubated overnight at 4˚C with diluted primary antibodies in PBS, and PBS was used to replace the primary antibody as a negative control. Anti-mouse IgG or anti-rabbit IgG [ready to use; Histofine SAB-PO (M) or (R) kit; Nichirei Biosciences Inc.] were used as secondary antibodies and were incubated at room temperature (20-25˚C). Diaminobenzidine was used as the chromogen. The sections were examined and photographed using a microscope (BX41; Olympus Corp.). For each case, three representative regions were randomly selected. Within each, three high-power fields (magnification, x400) were then randomly selected before the staining was evaluated by ImageJ (version. 1.53k; National Institutes of Health). In the present study, cases were classified as NEM-positive if they were positive for at least one NEM. Any PDC cases that expressed NEMs were re-evaluated by a pathologist (IK) with >17 years of experience in terms of their morphological features, to determine whether NEC was indeed present. All slices were deparaffinized and stained with hematoxylin and eosin (H&E) in advance according to the established protocol (8).

Statistical analysis. Statistical analyses were performed using the IBM SPSS Statistics software version 29.0 (IBM Corp.). Clinical and pathological characteristics were compared using Mann-Whitney U, Pearson's Chi-squared and Fisher's exact tests, as appropriate. Univariate and multivariate analyses were performed to identify prognostic factors. A Cox proportional hazards model was utilized to calculate hazard ratios, assessing the risk of mortality between groups. Statistical significance was set at $P<0.05$.

Results

Clinicopathological patient characteristics. Of 816 total CRC cases, 74 (9.1%) were identified as PDC. These were further divided into 13 that were positive for NEMs and 61 that were negative, based on the IHC staining results. The details of these 74 cases are presented in Table I. The median age of the patients with PDC was 68 years (range, 28-89 years). NEM-negative cases were more frequently observed among older patients ($P=0.007$). No significant differences were observed in terms of sex distribution among the patients. Primary tumors in the cecum, ascending colon, transverse colon and appendix were classified as right-sided colon (35.1%), whereas those in the descending colon, sigmoid colon, rectum and anus were classified as left-sided colon (64.9%). No significant differences were observed in terms of tumor location. In only eight cases (10.8%), the majority of the tumors consisted of PDC. In the remaining 66 cases (89.2%), WDC or MDC was predominant, with only portions of tumors exhibiting PDC. Lymph node metastasis was observed in 23 patients, including 10 that were NEM-positive. A statistically significant difference in lymph node metastasis was noted between NEM-positive and NEM-negative patients ($P<0.001$). Staging distribution was as follows: One patient (1.4%) was classified as stage I, 19 (25.7%) as stage II, 39 (52.7%) as stage III and 15 (20.2%) as stage IV. Regarding curability, 63 patients (85.1%) underwent R0 and R1 resections, while 11 patients (14.9%) underwent R2 resections. No statistically significant differences were observed in terms of resection rates.

IHC of NEMs. Among the 74 cases, 13 (17.5%) were NEM-positive, including four cases with diffuse staining and nine cases with focal staining. Representative images of the immunostaining for each are provided in Fig. 1. The summary of clinicopathological characteristics for the 13 cases classified as NEM-positive is presented in Table II. Among the 13 NEM-positive cases, PDC was primarily identified, accounting for 84.6% of these cases. The majority of patients (76.9%) were aged <68 years and 69.2% cases exhibited high proliferation rates (Ki-67 index >55%). Of note, two cases showed a loss of Rb. The detailed histopathological characteristics and IHC findings of these 13 cases are summarized in Table SI. Following re-examination of the 13 NEM-positive PDC sites, two cases were morphologically identified as NEC, including one large cell NEC (LCNEC) and one small cell NEC (SCNEC). H&E staining for these cases is shown in Fig. 2. The expression rates of CgA and Syn were 69.2% (9/13) each, while that of INSM1 reached 100% (13/13). All patients exhibited a Ki-67 index of >20%. Of the 13 cases, 10 had lymph node metastases, of which only one case was positive for NEMs within the lymph node metastases (Fig. 3). Liver metastasis was obtained from only one case and the sample tested negative for NEMs.

IHC of Rb. A total of two cases showed loss of Rb in PDC lesions (cases 1 and 2 in Table SI). Case 1 was a pure PDC with both NEM-positive and NEM-negative areas (Fig. 4). Of note, there was loss of Rb in the NEM-positive areas, whereas it remained positive in the NEM-negative ones. Case 2 had PDC with a predominant MDC area. Upon re-examination by a pathologist, the PDC area was reclassified as LCNEC. All three NEMs tested negative in the MDC area. Conversely, CgA expression was negative in the PDC area, whereas Syn and INSM1 were strongly positive (Fig. 5). Loss of Rb was detected in the PDC area, while Rb remained positive in the MDC area.

Table I. Clinicopathological characteristics of the cases classified as PDC (n=74).

Item	Total	NEMs+ (n=13)	NEMs- (n=61)	P-value
Age, years	68 (28-89)	60 (28-84)	70 (39-89)	0.007
Sex				0.602
Male	35 (47.3)	7 (53.8)	28 (45.9)	
Female	39 (52.7)	6 (46.2)	33 (54.1)	
Tumor location				>0.999
Right-sided colon	26 (35.1)	5 (38.5)	21 (34.4)	
Cecum	7 (9.5)	0 (0)	7 (11.5)	
Ascending colon	11 (14.9)	3 (23.1)	8 (13.1)	
Transverse colon	7 (9.5)	2 (15.4)	5 (8.2)	
Appendix	1 (1.4)	0 (0)	1 (1.6)	
Left-sided colon	48 (64.9)	8 (61.5)	40 (65.6)	
Descending colon	3 (4.1)	0 (0)	3 (4.9)	
Sigmoid colon	14 (18.9)	1 (7.7)	13 (21.3)	
Rectum	25 (33.8)	5 (38.5)	20 (32.8)	
Anus	6 (8.1)	2 (15.4)	4 (6.6)	
Histology				
PDC	8 (10.8)	1 (7.7)	7 (11.5)	>0.999
WDC>PDC	4 (5.4)	0 (0)	4 (6.6)	
MDC>PDC	44 (59.5)	9 (69.2)	35 (57.4)	
WDC+MDC>PDC	18 (24.3)	3 (23.1)	15 (24.6)	
Lymph node metastases				<0.001
+	23 (31.1)	10 (76.9)	13 (21.3)	
-	51 (68.9)	3 (23.1)	48 (78.7)	
Stage				0.761
I	1 (1.4)	0 (0)	1 (1.6)	
II	19 (25.7)	2 (15.4)	17 (27.9)	
III	39 (52.7)	8 (61.5)	31 (50.8)	
IV	15 (20.3)	3 (23.1)	12 (19.7)	
Curability				>0.999
R0, 1	63 (85.1)	11 (84.6)	52 (85.2)	
R2	11 (14.9)	2 (15.4)	9 (14.8)	

Values are expressed as n (%) or the median (range). PDC, poorly differentiated adenocarcinoma; WDC, well-differentiated adenocarcinoma; MDC, moderately differentiated adenocarcinoma; NEMs, neuroendocrine markers; NEMs+, NEM-positive; NEMs-, NEM-negative.

Prognostic factor analysis. Table III presents the results of uni- and multivariate analyses for the 74 CRC cases using clinical factors. In the univariate analysis, stage and curability emerged as potentially significant prognostic markers. NEM-positivity did not reach statistical significance (P=0.075). The multivariate analysis, incorporating significant markers from the univariate one, as well as NEM-positivity status, identified curability (P<0.0001) and NEM-positivity (P=0.017) as significant independent prognostic markers.

Discussion

In the present study, it was found that 17.6% of CRC tumors classified as PDC exhibited NEM expression, which represents a necessary condition for diagnosing NEC. In addition, 15.4% of these cases also showed Rb loss, which is an important feature of NEC. This suggests that, among CRC tumors that are morphologically classified as PDC, there may be cases that exhibit NEC characteristics as well.

According to the WHO classification of tumors, 5th edition, epithelial malignancies of the colon and rectum may be broadly classified into three types: Adenocarcinoma, neuroendocrine neoplasm (NEN) and mixed tumors that contain both (9). NEN can be further classified into NETs and NECs. The histological macro-classifications of epithelial malignancies of the colon and rectum are, therefore, adenocarcinoma, NET and NEC.

Adenocarcinomas represent the majority of CRC tumors, which may be divided into several distinct morphologic variants, >90% of which are WDCs or MDCs. According to the Multi-Institutional Registry of Large Bowel Cancer in Japan (10), ~95% of CRCs are adenocarcinomas. Among these, 93.5% are WDCs or MDCs. PDCs account for only

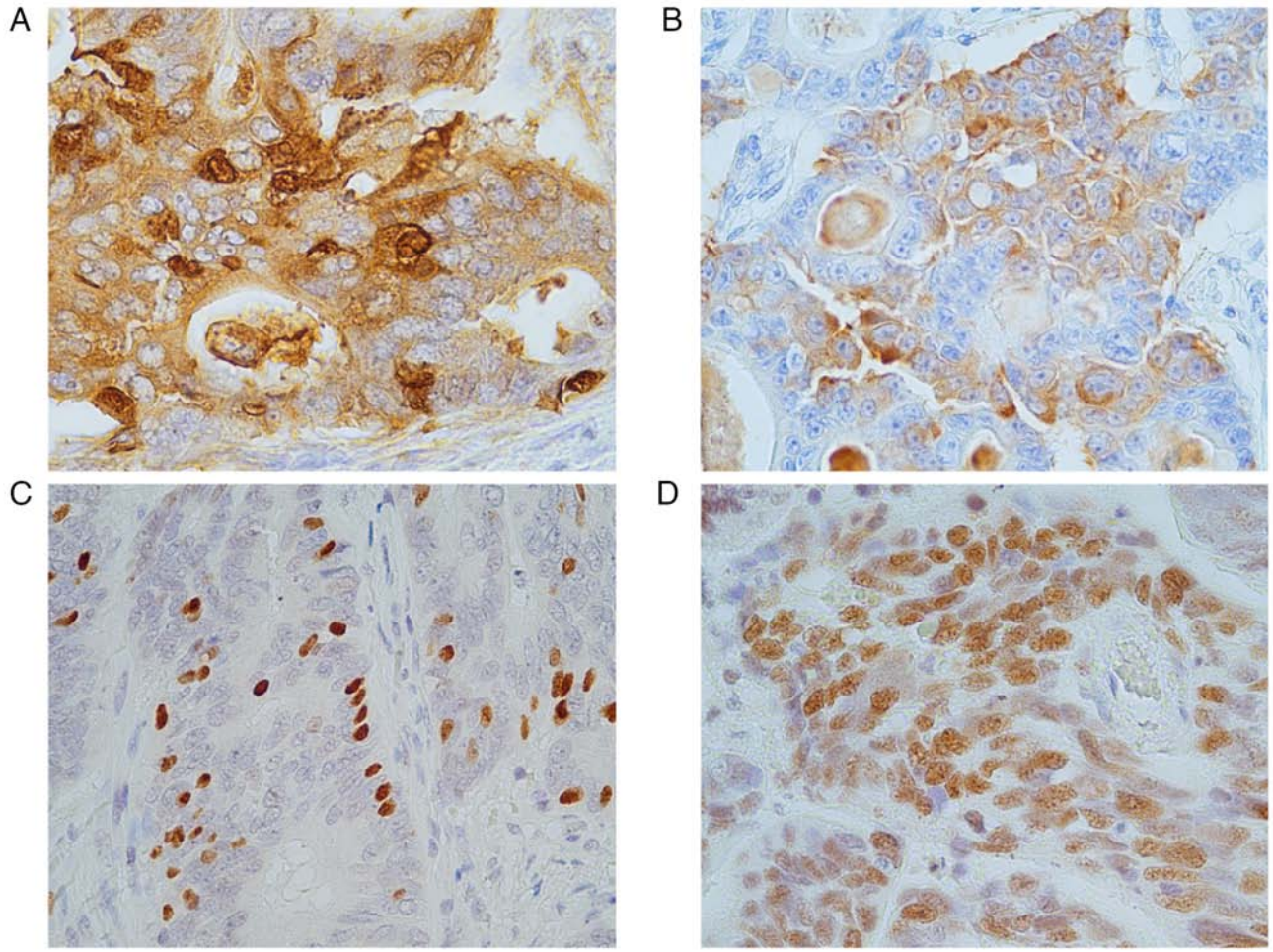

Figure 1. Representative images for each immunostain. (A) CgA; (B) Syn; (C) INSM1; and (D) Rb (magnification, x400). Cases were stained as either focal or diffuse-positive for cytoplasmic staining of tumor cells with CgA and Syn antibodies, while the staining reactions for INSM1 and Rb appeared in the cellular nuclei. CgA, chromogranin A; Syn, synaptophysin; INSM1, insulinoma-associated 1; Rb, retinoblastoma 1.

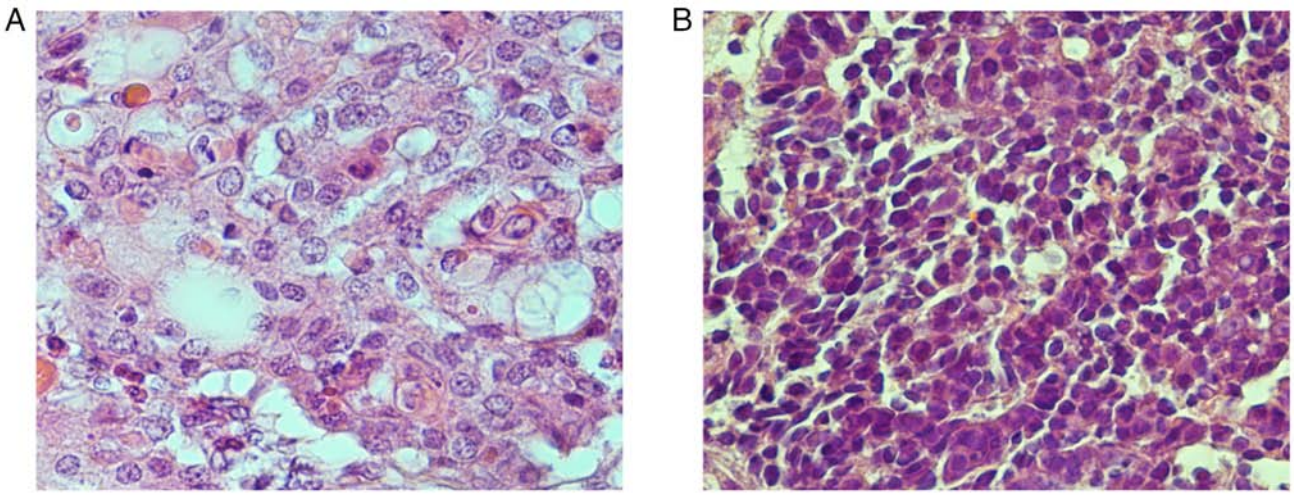

Figure 2. Histology images of two cases that were re-diagnosed as NEC based on re-examinations of the morphologies of their poorly differentiated adenocarcinoma sites, which were positive for neuroendocrine markers. (A) Large cell NEC (case 2 in Table SI). (B) small cell NEC (case 6 in Table SI) (H&E staining; magnification, x400). NEC, neuroendocrine carcinoma.

3.3% of all CRCs in Japan. In the present study, pure PDC was found in only eight cases (~1%). Ueno *et al* (11) indicated that PDC components can at times be found even within WDCs or MDCs and that even a small amount of PDC can impact the prognosis of CRC. Therefore, the present study included cases wherein only portions of the tumors exhibited

Table II. Clinicopathological characteristics of 13 cases classified as NEM-positive.

Clinicopathological characteristics	Total (n=13)
Age, years	
>68	3 (23.1)
<68	10 (76.9)
Morphological findings after re-examination	
PDC	11 (84.6)
LCNEC	1 (7.7)
SCNEC	1 (7.7)
NEMs	
CgA	
+	9 (69.2)
-	4 (30.8)
Syn	
+	9 (69.2)
-	4 (30.8)
INSM1	
+	13 (100)
-	0 (0)
Staining pattern	
Diffuse	4 (30.8)
Focal	9 (69.2)
Rb	
+	11 (84.6)
-	2 (15.4)
Ki-67, %	
>55	9 (69.2)
20-55	4 (30.8)
<20	0 (0)
NEMs of L/N meta	
CgA	
+	1 (7.7)
-	9 (69.2)
N/A	3 (23.1)
Syn	
+	0 (0)
-	10 (76.9)
N/A	3 (23.1)
INSM1	
+	1 (7.7)
-	9 (69.2)
N/A	3 (23.1)

Values are expressed as n (%). PDC, poorly differentiated adenocarcinoma; LCNEC, large cell neuroendocrine carcinoma; SCNEC, small cell neuroendocrine carcinoma; NEMs, neuroendocrine markers; CgA, chromogranin A; Syn, synaptophysin; INSM1, insulinoma-associated 1; Rb, retinoblastoma 1; L/N meta, lymph node metastases; N/A, not applicable.

PDC in order to analyze all sites with morphological PDC presentation.

According to the WHO classification of NENs from 2022 (3), NENs can be divided into two categories: Well-differentiated and poorly differentiated. Well-differentiated NENs are NETs including G1, G2 and G3 grades, while poorly differentiated NENs are NECs. Originally, in the 2010 WHO classification (12), NETs were classified into three categories (G1, G2 and NEC) based on cell proliferation. The main issue with this classification was that when the Ki-67 labeling index exceeds 20%, it becomes difficult to distinguish between NET-G3s and NECs (13). In the 2017 WHO classification (14), a solution to this issue was proposed for pancreatic NENs (pNENs) specifically by categorizing NET-G3s as well-differentiated NENs and NECs as poorly differentiated NENs. In the 2019 WHO classification, this categorization was expanded from pNENs to gastroenteropancreatic (GEP) NENs (15). Currently, NEC is positioned as a poorly differentiated cancer within the NEN category and serves as the counterpart to PDC in adenocarcinomas.

NECs are malignant tumors that can occur throughout the body. According to data from the SEER study (16), ~90% occur in the lungs and GEP-NECs account for ~4.2%. Among GEP-NECs, the colon represents the most common site, accounting for 29%. However, NECs of the colon and rectum are rare. NECs are typically classified as SCNECs or LCNECs. SCNECs are considered sufficiently distinctive for histological diagnosis, whereas it is often difficult to distinguish LCNECs from PDCs based solely on morphology (17,18). Furthermore, in the lungs, where the majority of cases occur, distinguishing SCNECs from LCNECs may at times be difficult, leading to misdiagnosis (19). However, distinguishing between PDCs and NECs based solely on morphology can be challenging. NECs may be present in certain patients with CRC who are diagnosed as PDC. In the present study, two cases of NEM-positive PDC were considered morphologically likely to be NECs after re-examination. In one other case, NEC was suspected based on morphology; however, because it was NEM-negative, the diagnosis remained PDC (data not shown). In the present study, three cases in which morphological distinction between PDC and NEC was difficult were also observed; however, this was a low percentage (4%).

The simplest method to differentiate NENs is to confirm NEM expression. According to the 2022 WHO classification, Syn, CgA and INSM1 are considered appropriate antibodies for NEMs. Syn has high sensitivity but low specificity, whereas CgA has high specificity but low sensitivity. INSM1, however, has high sensitivity as well as specificity (3). In the present study, out of the 13 patients who tested positive for NEMs, seven (53.8%) tested positive for all three antibodies. Furthermore, two patients (15.4%) tested positive for only one antibody and only INSM1 was positive in both instances. INSM1 was the only antibody that was positive in all 13 cases. The present results also suggest that INSM1 has the highest sensitivity for detecting NEC features.

Ki-67 and Rb are also important factors in the characterization of NENs. Ki-67 is an important factor in NET grading. A Ki-67 labeling index of ≥20% serves as the diagnostic criterion for NET-G3 or NEC. According to the 2022 WHO classification (3), Ki-67 is often ≥55% in NEC, whereas it is typically

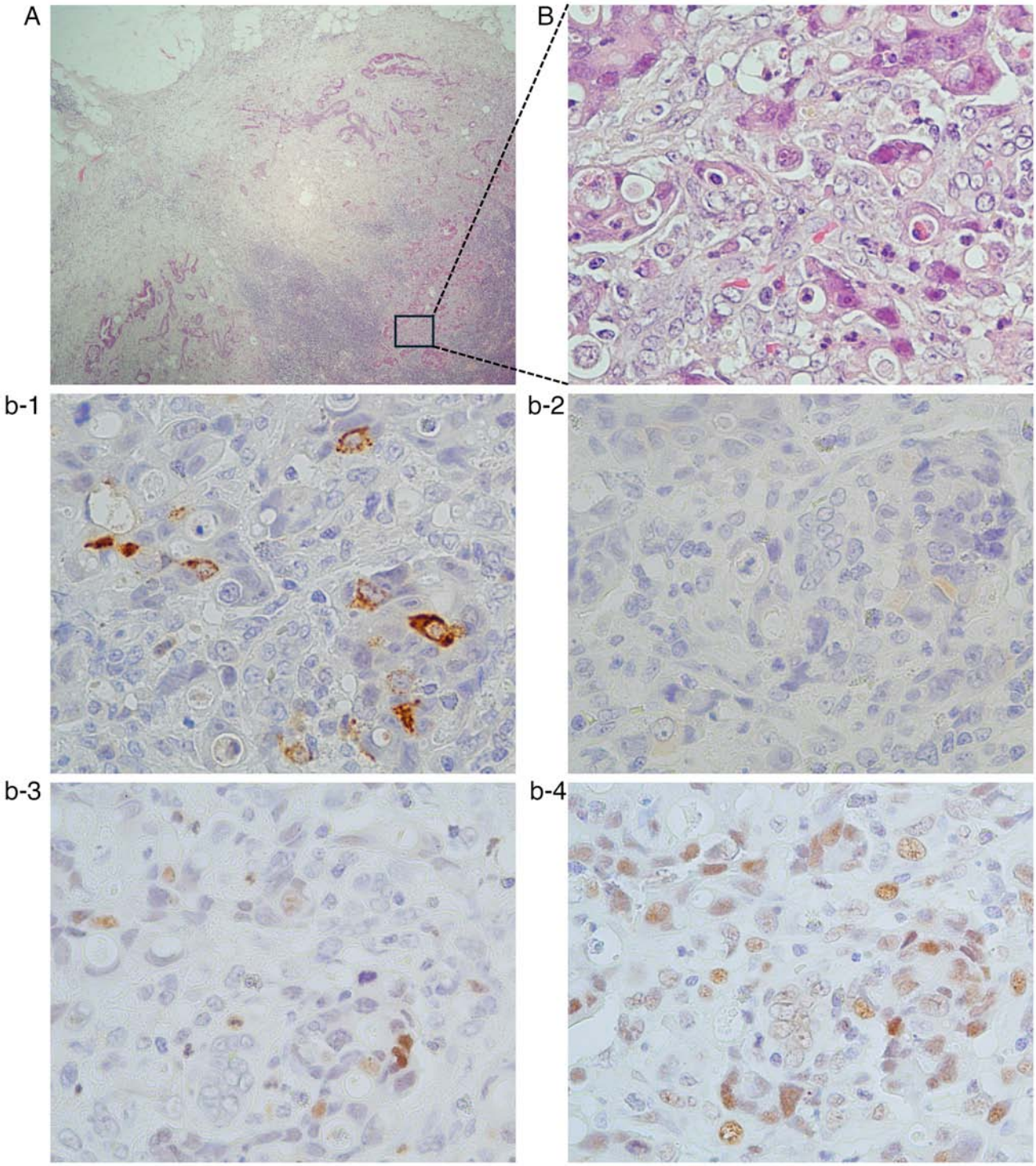

Figure 3. Analysis of metastatic lymph node (case 7 in Table SI). (A) Metastatic lymph node (H&E staining; magnification, x40) and (B) the PDC area (H&E staining). b-1 - 4 were the corresponding areas in B. Immunohistochemistry indicated that (b-1) CgA was clearly positive, (b-2) Syn was negative, (b-3) INSM1 was weakly positive and (b-4) Rb was clearly positive (magnification, x400). PDC, poorly differentiated adenocarcinoma; CgA, chromogranin A; Syn, synaptophysin; INSM1, insulinoma-associated 1; Rb, retinoblastoma 1..

lower in NET-G3. A Ki-67 level of 55% as a cut-off was proposed in the Nordic NEC study, which focused on NECs with Ki-67 labeling indices of >20%. It has been shown that NECs with Ki-67 indexes of ≥55% have poor prognoses but are highly sensitive to platinum-based chemotherapy. On the other hand, NECs with Ki-67 indexes of <55% do not respond to platinum-based chemotherapy, but have much better prognoses (20). In typical CRCs, the median Ki-67 labeling index is ~40% (21), with ~40% having Ki-67 indexes of ≤50% (22). In the present study, the Ki-67 labeling index of NEM-positive areas was >55% in 9 cases, many of which met the criteria for NEC.

The tumor suppressor gene Rb is known to cause cancer when inactivated. Inactivation of Rb occurs at a high rate in small-cell lung cancer, with reports of 60% (23) and 89% (24). Similarly, inactivation also occurs in ~50% of GEP-NECs (25). Loss of Rb is an important feature of NEC that can be used

Table III. Prognostic factor analysis of 74 PDC cases.

	Univariate	Multivariate	
Factor	P-value	P-value	HR (95% CI)
Sex (male vs. female)	0.253		
Age (<68 vs. >68 years)	0.408		
Predominant histology (PDC vs. MDC and/or WDC)	0.360		
Stage (I/II vs. III/IV)	0.005	0.109	1.604 (0.895-2.996)
Curability (R0, 1 vs. R2)	<0.0001	<0.0001	7.072 (2.667-18.762)
CEA (<6 vs. >6 ng/ml)	0.053	0.693	0.999 (0.992-1.005)
NEMs+ (yes vs. no)	0.075	0.017	3.135 (1.231-7.981)

HR, hazard ratio; PDC, poorly differentiated adenocarcinoma; WDC, well-differentiated adenocarcinoma; MDC, moderately differentiated adenocarcinoma; NEMs, neuroendocrine markers; NEMs+, NEM-positive.

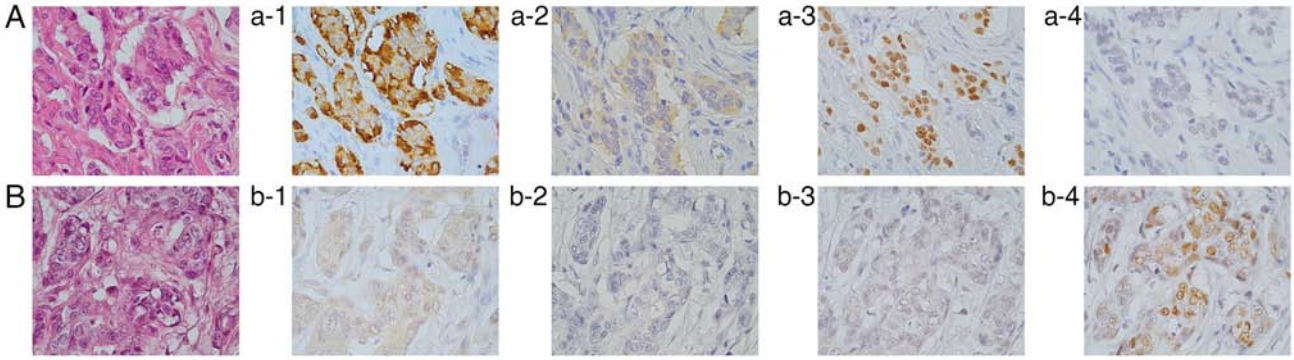

Figure 4. Case of Rb loss (case 1 in Table SI). Histology of (A) NEM-positive and (B) NEM-negative area (H&E staining). Immunohistochemistry of (a-1-4) the NEM-positive and (b-1-4) the NEM-negative areas. (a-1) CgA, (a-2) Syn and (a-3) INSM1 showed strong positive staining. (a-4) Rb was clearly negative. (b-1) CgA, (b-2) Syn and (b-3) INSM1 were clearly negative. (b-4) Rb was clearly positive (magnification, x400). NEM, neuroendocrine marker; CgA, chromogranin A; Syn, synaptophysin; INSM1, insulinoma-associated 1; Rb, retinoblastoma 1.

to distinguish it from NET-G3 (26,27). On the other hand, in CRC, the rate of inactivation has been reported to be low, at 0.21% (28). In the present study, Rb loss was observed in two cases. Of note, it was only observed in NEM-positive areas, whereas Rb expression was maintained in NEM-negative areas in the same cases (cases 1 and 2 in Table SI). Even in the other 11 cases where Rb expression was maintained, there was almost no NEM expression in the predominant areas, such as the WDCs and MDCs. NEM-positive and NEM-negative areas were confirmed in the same specimen. In case 1 (Table SI), despite being morphologically the same PDC tissue, there were areas that were NEM-negative and Rb-positive, as well as some that were NEM-positive and showed Rb loss. Colorectal NEC is typically associated with overlying adenomas or adenocarcinomas rather than NETs (29). Ogimi *et al* (30) analyzed the distribution of NEMs in CRC and normal mucosal tissues and suggested that NECs may originate from preexisting adenocarcinomas. Iijima *et al* (31) explored the histogenesis of combined pulmonary NECs by examining EGFR and p53 mutations and found that some combined NECs arose from non-NEC components. In the present study, particularly in cases 1 and 2, a similar situation was suggested, wherein NECs may have arisen from adenocarcinomas.

A few studies have reported that CRCs with neuroendocrine differentiation, or NEM-positive CRCs, have poor prognoses (32,33). In the present study, NEM-positive PDC was found to be a poor prognostic factor. The rate of lymph node metastasis was significantly higher in NEM-positive cases vs. NEM-negative ones. In the present study, among the 10 cases with lymph node metastasis, only one showed metastasis of NEM-positive cells in the metastatic lymph nodes. A liver metastasis was obtained as the distant metastatic tissue of NEM-positive PDC in one case. However, the cancer cells in this metastatic site were also NEM-negative. Therefore, it cannot be concluded that NEM-positive cells are more malignant.

The present study had several limitations. First, the small sample size, comprising only 13 NEM-positive cases of PDC, may have limited the generalizability and statistical significance of the findings. In addition, the study did not account for all variables that could have influenced prognosis (e.g., the patients' lifestyle habits and comorbidities), which may have potentially affected the results. Second, the absence of comprehensive genetic testing across all of the analyzed cases precluded a full exploration of the genetic associations between NEC and other cancer types. Future research with

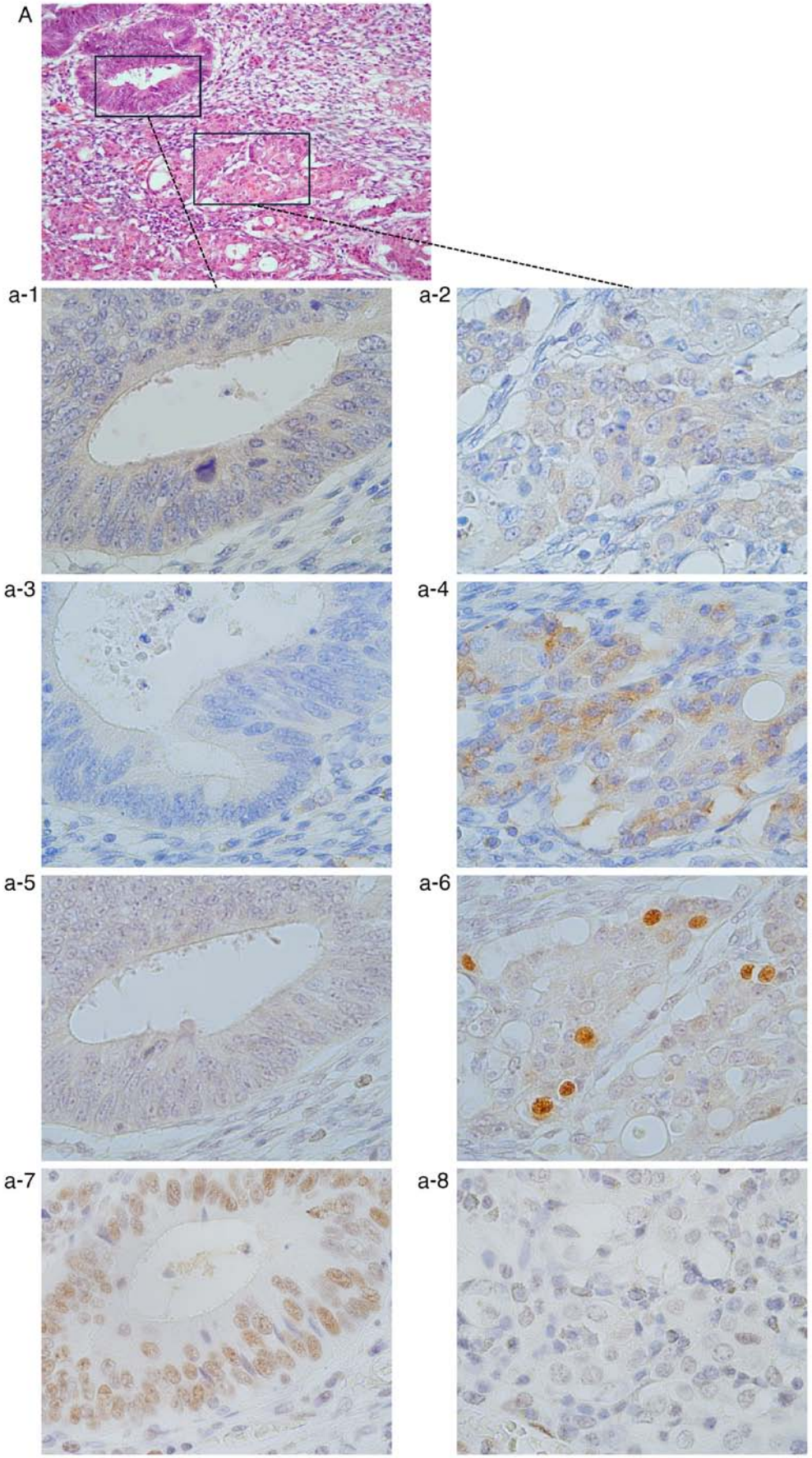

Figure 5. Case of Rb loss (case 2 in Table SI). (A) H&E staining (magnification, x200). Immunohistochemistry of (a-1, a-3, a-5 and a-7) the MDC areas and (a-2, a-4, a-6 and a-8) the PDC areas. In the MDC areas, (a-1) CgA, (a-3) Syn and (a-5) INSM1 were clearly negative. (a-7) Rb was positive. In the PDC areas, (a-2) CgA was negative, while (a-4) Syn and (a-6) INSM1 were positive. (a-8) Rb was negative (magnification, x400). PDC, poorly differentiated adenocarcinoma; MDC, moderately differentiated adenocarcinoma; CgA, chromogranin A; Syn, synaptophysin; INSM1, insulinoma-associated 1; Rb, retinoblastoma 1.

larger patient populations is necessary. In addition, the development of more precise diagnostic tools and targeted therapies and a deeper understanding of the molecular mechanisms underlying NEC and PDC are imperative to enhance the prognosis for these patients.

The mechanisms underlying the development of PDC and NEC in CRCs remain largely elusive. It has been demonstrated that small-cell prostate cancer can emerge during the progression of prostate adenocarcinoma (34). In such cases, a distinct treatment approach from that used for adenocarcinoma is necessary. The current findings indicate that PDC in CRCs may include components with NEC characteristics. These results underscore the need to reevaluate existing treatment protocols for CRC to more effectively address the distinct challenges presented by NEC and PDC. This could potentially lead to more personalized and effective treatment strategies. Although the carcinogenic processes leading to prognostically poor NEC in the colon remain largely elusive, the present study provides a preliminary exploration toward their elucidation. Further research is essential to decipher the molecular mechanisms in CRC cases that exhibit features of both PDC and NEC.

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Authors' contributions

YI, YR and IK designed the study, the main conceptual ideas and the proof outline. NO, ST, NK, KN, MO, JW and AI collected the data. YR and ST assembled the data. IK, SY, SF and IE provided expert advice as pathologists and surgeons, and were involved in treating some of the patients. YR wrote the manuscript with support from YI, IK and EK. IE and YI confirm the authenticity of all the raw data. All of the authors discussed the results, commented on the manuscript and have read and approved the final manuscript.

Ethics approval and consent to participate

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. It was approved by the Ethics Committee of Yokohama City University (Yokohama, Japan; approval no. B200700086).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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