Clinicopathological features and menin expression of pancreatic neuroendocrine neoplasm associated with multiple endocrine neoplasia type 1

Akari Sonoda[1] | Yo-ichi Yamashita[1] | Tatsuya Kondo[2] | Hiromitsu Hayashi[1] | Katsunori Imai[1] | Takaaki Higashi[1] | Kensuke Yamamura[1] | Takanobu Yamao[1] | Daisuke Hashimoto[1] | Hideo Baba[1]

[1]Department of Gastroenterological Surgery, Graduate School of Life Sciences, Kumamoto University, Kumamoto, Japan

[2]Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Correspondence
Hideo Baba, Department of Gastroenterological Surgery, Kumamoto University Graduate School of Medical Sciences, 1-1-1 Honjo, Kumamoto 860-8556, Japan.
Email: hdobaba@kumamoto-u.ac.jp

Abstract

Background/Purpose: We examined therapeutic strategies for pancreatic neuroendocrine neoplasm (pNEN) associated with MEN1 (M-pNEN) by investigating clinicopathological features and menin expression.

Methods: Seventy-seven patients who underwent resection of pNEN at our department from January 2001 to December 2017 were retrospectively analyzed. Immunohistochemical analysis of menin was performed using resected specimens.

Results: Seven patients (9%) met the diagnostic criteria for MEN1. M-pNEN had more tumors ($P < .01$), a higher recurrence rate ($P = .028$), and higher residual pancreatic recurrence ($P < .01$) than sporadic pNEN (S-pNEN). There were no significant differences in tumor size, lymph node metastasis, or World Health Organization grade between the two groups. Reduced menin staining in the tumor nuclei was found in 86% of M-pNEN; whereas only 34% of S-pNEN showed decreased nuclear staining. The remainder (66%) showed strong nuclear staining similar to normal islet cells ($P = .0071$). Furthermore, four patients (57%) with MEN1 had many microadenomas with reduced nuclear menin staining. Overall survival of M-pNEN patients was significantly better than S-pNEN patients ($P = .049$).

Conclusion: M-pNEN patients tend to develop spatially and temporally multifocal pNENs. However, M-pNEN patient prognosis is good with repeated surgeries at recurrence. Therefore, minimal resection with strict follow-up is recommended rather than extensive pancreatic resections for consideration of recurrence in M-pNEN.

KEYWORDS

menin, multiple endocrine neoplasia type 1, pancreatic neuroendocrine neoplasm

1 | INTRODUCTION

Pancreatic neuroendocrine neoplasm (pNEN) is a rare tumor, comprising 1%-2% of all pancreatic neoplasms, but recently the diagnostic ability of computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has improved, and the overall incidence of pNEN has increased.[1] PNEN occurs sporadically

or genetically, and representative hereditary diseases include von Hippel-Lindau (VHL) disease, von Recklinghausen disease, and multiple endocrine neoplasia type 1 (MEN1).[2]

The most common hereditary syndrome associated with pNENs is MEN1, an autosomal dominant hereditary manner. MEN1 is characterized by NENs in several of three major organs, the pituitary gland, parathyroid gland, and endocrine pancreas. According to an epidemiological study conducted in Japan, 4.3%–10% of all pNEN patients are related to MEN1.[3,4] Patients with MEN1 have been reported to have multiple microadenomas and a high rate of recurrence; however, there is no detailed literature on the clinical course of pNEN associated with MEN1 (M-pNEN) treatment. In addition, there are few reports of direct comparisons between M-pNEN and sporadic pNEN (S-pNEN). Surgical excision is the only curative treatment choice for pNEN, but surgical strategies for M-pNEN have not yet been fully established.

The cause of MEN1 is several mutations in a tumor suppressor gene, *MEN1*. And menin is protein encoded by *MEN1*.[5] In pNEN development, menin has been reported to be both an activator and repressor of gene transcription and has been associated with several pathways of tumor development.[6] To date, there have been few reports that have analyzed M-pNEN focusing on the expression of menin in pNEN. Investigations of clinicopathological differences in between M-pNEN and S-pNEN may help in determining treatment strategies.

2 | MATERIALS AND METHODS

2.1 | Patients

Seventy-eight patients who underwent pNEN resection at our department from January 2001 to December 2017 were included. Seven patients (9%) met the diagnostic criteria for MEN1. Seventy-one patients were not associated with MEN1, but one (1.4%) was excluded because of VHL disease. A total of 77 patients were retrospectively analyzed. Our institutional ethics committee approved this study (Registry No. 1291). Written informed consent was obtained from all patients, and this study was conducted according to the Declaration of Helsinki.

2.2 | MEN1 diagnosis criteria

At least one of the following criteria had to be present for inclusion in the registry[7]:

1. Confirmation of neoplastic disease in at least two of the commonly affected organs: parathyroid, endocrine pancreas, and anterior pituitary.

2. Evidence of one lesion described previously and a family history of MEN1.
3. Identification of a pathological germline mutation in the *MEN1* gene.

All seven patients of this study met the criteria of (1). Six patients met the criteria of (2), and one patient was identified as having a *MEN1* mutation (3).

2.3 | Immunohistochemical staining

Immunohistochemical evaluation was performed in 69 cases (M-pNEN, seven patients; S-pNEN, 62 patients) for which resected specimens were available.

Immunohistochemical staining of menin, Ki-67, and pancreatic endocrine hormones (insulin, glucagon, gastrin, and pancreatic polypeptide) was performed. We used primary antibodies against insulin (A0564; Dako), glucagon (A0565; Dako), somatostatin (A0566; Dako), gastrin (A0568; Dako) and pancreatic polypeptide (A0619; Dako). Staining was judged to be positive if it occurred in part of the tumor. Ki-67 staining was evaluated according to the 2019 World Health Organization (WHO) grading.[8] Menin staining used commercially available anti-menin antibody (A300-105A) manufactured by Bethyl Laboratories.[9] The staining intensity of tumor cell nucleus and cytoplasm was classified into four grades as follows: 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). Grade 0/1 of nucleus was judged as a decrease in staining and grade 2/3 of cytoplasm was judged as an increase in staining.

2.4 | Definition of recurrence in M-pNEN patients

We defined the recurrence of M-pNENs in this study as "the emergence of new pNEN requiring resections" and "the occurrence of NEN at distant organs excluding the pituitary gland and adrenal glands which was considered to be metastasized of pNEN".

2.5 | Statistics

Statistical comparisons between the M-pNEN and S-pNEN groups were conducted using the χ^2 test, Pearson's correlation coefficient, or Student's *t*-test. Survival curves were generated using the Kaplan–Meier method and were analyzed by the log-rank test. A result was considered significant if $P < .05$. All statistical analyzes were performed using JMP 12 software (SAS Institute).

3 | RESULTS

3.1 | Clinicopathological features of M-pNEN and S-pNEN patients

The clinicopathological features of seven M-pNEN patients and 70 S-pNEN patients were compared and summarized (Table 1). Patients with M-pNEN had higher numbers of tumors ($P < .001$), a higher rate of recurrence ($P = .028$), and a higher rate of residual pancreatic recurrence ($P < .001$). However, there were no significant differences in age at first surgery, tumor size, rate of lymph node metastasis, and 2019 WHO grading between the two groups.

3.2 | Detailed characteristics of M-pNEN patients

Table 2 shows the detailed clinical information of the seven M-pNEN patients. These patients consisted of three men and four women, all of whom had endocrine tumors in several different organs: the pancreas, pituitary gland, parathyroid

TABLE 1 Clinicopathological features of pNEN

Clinicopathological features	M-pNEN (n = 7)	S-pNEN (n = 70)	P-value
Age (years)	43.3 ± 22.8	56.7 ± 16.8	.055
Gender			.665
Male	3	34	
Female	4	36	
Diagnosis			.109
Non-functional	2	42	
Functional	5	28	
Insulinoma	3	25	
Glucagonoma	0	1	
Gastorinoma	2	1	
VIPoma		1	
Operation (First time)			.608
PD	1	17	
DP	3	32	
EN	1	14	
Others	2	7	
Tumor size (mm),	14.5 ± 7.3	20.7 ± 17	.350
Number of tumor			**<.001**
Single	2	61	
Multiple	5	9	
WHO grade (2019)			.604
NET, G1	7	57	
NET, G2	0	10	
NET, G3	0	2	
MiNEN	0	1	
Lymph node metastasis			.827
Positive	1	8	
Negative	6	49	
D0		13	
Recurrence	4 (57.1%)	12 (17.1%)	**.028**
Recurrent organ			
Liver	0	11	.126
Pancreas	4 (57.1%)	1 (1.4%)	**<.001**

Bold values indicate statistically significant values.

Abbreviations: DP, distal pancreatectomy; EN, enucleation; MiNEN, mixed neuroendocrine-non-endocrine neoplasm; PD, pancreaticoduodenectomy.

TABLE 2 Clinical information of M-pNEN

	Age	Sex	Tumor location	Familial history	Operation	Number of operations for pancreas	Single/ multiple	WHO grading	menin IHC nuclear staining	SASI test
1	68	F	Pancreas Pituitary Thyroid	○	DP	Once	Single	G1	Normal	×
2	53	M	Pancreas Parathyroid Thyroid	○	PD	Once	Single	G1	Aberrant	×
3	49	M	Pancreas Pituitary Parathyroid	×	PP PD EN	Three times	Multiple	G1	Aberrant	×
4	63	F	Pancreas Parathyroid Adrenal gland	○	PP PD	Twice	Multiple	G1	Aberrant	×
5	17	F	Pancreas Parathyroid Pituitary Thymus	○	DP PP	Twice[a]	Multiple	G1	Aberrant	○
6	13	M	Pancreas Parathyroid Pituitary	○	EN DP+EN	Twice[a]	Multiple	G1	Aberrant	○
7	35	F	Pancreas Pituitary	○	DP	Once	Multiple	G1	Aberrant	○

Abbreviations: F: female, M: male, DP: distal pancreatectomy, PD: pancreaticoduodenectomy, PP: partial pancreatectomy, EN: enucleation, NR: denotes data not reported, SASI test: selective arterial secretin injection test.

*Initial surgery performed at another hospital

gland, thyroid gland, and adrenal gland. Two (29%) were diagnosed with non-functional pNEN, three (43%) with insulinoma, and two (28%) with gastrinoma. Six (86%) patients had a family history of MEN1. One patient underwent germline genetic testing and a *MEN1* mutation was found. Four patients (57%) had undergone multiple pancreatic resections for pNEN. All tumors showed G1 WHO grading.

3.3 | Immunohistochemical analysis for M-pNEN: pancreatic hormones and menin

Sixty-nine patients (M-pNEN, seven patients; S-pNEN, 62 patients) were subjected to pathological evaluation.

First, in the staining of pancreatic endocrine hormones, two patients with MEN1 (case 1 and 2) had a single tumor, and the tumors had the same pancreatic hormone staining as the diagnosis. Five patients with MEN1 (cases 3–7) had multiple pNENs. In case 3 and case 5, pNENs had different hormone staining in each tumor. Case 3, diagnosed as glucagonoma, had multiple duodenal NENs producing gastrin and pancreatic NENs producing glucagon (Figure 1). In case 5, diagnosed as insulinoma, there were multiple pNENs that showed insulin or gastrin staining and pNENs that did not show hormone staining (Figure 2).

Next, we evaluated menin expression distribution in pNENs by immunohistochemical staining. The typical findings of

menin staining are shown in Figure 3. Six (86%) of seven M-pNEN patients showed decreased nuclear menin staining (aberrant pattern), whereas 21 (34%) of 62 S-pNENs showed decreased nuclear menin staining. These distributions differed significantly between the two groups ($P = .0071$). On the other hand, only two M-pNEN patients (29%) showed increased cytoplasmic expression of menin, and there was no difference in the cytoplasmic expression of menin between M-pNENs and S-pNENs ($P = .2477$). The multiple pNENs were examined in case 3 and case 5 of M-pNEN, and all pNENs showed decreased nuclear staining of menin (Figures 1, 2).

3.4 | Patients' prognosis

Figure 4 shows Kaplan–Meier survival curves for M-pNEN and S-pNEN patients. The 5- and 10-year survival rates of M-pNEN patients were both 100%. The 5-and 10-year survival rates of S-pNEN patients were 90% and 72%, respectively. M-pNEN patients had significantly better overall survival ($P = 0.049$).

4 | DISCUSSION

Several studies have reported that pNENs occurring in MEN1 patients differ in characteristics from other pNENs.[10,11] The European Neuroendocrine Tumor Society (ENETS)
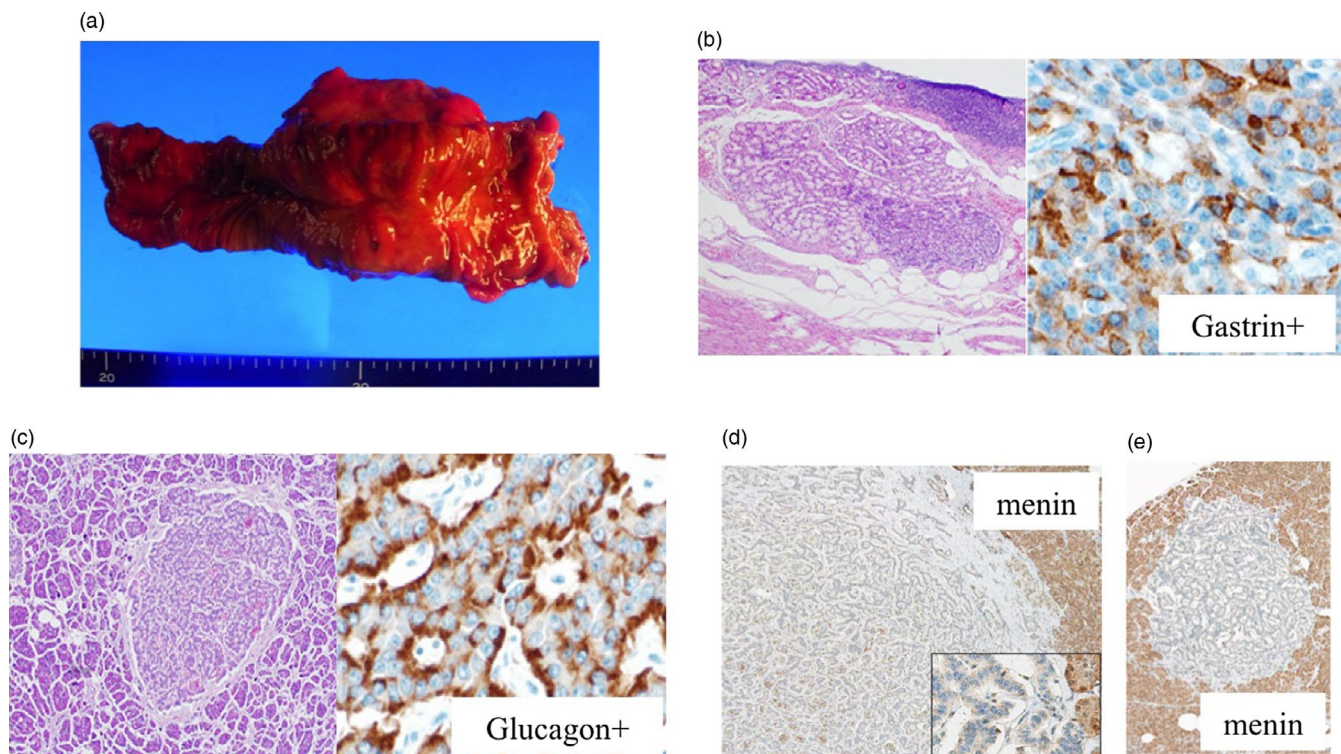

FIGURE 1 Gross and immunohistochemical evaluation of case 3. (A) Resected specimen in case 3. (B) Immunohistochemical staining using gastrin antibody was performed in duodenal NEN. (C) Immunohistochemical staining using glucagon antibody was performed in pNEN. (D and E) Immunohistochemical staining using menin antibody was performed in several pNETs
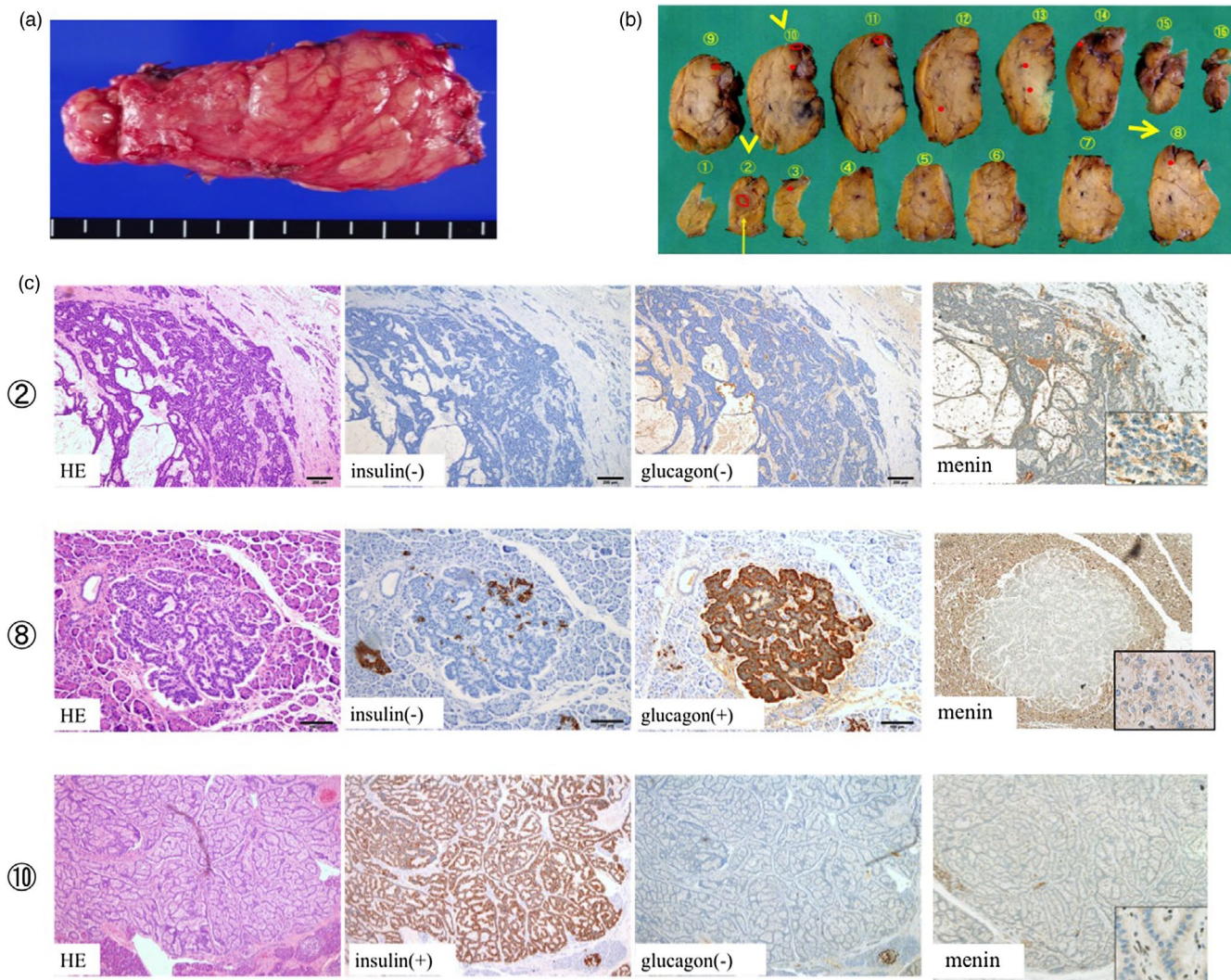
FIGURE 2 Gross and immunohistochemical evaluation of case 5. (A and B) Resected specimen of pancreas and its dissected section in case 5. Multiple pNENs are detected in the pancreas (red circle and yellow arrow). (C) Immunohistochemical staining using insulin, glucagon, or menin was performed in different pNENs (No. 2, 8, and 10 specimens)
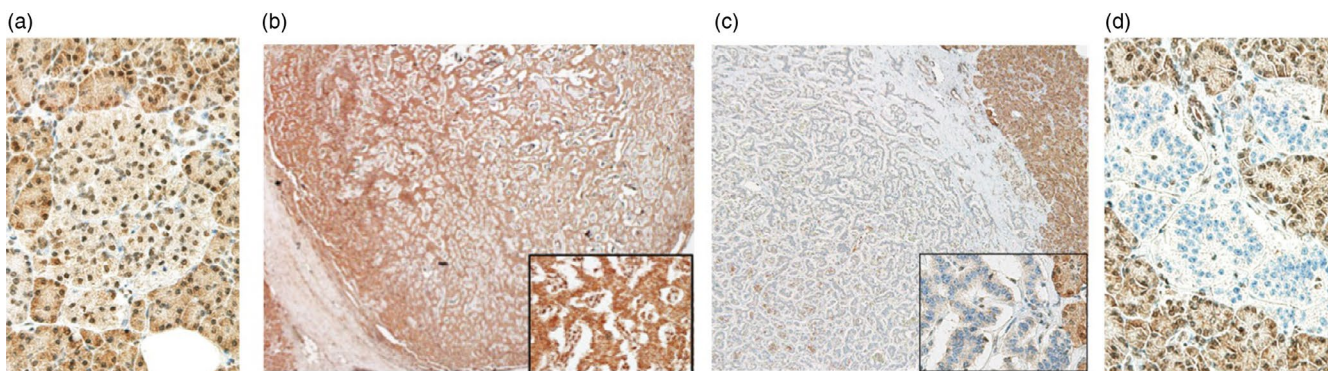

FIGURE 3 Immunohistochemical staining of menin. (A) Representative islet stained with menin. (B) Menin immunostaining was performed in representative S-pNEN (menin positive). (C and D) Menin immunostaining was performed in representative M-pNEN (menin downregulated)

treatment guidelines indicate differences in the indications for gastrinoma and non-functional (NF)-pNEN surgery. For functional pNENs excluding gastrinoma, minimal excision is recommended regardless of the presence of MEN1.[12] The Japanese Neuroendocrine Tumor Society (JNETS) guidelines recommend surgery for all NF-pNENs without MEN1, but
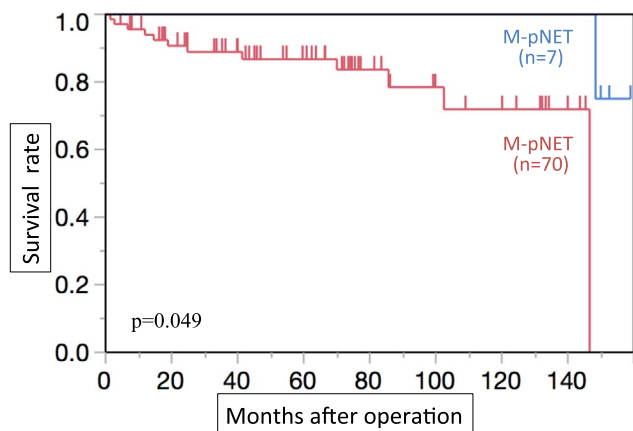
FIGURE 4 Overall survival curves of M-pNEN and S-pNEN patients

follow-up for NF-pNENs with MEN1 when growth is slow and <2 cm is advocated.[13] In the case of multiple tumors, a feature of M-pNEN, it is difficult to select a surgical procedure. Therefore, we believe that detailed treatment reports for M-pNEN are necessary. In our study, M-pNEN was found to have a higher incidence of multiple tumors and a higher risk of recurrence than those with S-pNEN. Of note, M-pNEN had significantly higher recurrence in the residual pancreas compared with S-pNEN (47% vs. 1.4%; $P < .001$). Only one case of S-pNEN had a recurrence in the remnant pancreas, and a second resection was performed; however, the possibility of surgical stump recurrence cannot be ruled out. Therefore, in our series, almost all the remaining pancreatic recurrences of pNEN appeared to be exclusively developed in MEN1.

We present the treatment course of four M-pNEN patients who have undergone multiple surgeries for pNEN and residual pancreatic recurrence. Of these, all three patients who had functional pNEN exhibited improved symptoms immediately after their initial surgery, but they gradually became symptomatic due to excessive hormones such as insulin (hypoglycemia) and gastrin (gastrointestinal ulcers) because of the recurrent pNENs. If there are many tumors present at the first diagnosis, it is very important to identify the exact location of tumors that produce the hormones associated with corresponding symptoms. In case 5 and case 6, many tumors were detected at the time of recurrence, so a selective arterial secretagogue injection (SASI) test was performed before surgery to examine the location of responsible tumors (Table 2). The SASI test can detect tumor locations that cause symptoms using the feeding arteries to the pancreas.[14,15] By performing it before surgery, it may be possible to determine the region of the tumor that needs to be removed. MEN1 patients often have multiple pNENs or microadenomas that cannot be detected by radiological imaging; therefore, we consider SASI testing is essential for appropriate surgery, especially in M-pNEN[16]. To completely reduce the risk of developing

pNEN in M-pNENs patients in the future, total pancreatectomy (TP) could be considered. However, M-pNEN patients was young such as 43.3 years in our series; therefore, lifelong insulin injection and complete loss of pancreatic exocrine functions after TP would have a big impact on QOL. Therefore, we selected function-preserving minimal surgery for pNEN in M-pNEN patients with strictly follow-up for developing pNEN after surgeries.

To investigate the genetic background of the frequent occurrence of M-pNEN, menin, which is encoded by the causative gene (*MEN1*), was stained in our series. To date, there have been reports that menin translocation to the nucleus is inhibited in M-pNEN, resulting in increased cytoplasmic staining and decreased nuclear staining.[9] On the other hand, the increased cytoplasmic staining of menin in M-pNEN was not found in our series irrespective of using the same antibody of menin. The previous report[9] referred the relationship between the MEN1 gene mutations and the immunohistochemical expressions of menin. Therefore, it is likely that the S-pNEN group includes a considerable number of MEN1 gene mutation cases. This may lead to different results of staining pattern of menin. Although an increase in the cytoplasm was not clear in our series, a decrease in nuclear staining was observed in many cases of M-pNEN (86%). This is the first report to describe a significant difference in the rate of nuclear staining of menin between M-pNEN and S-pNEN; therefore, it is a valuable data set.

Interestingly, multiple pNENs that occurred in one patient with MEN1 had different hormone patterns in each tumor; however, they showed similar menin staining patterns. This result suggests that MEN1 patients have lost one *MEN1* allele since their birth, but that damage to the other allele occurred after each islet cell had differentiated and become tumorous.[17] Further research is required regarding the tumor development of M-pNEN. In addition, all microadenomas occurring in M-pNEN patients showed a similar staining pattern, which is more easily detected by immunohistochemical staining of menin; thus, diagnosing M-pNEN may be helped by detecting menin downregulation in microadenomas of the resected pancreas.

This research has some limitations. As a result of this single-center retrospective study, the number of M-pNEN cases is very limited. There is also the slight possibility that patients who have not yet been diagnosed as MEN1 were grouped into the S-pNEN group. The evaluation of immunohistochemical staining was performed with a single reagent, and few cases have been verified.

In conclusion, M-pNEN patients tend to develop spatially and temporally multifocal pNENs. The expression of pancreatic hormones was different in each M-pNEN in our series; therefore, the SASI test is recommended for appropriate resection in symptomatic multiple M-pNENs. The prognosis of M-pNEN patients is favorable with repeated

surgeries at recurrence. Therefore, minimal resection with strict follow-up can be recommended rather than extensive pancreatic resections conducted in case of M-pNEN recurrences.

CONFLICT OF INTEREST
Akari Sonoda and coauthors have no conflict of interest in relation to this article.

ORCID
Akari Sonoda https://orcid.org/0000-0002-6610-3075
Yo-ichi Yamashita https://orcid.org/0000-0001-8439-6660
Daisuke Hashimoto https://orcid.org/0000-0002-7880-889X

REFERENCES
1. YaoJC, HassanM, PhanA, DagohoyC, LearyC, MaresJE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008;26:3063–72.
2. StevensonM, LinesKE, ThakkerRV. Molecular genetic studies of pancreatic neuroendocrine tumors: new therapeutic approaches. Endocrinol Metab Clin North Am. 2018;47:525–48.
3. ItoT, SasanoH, TanakaM, OsamuraRY, SasakiI, KimuraW, et al. Epidemiological study of gastroenteropancreatic neuroendocrine tumors in Japan. J Gastroenterol. 2010;45:234–43.
4. ItoT, IgarashiH, NakamuraK, SasanoH, OkusakaT, TakanoK, et al. Epidemiological trends of pancreatic and gastrointestinal neuroendocrine tumors in Japan: a nationwide survey analysis. J Gastroenterol. 2015;50:58–64.
5. ThakkerRV. Multiple endocrine neoplasia–syndromes of the twentieth century. J Clin Endocrinol Metab. 1998;83:2617–20.
6. IyerS, AgarwalSK. Epigenetic regulation in the tumorigenesis of MEN1-associated endocrine cell types. J Mol Endocrinol. 2018;61:R13–R24.
7. BrandiML, GagelRF, AngeliA, BilezikianJP, Beck-PeccozP, BordiC, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab. 2001;86:5658–71.
8. KlimstraD, KloppelG, La RosaS, RindiG. Classification of neuroendocrine neoplasms of the digestive system. In: WHO Classification of Tumours: Digestive System Tumours, 5th ed, WHO Classification of Tumours Editorial Board (Ed), International Agency for Research on Cancer, Lyon; 2019.
9. CorboV, DalaiI, ScardoniM, BarbiS, BeghelliS, BersaniS, et al. MEN1 in pancreatic endocrine tumors: analysis of gene and protein status in 169 sporadic neoplasms reveals alterations in the vast majority of cases. Endocr Relat Cancer. 2010;17:771–83.
10. ChristakisI, QiuW, Silva FigueroaAM, HydeS, CoteGJ, BusaidyNL, et al. Clinical features, treatments, and outcomes of patients with thymic carcinoids and multiple endocrine neoplasia type 1 syndrome at MD Anderson Cancer Center. Horm Cancer. 2016;7:279–87.
11. GiudiciF, CavalliT, GiustiF, GronchiG, BatignaniG, TonelliF, et al. Natural history of MEN1 GEP-NET: single-center experience after a long follow-up. World J Surg. 2017;. https://doi.org/10.1007/s00268-017-4019-2.
12. FalconiM, ErikssonB, KaltsasG, BartschDK, CapdevilaJ, CaplinM, et al. ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. Neuroendocrinology. 2016;103:153–71.
13. Japan Neuroendocrine Tumor Society. Clinical Guideline for Pancreatic and Gastrointestinal Neuroendocrine Tumors, 2nd edn. Tokyo: Kanehara; 2019. (in Japanese).
14. ImamuraM, TakahashiK, AdachiH, MinematsuS, ShimadaY, NaitoM, et al. Usefulness of selective arterial secretin injection test for localization of gastrinoma in the Zollinger-Ellison syndrome. Ann Surg. 1987;205:230–9.
15. WiesliP, BrandleM, SchmidC, KrahenbuhlL, FurrerJ, KellerU, et al. Selective arterial calcium stimulation and hepatic venous sampling in the evaluation of hyperinsulinemic hypoglycemia: potential and limitations. J Vasc Intervent Radiol. 2004;15:1251–6.
16. NiinaY, FujimoriN, NakamuraT, IgarashiH, OonoT, NakamuraK, et al. The current strategy for managing pancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1. Gut Liv. 2012;6:287–94.
17. MaxwellJE, ShermanSK, HoweJR. Translational diagnostics and therapeutics in pancreatic neuroendocrine tumors. Clin Cancer Res. 2016;22:5022–9.