Ossification in pancreatic neoplasms: A report of three cases of neuroendocrine tumor, mucinous cystic neoplasm, and intraductal papillary mucinous neoplasm with review of the literature

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Abstract. Heterotopic ossification (HO) is the formation of mature bone tissues in non-skeletal tissues and has been reported in various types of neoplasms. The presence of HO in pancreatic tumors is rare, with only 16 cases reported in the English literature, and the mechanism of HO formation remains unclear. In the present study, the 17th to 19th cases of HO in pancreatic neoplasms were described and the literature on HO in pancreatic neoplasms was reviewed. Three Japanese women with pancreatic masses on imaging underwent laparoscopic distal pancreatectomy. The three pancreatic masses were histopathologically diagnosed as a pancreatic neuroendocrine tumor (NET), mucinous cystic neoplasm (MCN), and intraductal papillary mucinous neoplasm, and HO was found in all of them. Neither pancreatic NET with HO nor MCN with HO has yet been reported in the English-language literature, and to our knowledge, these are the first such reports. Although rare, the knowledge that various types of pancreatic tumors, including NETs and MCNs, are associated with HO may be useful in daily practice, and the significance of tumor-associated HO is discussed.

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Abbreviations: CT, computed tomography; HO, heterotopic ossification; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; NET, neuroendocrine tumor; PDAC, pancreatic ductal adenocarcinoma; SPN, solid pseudopapillary neoplasm

Key words: HO, pancreatic neoplasm, pancreatic NET, MCN, IPMN

Introduction

Heterotopic ossification (HO) is the formation of mature bone tissues in non-skeletal tissues (1). HO is classified into genetic and non-genetic forms. The non-genetic form is usually associated with trauma or tissue injury and is most frequently observed in atherosclerotic plaques and soft tissues surrounding the joint (1,2). Moreover, intra- or peritumoral HO has been reported in various types of benign and malignant lesions, including meningiomas, melanocytic nevi (osteonevus of Nanta), epidermal cysts, thyroid tumors and lung tumors (2-9). The frequency of HO may depend on the origin or histology of the tumor (6,7,10). HO is relatively common in papillary thyroid cancer (13%), lung carcinoid (up to 10%), osteonevus of Nanta (1.56%), and meningiomas (1%) and rare in others. Although the mechanism of HO formation remains unclear, it may be related to the tumor microenvironment and influence tumor behavior (10).

In the digestive system, HO is most frequently observed in colorectal tumors (10-13). The most frequent benign polyps with HO were juvenile polyps, followed by tubulovillous adenomas and traditional serrated adenomas. By contrast, the most common histological subtype of colorectal carcinoma with HO was conventional adenocarcinoma, followed by mucinous adenocarcinoma and serrated adenocarcinoma (10). The presence of HO in pancreatic neoplasms is rare, with only 16 patients with pancreatic neoplasms accompanying HO reported in the English-language literature (14-25). The most common histological type of pancreatic neoplasm associated with HO is the solid pseudopapillary neoplasm (SPN), followed by intraductal papillary mucinous neoplasm (IPMN). Pancreatic ductal adenocarcinoma (PDAC) and intrapapillary tubulopapillary neoplasms have rarely been associated with HO. In the present study, three patients with pancreatic neuroendocrine tumor (NET), mucinous cystic neoplasm (MCN),

and IPMN with HO were described, and the clinicopathological features of pancreatic neoplasms with HO were discussed, as well as the current status of the diagnosis and significance of tumor-associated HO.

Case report

Patient 1. A 64-year-old Japanese female underwent plain chest radiography during a medical checkup and was found to have a nodular shadow in her left pulmonary hilum. Her medical history included a total hysterectomy for uterine leiomyoma 31 years prior to presentation. For a close examination, the patient presented to Osaka Medical College Health and Science Clinic (Takatsuki, Japan) in July 2020 (as a supplement, the name of this college was changed to Osaka Medical and Pharmaceutical University in April 2021). The patient underwent computed tomography (CT), which suggested that the shadow was of vascular origin and coincidentally detected a mass measuring 1.5 cm in diameter with calcification in the pancreatic tail (Fig. 1A and B). The patient was referred to the Department of Gastroenterology at Osaka Medical College Hospital (Takatsuki, Japan) for further examination. As the mass was small and showed no tendency to enlarge, the patient was followed up for 17 months starting in July 2020. Although tumor size did not increase during this period, repeat CT showed that the tumor had solidified, suggesting possible malignancy. Thus, the patient underwent endoscopic ultrasonography-guided fine-needle aspiration, and pathological examination of the specimen revealed loosely cohesive clusters of small round cells, suggesting a pancreatic NET. The patient underwent a laparoscopic distal pancreatectomy in February 2022.

Histopathological examination of the surgically resected specimens demonstrated that the tumor was covered by a fibrous capsule and was well-circumscribed from the surrounding pancreatic parenchyma. Neoplastic cells showing trabecular or ribbon-like growth had small round nuclei and inconspicuous nucleoli (Fig. 2A and B). Necrotic or mitotic cells were not observed. Immunohistochemical analysis demonstrated that the neoplastic cells expressed chromogranin A and synaptophysin with a Ki-67 labelling index of 1%. Based on these findings, NET G1 was diagnosed according to the diagnostic criteria of the World Health Organization Classification 2019 (26). A peculiar finding of the present tumor was the presence of mature bone tissue, which was composed of lamellar bone and osteocytes without nuclear atypia within the fibrosclerotic stroma and with no cartilaginous components (Fig. 2A and B). The postoperative course was uneventful, and the patient was free from tumor recurrence for 3 years after surgery without any treatment.

Patient 2. A 62-year-old Japanese female visited Hokusetsu General Hospital (Takatsuki, Japan) in May 2022 with a complaint of epigastric pain and was hospitalized for 10 days with a diagnosis of acute pancreatitis. Her medical history included cholecystectomy for cholecystolithiasis ~20 years prior to presentation, hyperlipidemia and *Helicobacter pylori* (*H. pylori*) infection. CT revealed a cystic lesion measuring 4 cm in diameter with calcification in the pancreatic tail (Fig. 3A and B), which was suggested to be MCN. The patient

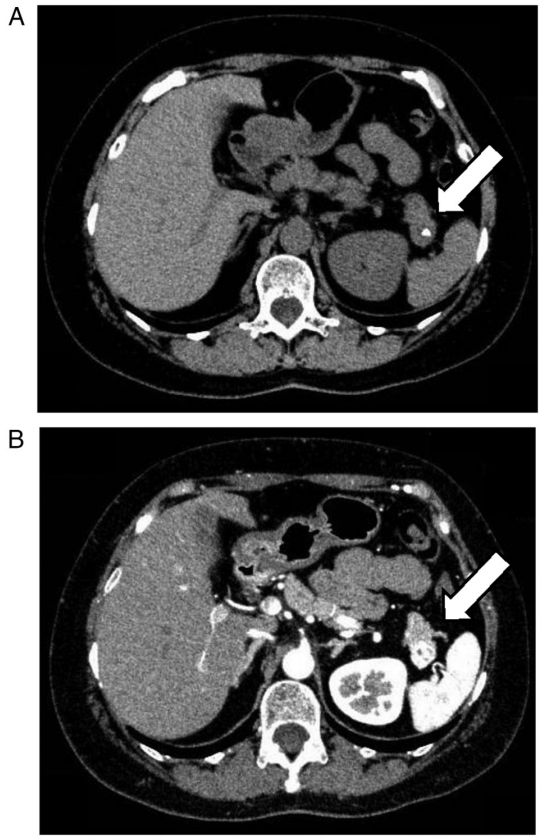

Figure 1. Abdominal plain (A) and contrast-enhanced (B) computed tomography. A low-density mass with calcification in the pancreatic tail (white arrows).

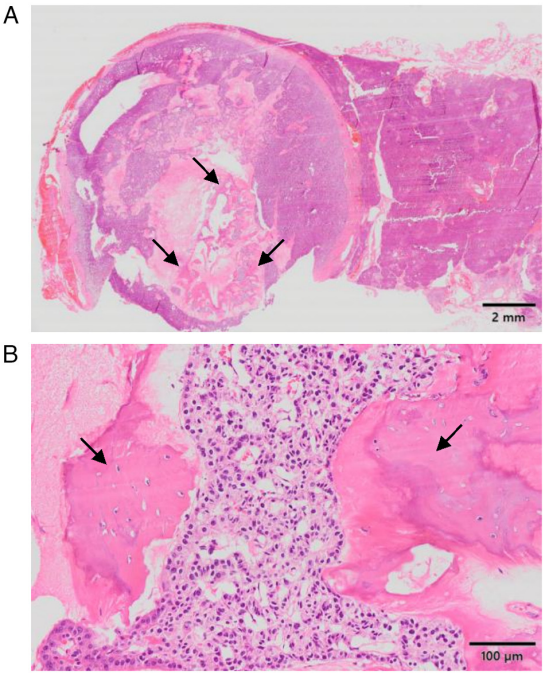

Figure 2. Histopathological features of patient 1. (A) The tumor is well circumscribed by fibrous capsule from the surrounding pancreatic parenchyma, and mature bone tissue (black arrows) is noted within the tumor (hematoxylin and eosin staining, x4). (B) A magnified image of (A). Trabecular and ribbon-like neoplastic growth composed of neoplastic cells with small round nuclei is observed. Mature bone tissue with osteocytes (black arrows) is also noted within the tumor with no cartilaginous components (hematoxylin and eosin staining; magnification, x100).

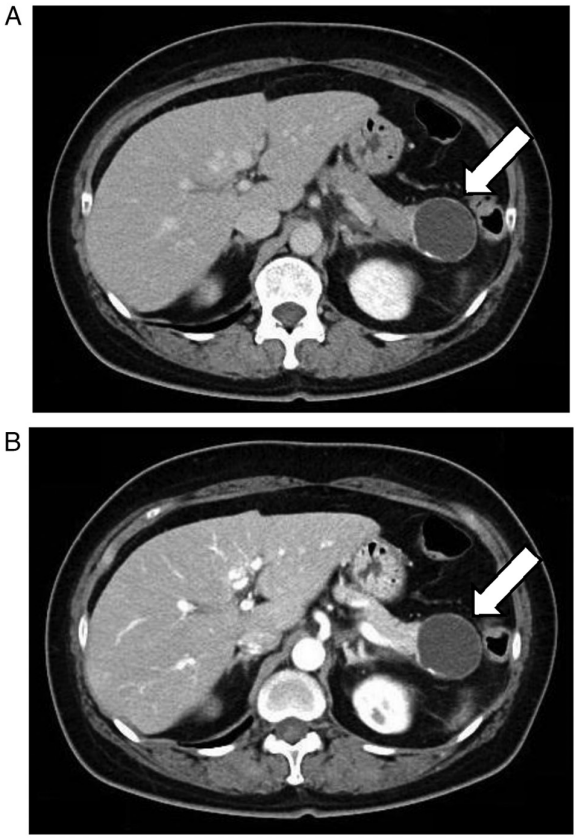

Figure 3. Abdominal contrast-enhanced computed tomography (A and B) A cystic lesion with calcification in the pancreatic tail (white arrows).

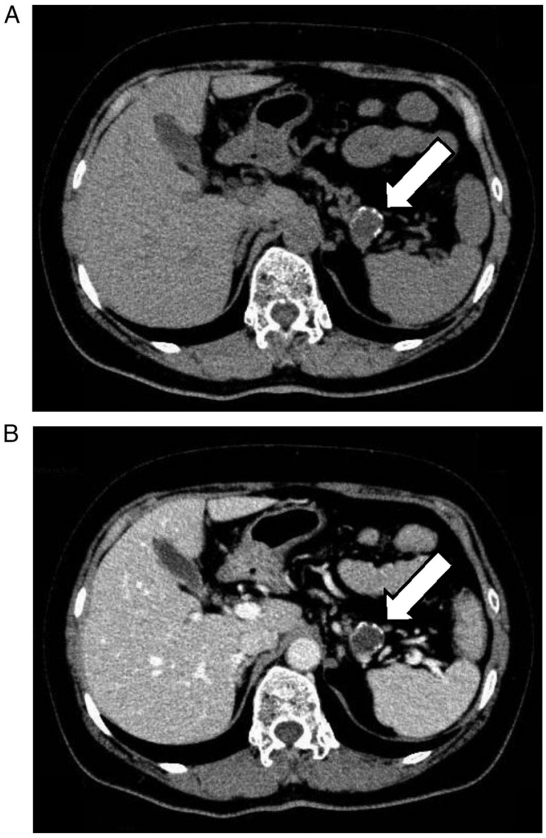

Figure 5. Abdominal plain (A) and contrast-enhanced (B) computed tomography. A cystic lesion in the pancreatic tail with calcification (white arrows).

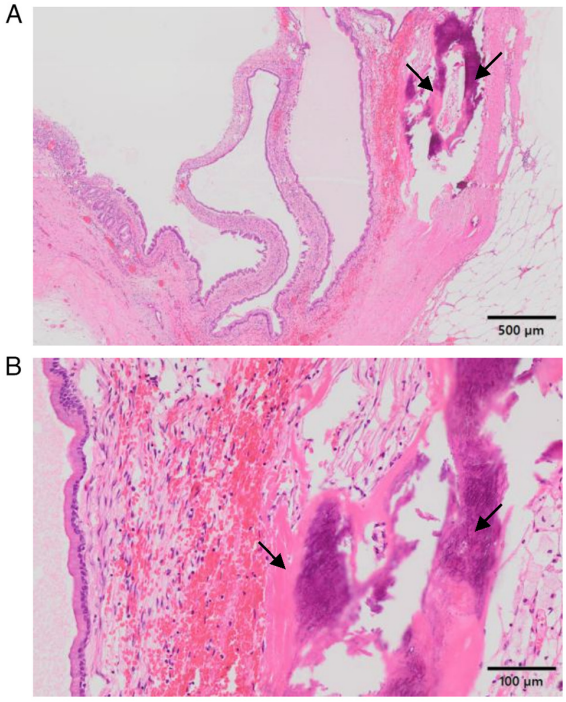

Figure 4. Histopathological features of patient 2. (A) A well-circumscribed cystic lesion is present, and bone formation is noted in the cystic wall (black arrows) (hematoxylin and eosin staining, x20). (B) A magnified image of (A). The cyst is covered by the mucus-containing columnar cells without nuclear atypia, and ovarian-like stromal cells are present around columnar cells. Mature bone tissue with osteocytes is also observed within the cystic wall with no cartilaginous components (black arrows) (hematoxylin and eosin staining, x100).

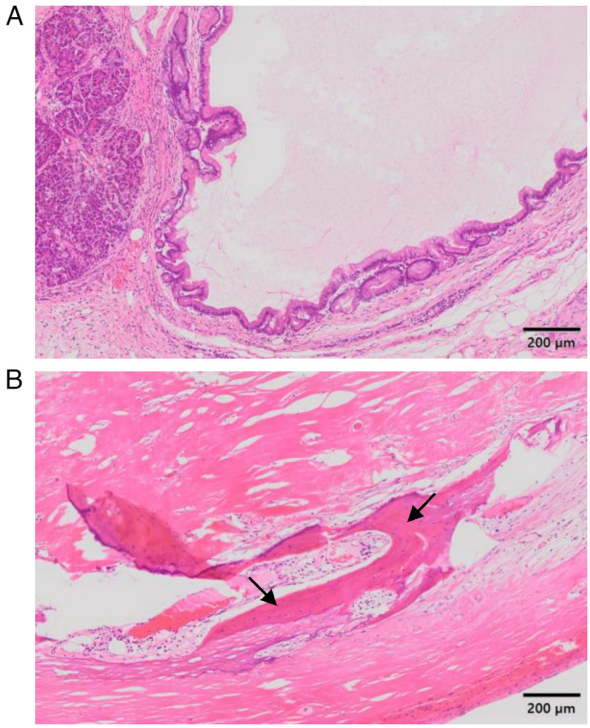

Figure 6. Histopathological features of patient 3. (A) Proliferation of the mucus-containing columnar cells without nuclear atypia within the dilated pancreatic duct (hematoxylin and eosin staining, x40). (B) Ossification with no cartilaginous components is noted within the sclerotic fibrous tissue around the pancreatic duct (black arrows) (hematoxylin and eosin staining, x40).

Table I. Pancreatic neoplasms with heterotopic ossification in English-language literature.

Case no.	Age/ Sex	Location	Histological type	Reason for detection	Diagnostic imaging	Postoperative outcome	Follow-up duration	(Refs.)
1	44/M	Tail	SPN	Abdominal pain	Calcification/PR and CT	Alive without recurrence (AWR)	4 years and 3 months	(14)
2	39/M	Body	SPN	Incidental	Calcification/PR and CT	AWR	3 years and 2 months	(14)
3	51/F	Tail	SPN	Incidental	Calcification/PR and CT	AWR	3 years	(14)
4	44/F	Body	SPN	Abdominal pain	Calcification/PR and CT	AWR	2 years and 9 months	(14)
5	34/F	Tail	SPN	Incidental	Calcification/PR, CT, and US	AWR	8 months	(16)
6	36/F	Tail	SPN	Dyspepsia and chest pain	Calcification/CT	AWR	10 months	(17)
7	34/F	Tail	SPN	Incidental	Calcification/CT	AWR	3 months	(17)
8	25/F	Body	SPN	Incidental	Ossified lesion (ossific focus)/ CT, US, EUS, MRI, and ERP	AWR	2 years	(18)
9	38/F	Head	SPN	Abdominal discomfort	Calcification/CT, US, and EUS	AWR	6 months	(20)
10	66/F	Body	SPN	Abnormal genital bleeding	Calcification/CT, EUS, and MRI	Died of concurrent PDAC	2 months	(23)
11	71/F	Body	PDAC	Epigastric fullness, discomfort and pain in the upper abdomen and back, and loss of weight	Calcification/CT	Not surgically removed; diagnosed at autopsy		(15)
12	39/M	Body	PDAC	Epigastric pain	Calcification/CT and EUS	Not available (NA)	NA	(24)
13	66/M	Tail	ITPN	Epigastric pain	Calcification/CT	AWR	15 months	(19)
14	56/M	Head	IPMN high	Incidental	High-density area/CT, high signal lesion/MRI T1-weighted	AWR	2 years and 9 months	(21)
15	70/M	Tail	IPMN low	Abdominal discomfort and pain	Calcification/CT	NA	NA	(22)
16	58/F	Body	IPMN	Upper abdominal discomfort	Mural nodule/multidetector CT	Died of other causes	2 years and 4 months	(25)
17	71/F	Tail	IPMN low	Incidental	Calcification/CT and EUS	AWR	1 years and 8 months	Present case
18	62/F	Tail	MCN low	Epigastric pain	Calcification/CT and EUS	AWR	2 years and 3 months	Present case
19	64/F	Tail	NET	Incidental	Calcification/CT, EUS, and MRI	AWR	2 years and 10 months	Present case

M, male; F, female; PR, plain radiography; CT, computed tomography; AWR, alive without recurrence; NA, not available; yr, years; mo, months; US, ultrasonography; EUS, endoscopic ultrasonography; MRI, magnetic resonance imaging; ERP, endoscopic retrograde pancreatography; SPN, solid pseudopapillary neoplasm; PDAC, pancreatic ductal adenocarcinoma; ITPN, intraductal tubulopapillary neoplasm; IPMN, intraductal papillary-mucinous neoplasm; IPMN high, IPMN with high-grade dysplasia; IMPN low, IPMN with low-grade dysplasia; MCN low, mucinous cystic neoplasm with low-grade dysplasia; NET, neuroendocrine tumor.

was referred to the Department of Gastrointestinal Surgery at Osaka Medical and Pharmaceutical University Hospital (Takatsuki, Japan). Laparoscopic distal pancreatectomy was performed in September 2022.

Histopathological examination of the surgically resected specimens revealed a well-circumscribed cystic lesion covered by mucus-containing columnar cells without nuclear atypia or invasive growth (Fig. 4A and B). Characteristically, ovarian-like stroma was observed around the lesion (Fig. 4B). Moreover, mature bone formation with osteocytes was noted within the cystic wall, with no cartilaginous components (Fig. 4A and B). Accordingly, MCN with low-grade dysplasia accompanied by HO was diagnosed. The postoperative course was uneventful, and the patient was free of tumor recurrence 27 months after surgery without any treatment.

Patient 3. A 71-year-old Japanese female was treated for diabetes mellitus by her primary care physician for 7 years. For further evaluation of elevated levels of serum amylase (274 U/l; range 44-132) and hemoglobin A1c (9.5%; range 4.9-6.0), the patient was referred to the Department of Diabetes, Metabolism and Endocrinology at Osaka Medical and Pharmaceutical University Hospital in November 2022. In addition to diabetes, her medical history included postoperative cataract, hypertension, hyperlipidemia, and *H. pylori* infection. Coincidentally, a CT scan revealed multiple cystic changes from the pancreatic head to the tail, and the cystic lesion in the tail was 1.7 cm in diameter with scattered calcifications (Fig. 5A and B). It was strongly suspected to be an IPMN with high-grade dysplasia or pancreatic NET, and laparoscopic distal pancreatectomy was performed in April 2023.

Histopathological examination of the surgically resected specimens revealed that the main and branch pancreatic ducts were cystically dilated, and the proliferation of mucus-containing columnar cells without nuclear atypia or invasive growth was noted (Fig. 6A). Ossification composed of lamellar bone and osteocytes without nuclear atypia was present, with sclerotic fibrosis but no cartilaginous components around the cyst (Fig. 6B). Accordingly, IPMN with low-grade dysplasia containing HO was diagnosed. The postoperative course was uneventful. Multiple cystic lesions in the remnant pancreas were followed up with periodic CT, and no tumor progression was noted 20 months after surgery.

Discussion

In the present study, the cases of three patients with pancreatic neoplasms and HO were reported. Only 16 patients with pancreatic neoplasms accompanying HO have been reported in the English-language literature (14-25), which is considered rare. The clinicopathological features of the previously reported cases as well as those of the three present cases are summarized in Table I. There was a slight female predominance (13 females and 6 males), with the tail region being the most common site of occurrence (10 cases in the tail and 9 in the head and body). Regarding its histology, HO of pancreatic neoplasms can be observed in benign and malignant forms, as observed in colorectal tumors (10). HO is observed in benign tumors, such as IPMN with low-grade dysplasia and MCN with low-grade dysplasia; low-grade malignant tumors, including SPN and NET G1; and malignant tumors, such as IPMN with high-grade dysplasia and PDAC. The most frequent histological subtype of pancreatic neoplasms with HO is SPN (10 of 19 patients), followed by IPMN (4 patients) and PDAC (2 patients). To the best of the authors' knowledge, this is the first report of a pancreatic NET and MCN with HO. The reasons for the differences in the frequency of HO among histological types of pancreatic neoplasms remain unclear. The reported cases of SPN suggest that the presence of HO in the abundant fibrous stroma reflects a reactive or metaplastic process (14,17). In our current cases of IPMN, MCN and NET, HO was also observed adjacent to fibrous components, which may further support the involvement of the aforementioned process. Alternatively, HO has been reported in two patients with PDAC (15,24), which might lead to the hypothesis that HO is associated with proliferative potential. However, HO also occurs in tumors with low proliferative activity, such as IPMN with low-grade dysplasia, MCN with low-grade dysplasia, and NET G1, indicating that the causal relationship between proliferative capacity and HO remains unclear. Regarding the relationship with prognosis, Table I may suggest that the presence of HO is not associated with poor prognosis or is not a prognostic factor; however, there are limitations to drawing further conclusions.

In daily practice, HO is most likely to be recognized as a simple calcification on radiographs, as is the case in all three presentations here, and is therefore underestimated. The frequency of calcification depends on the histological type; acinar cell carcinoma (6-50%), NET (30%), pancreatoblastoma (30%), serous cystic neoplasm (30%), SPN (22%) and MCN (15%) are relatively common, whereas other types, such as IPMN and pancreatic ductal carcinoma, are rare. Comparing the frequency of calcification by histological type of pancreatic tumor to that of HO, the two are somewhat discrepant and thus do not appear to be directly related. From a mechanistic perspective, although not yet fully understood, the activation of bone morphogenetic protein signaling pathways has been implicated in HO in some pancreatic tumors, again supporting the distinction between HO and dystrophic calcification (18,21). The involvement of BMP in HO formation associated with tumors has been reported not only in pancreatic tumors but also in colorectal cancer, pulmonary carcinoid and papillary thyroid carcinoma (7,9,10). Does it make sense to distinguish HO from calcifications? One possible significance of HO is its potential as a prognostic marker for pancreatic tumors. Matsunou *et al* (14) reported that SPN cases with ossification and calcification had fibrotic areas in their centers and showed infiltrative growth patterns and pleomorphic atypia, indicating that they are aggressive. Nakamura *et al* (16) also reported a case of SPN with HO, arguing that this may have a high malignant potential because of its infiltrating growth pattern. However, the prognostic significance of HO in SPN has not been established, as this finding is rare, and recurrence or metastasis in such cases has not been reported (Table I). HO may also provide clues for the differential diagnosis of pancreatic tumors, which may require advances in diagnostic modalities to identify HO accurately.

In conclusion, the present study describes three patients with pancreatic neoplasms and HO. Although rare, clinicians should recognize that several types of pancreatic neoplasms including NET and MCN can accompany HO. Additional studies, which are based on increased awareness and interest

in HO and improved accuracy of its identification in diagnostic imaging, are required to clarify the prognostic significance and underlying mechanisms of it in pancreatic neoplasms.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Authors' contributions

YF, MI and YH drafted the manuscript and figures and contributed to conception, design, data collection and data analysis. EY, KN, SK, AT and MA contributed to data collection and data analysis. YF and YH confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted according to the principles of the Declaration of Helsinki. All data were anonymized. Written consent for these case reports was obtained from all patients.

Patient consent for publication

Written consent for publication of patient data and associated images was obtained from the patients.

Competing interests

The authors declare that they have no competing interests.

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