

ORIGINAL

Histological and immunohistochemical analysis of epithelial cells in epidermoid cysts in intrapancreatic accessory spleen

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Abstract : Background : Epidermoid cysts in intrapancreatic accessory spleen (ECIPAS) are a rare lesion. Its pathogenesis, including the origin of cystic epithelium, is not well established. We aimed to elucidate new aspects of the pathological features of ECIPAS to clarify its pathogenesis. **Methods :** Six cases of ECIPAS were included in this study. As well as histopathological analysis, to elucidate the features and nature of cystic epithelial cells, immunohistochemical analysis including Pbx1 and Tlx1 and imaging mass spectrometry was performed. **Results :** Histologically, the cysts were covered by either monolayered or multilayered epithelium. Immunohistochemistry revealed that the epithelial cells in multilayered epithelium exhibited different attributes between the basal and superficial layers. Few epithelial cells had abundant clear cytoplasm and were immunohistochemically positive for adipophilin, suggesting lipid-excreting function. The intracystic fluid contained cholesterol clefts and foamy macrophages, and imaging mass spectrometry revealed the accumulation of lipids. Immunohistochemical analysis indicated that the epithelial cells were positive for Pbx1 in some cases. **Conclusion :** Novel histological features of epithelial cells of ECIPAS were indicated. Although more cases need to be evaluated, we propose that the cause of ECIPAS may be different from that of pancreatic ductal origin. *J. Med. Invest.* 70:251-259, February, 2023

Keywords : Epidermoid cyst in intrapancreatic accessory spleen, imaging mass spectrometry, lipid excretion, Pbx1, Tlx1

INTRODUCTION

Epidermoid cyst in intrapancreatic accessory spleen (ECIPAS) is a rare cystic lesion in the pancreas. Since Davidson *et al.* reported the first case of ECIPAS in 1980 (1), 63 cases have been reported (2-4). ECIPAS is considered a benign lesion, with only a single case having been reported to undergo malignant transformation (5), and usually does not require surgical treatment. However, elevation of a tumor marker for pancreatic adenocarcinoma (serum CA19-9 levels) is detected in some cases (6). ECIPAS is sometimes difficult to differentiate from neoplastic diseases such as mucinous cystic neoplasm, intraductal papillary mucinous neoplasm, solid pseudopapillary tumor, or cystic pancreatic neuroendocrine tumor by imaging (7, 8). Thus, ECIPAS can mimic potentially malignant neoplasms, resulting in surgical resection.

The pancreatic tail is known as one of the common locations of the accessory spleen, and its occurrence is reported to be 9.3% (9). Most epidermoid cysts in the accessory spleen exist in the pancreatic tail, with one reported case in the pancreatic head (2) and another in the extrapancreatic accessory spleen (10). Normal spleens, including accessory spleens, do not contain epithelial tissue. The reason why epidermoid cysts are generated in the spleen without epithelial components is unknown. While

several hypotheses regarding the pathogenesis of ECIPAS have been proposed, its origin remains poorly understood. Moreover, the relationship between the pathogenesis of ECIPAS and the organogenesis of accessory spleen has not been discussed in detail. In this study, we examined the pathological features of ECIPAS in an effort to shed light on its origin. We focused on the organogenesis of the spleen by examining the expression of Pbx1 and Tlx1, transcription factors required for organogenesis of the spleen (11). The invasion of epithelial and pluripotent cells expressing Pbx1 or Tlx1 into the splenopancreatic mesenchyme during tissue development could give rise to the formation of a spleen around the pancreatic tail epithelial primordium.

METHODS

Cases and sample preparation

This study enrolled six surgically resected ECIPASs between January 2009 and March 2021 at Tokushima University Hospital. All cases had undergone routine histological diagnosis. Clinical information was extracted from electronic medical records. Formalin-fixed and paraffin-embedded (FFPE) blocks, hematoxylin-eosin (HE) stained slides of all 6 cases used for routine diagnostic workup were retrieved from the archives of the Department of Pathology, Tokushima University Hospital. This study was conducted retrospectively from data and material obtained during ordinary clinical practice. This study was in accordance with the principles of the Helsinki Declaration and later amendments or comparable ethical standards. This study was approved by the Ethical Review Board of Tokushima University Hospital (No. 3891).

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Immunohistochemistry

To confirm the existence of littoral cells in splenic tissue, 2 μm sections of FFPE blocks of case #2 were subjected to immunohistochemical staining for CD8. To elucidate the features of epithelial cells in ECIPAS, 2 μm sections of FFPE blocks from all 6 cases were also subjected to immunohistochemical staining. To examine the expression pattern of Pbx1 and Tlx1, neighboring section was subjected to immunohistochemical staining for Pbx1 and Tlx1. Primary antibodies used for immunohistochemistry are shown in Table 1. According to the manufacturer's instructions, immunohistochemical staining was performed using a Bond-Max automated immunostainer (Leica Microsystems, Wetzlar, Germany). As previously reported, the immunohistochemistry results were evaluated separately in the superficial and basal layers of the stratified epithelium and monolayered epithelium (12). Immunohistochemistry for αSMA (clone 1A4; dilution 1:100; Dako; stained with Ventana Benchmark Ultra, Roche Diagnostic, Basel, Switzerland) had been performed during the routine diagnostic workup for case #3, and the immunohistochemically stained slide was retrieved from the archives of the Department of Pathology, Tokushima University Hospital.

Before submitting FFPE sections for imaging mass spectrometry, described below, additional immunohistochemistry for adipophilin (clone AP125; undiluted; Progen) was performed by Ventana Benchmark Ultra according to the manufacturer's instructions.

For comparison of Pbx1 and Tlx1 expression between ECIPAS and non-ECIPAS pancreatic tissue, 2 μm sections of FFPE blocks of the pancreatic head, body, and tail collected from 3 autopsy cases were also subjected to immunohistochemical staining. Details of the 3 autopsy cases are shown in Supplementary Table 1.

Imaging mass spectrometry

The FFPE sections of ECIPAS tissue (5 μm thick) were mounted onto indium tin oxide-coated slides (Bruker Daltonics, Billerica, MA). Optical images of the sections were obtained using a scanner (GTX830; Epson, Tokyo, Japan) before analysis by imaging mass spectrometry (IMS). The matrix solution, which contained 40 mg of α -cyano-4-hydroxycinnamic acid (Nacalai Tesque, Kyoto, Japan) in 6 mL of acetonitrile/water/trifluoroacetic acid [70/29.9/0.1(v/v)], was sprayed onto the tissue sections and ionization, and imaging of the phospholipids was performed using a

Table 1. Primary antibodies used for immunohistochemical staining.

Antibody	Dilution	Clone	Source
CD8	1:40	4B11	Leica
CK7	1:50	OV-TL 12/30	Dako
CK20	1:50	KS20.8	Dako
CK5/6	1:100	D5/16 B4	Dako
p63	1:50	4A4	Nichirei
CA19-9	1:50	116-NS-19-9	Dako
CEA	1:50	II-7	Dako
MUC1	Ready to use	Ma695	Leica
MUC4	1:100	8G7	Santa Cruz
WT-1	1:50	6F-H2	Dako
D2-40	1:100	D2-40	Dako
Adipophilin	1:800	Polyclonal	Arigo
SOX9	1:150	Polyclonal	Atlas Antibodies
Pbx1	1:200	4A2	Abnova
Tlx1	1:400	Polyclonal	Abcam

matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF-MS, rapid flex; Bruker Daltonics). Flex-Imaging software (version 5.0; Bruker Daltonics) was used for the data analysis. Data were analyzed, and candidate lipids were identified using the LIPIDMAPS (<https://www.lipidmaps.org/>) (accessed on Oct 8, 2021) lipid database.

RESULTS**Clinicopathological findings**

Table 2 summarizes the clinicopathological features of the six ECIPAS samples. The median age of the patients with ECIPAS was 57.8 years (range 41-67 years), among whom 3 (50%) were male, and 3 (50%) were female. Case #4 was symptomatic, but other cases were asymptomatic. Only one case (case #4) showed elevated serum CA19-9 levels. In case #6, serum CA19-9 was 29 U/mL two months before distal pancreatectomy (DP), although elevated levels (1875.9 U/mL) were recorded 6 months before DP. ECIPAS in all cases were located in the pancreatic tail, and all

Table 2. Clinicopathological findings of ECIPAS.

Case	Age	Sex	Symptom	Serum CA19-9 (U/mL)	Location	Surgery	Size (mm)	Cyst	Epithelial lining
#1	57	M	None	26	Tail	DP	19	Multilocular	Multilayer and monolayer
#2	63	F	None	11	Tail	DP	21	Multilocular	Multilayer and monolayer
#3	55	M	None	11	Tail	DP	65	Unilocular	Multilayer and monolayer
#4	67	F	Occasional back pain	52	Tail	DP	10	Unilocular	Multilayer and monolayer
#5	41	F	None	9	Tail	DP	22	Unilocular	Multilayer and monolayer
#6	64	M	None	29	Tail	DP	23	Multilocular	Multilayer and monolayer

Normal range of serum CA19-9: < 37 U/mL

Abbreviations: DP: Distal pancreatectomy, F: Female, M: Male

patients underwent distal pancreatectomy. The median size of the cysts was 21.5 mm (range 10-65 mm).

Histopathological findings

Among the 6 cases, cysts in 3 cases were multilocular and the remaining 3 cases were unilocular. Typical gross findings and loupe images of ECIPAS are shown in Figure 1a,b; the case shown in Figure 1a,b is case #2, which was composed of multilocular cysts. The epithelium was found to be either monolayered or stratified. Epithelial cells were squamous- or urothelial-like, or cuboidal (Figure 1c). No cases showed keratinizing or atypical epithelia. Several epithelial cells showed clear and abundant cytoplasm, implying lipid accumulation (Figure 1d). Intracystic fluids were serous or gelatinous. Histologically, intracystic fluid contained cholesterol clefts and foamy macrophages; it was indicated that epithelial cells with clear and abundant cytoplasm could excrete lipid. The cyst in case #3 contained a 19mm-sized yellowish solid mass (Figure 1e). The solid mass consisted of cholesterol and foamy macrophages with an eosinophilic amorphous matrix, implying similarity to the intracystic fluid (Figure 1f, g). Smooth muscle was detected in the cyst wall of case #3 (Figure

1h-j).

Splenic tissue components surrounding cysts contained red pulp and white pulp, and the presence of littoral cells was confirmed by immunohistochemistry for CD8 (Figure 1k).

Immunohistochemical findings

Immunohistochemical staining was evaluated separately in the superficial and basal layers of the multilayered and monolayered epithelium. Representative images of immunohistochemistry are shown in Figure 2. The superficial layer showed a positive result for CK7, CK20, CA19-9, and MUC4 in all 6 cases (100%), CK5/6, CEA, and MUC1 in 4 cases (67%), and SOX9 in 3 cases (50%). On the other hand, the basal layer showed a positive result for CK5/6, p63, MUC1, and D2-40 in all 6 cases (100%; expression of D2-40 was weak in all cases), but was negative for CK20, CA19-9, and CEA in all cases. Monolayered epithelium showed a positive result for CK7, CK5/6, CA19-9, MUC1, and MUC4 in all 6 cases (100%), p63, CEA, SOX9 in 3 cases (50%), CK20 in 2 cases (33%), and a negative result for WT-1, D2-40, and c-kit in all 6 cases. Several epithelial cells with clear cytoplasm showed positive result for adipophilin,

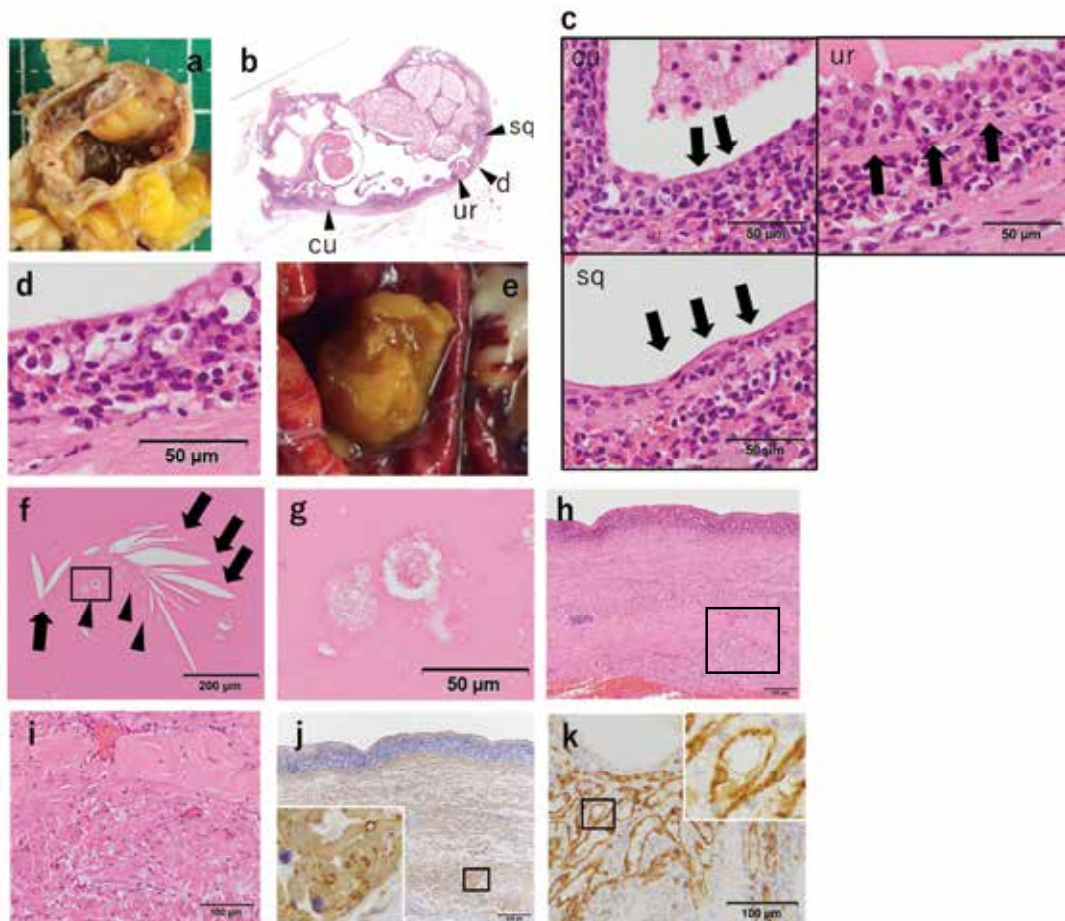


Fig 1. (a) Macroscopic and (b) histological images of epidermoid cyst in intrapancreatic accessory spleen (ECIPAS). ECIPAS in case #2 was 21mm-sized, and was composed of multiple cysts. Solid component consisted of epithelial cells was seen. (c) Magnified image of figure 1b. Multiple cysts were covered by stratified squamous- (“sq”) or urothelial-like (“ur”), or monolayered cuboidal (“cu”) epithelium. “sq”, “ur”, and “cu” correspond with each arrowhead in figure 1b. (d) Magnified image of area indicated by arrowhead “d” in figure 1b. Epithelial cells with clear and abundant cytoplasm, resembling sebaceous gland. (e) A 19mm-sized yellowish mass was detected in the cyst. This image was obtained before formalin fixation. (f) Histologically, the mass consisted of cholesterol (arrow), foamy macrophages (arrowhead) and an eosinophilic amorphous matrix. (g) Magnified image of box shown in figure 1f. (h) A layer of smooth muscle was detected in the stroma of the cyst wall. (i) High power view of box shown in figure 1h. (j) Smooth muscle was confirmed by immunohistochemistry for αSMA. (k) Littoral cells were confirmed by immunohistochemistry for CD8. Figure (a-d, k) show image of case #2, and (e-j) show image of case #3.

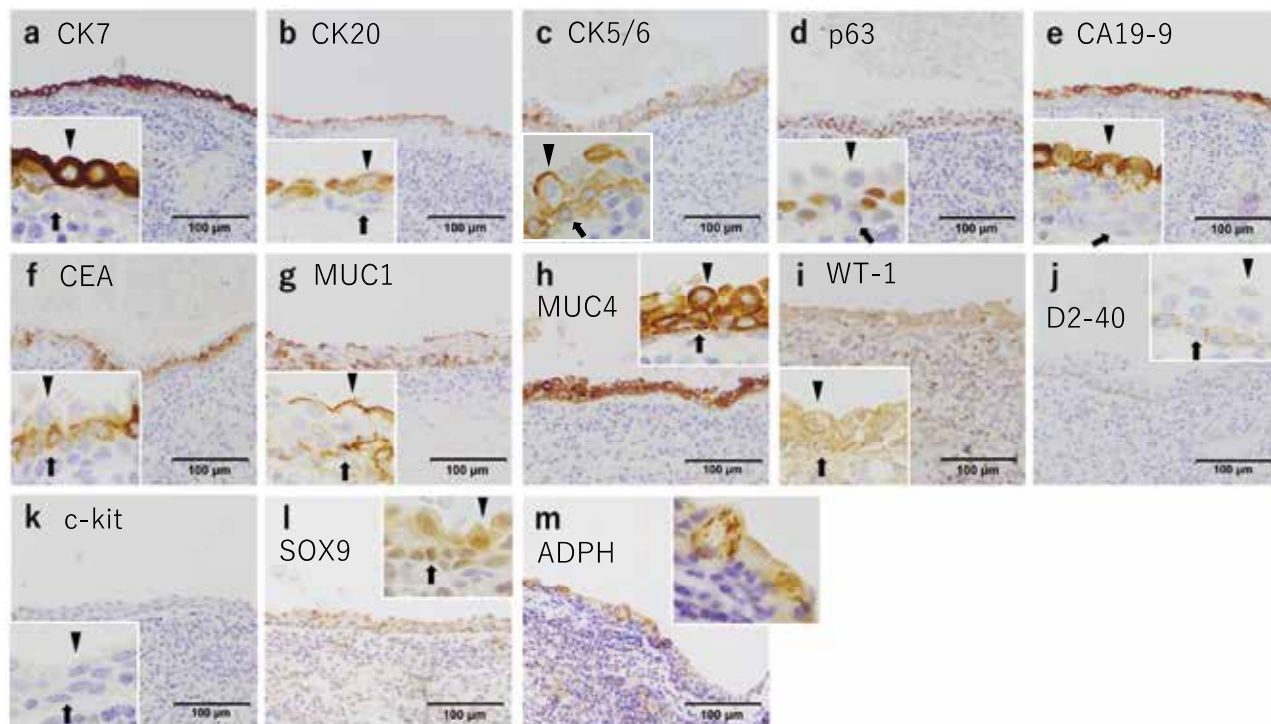


Fig 2. Representative immunohistochemistry images for (a) CK7, (b) CK20, (c) CK5/6, (d) p63, (e) CA19-9, (f) CEA, (g) MUC1, (h) MUC4, (i) WT-1, (j) D2-40, (k) c-kit, (l) SOX9, and (m) adipophilin (ADPH). In figure (a-l), each arrowhead shows superficial layer, and each arrow shows basal layer. Epithelium in figure (m) is monolayered epithelium. Figures 2 a-f and h-l show the result of case #5, Figure 2g shows the result of case #2, and Figure 2m shows the result of case #4, respectively.

with epithelial cells in the superficial layer in 2 cases (33%) and monolayered epithelium in 4 cases (67%). The coexistence of p63-positive cells in the basal layer and CA19-9-positive cells in the superficial layer was detected. Table 3 summarizes the results of the immunohistochemical staining.

Imaging mass spectrometry

As shown above, case #2 contained several epithelial cells with clear and abundant cytoplasm, which were positive for adipophilin (Supplementary Fig 1), implying lipid excretion. For different approaches for its features, FFPE section of case #2 was subjected to imaging mass spectrometry (IMS) analysis. Areas shown by white dashed squares were analyzed with IMS. IMS analysis indicated that a protonated molecular ion at m/z 712.5 ($[M+H]^+$) was observed in the intracystic fluid, indicating the accumulation of lipids (Figure 3). A protonated molecular ion at m/z 712.5 ($[M+H]^+$) was also weakly observed in epithelial cells; these epithelial cells had clear and abundant cytoplasm, though they did not resemble sebaceous gland cells. Twenty-nine candidate lipids were identified, including glycerol-phosphocholines and glycerophosphoethanolamines (Supplementary Table 2).

Immunohistochemical analysis of Pbx1 and Tlx1

The immunohistochemical expression of the transcription factors Pbx1 and Tlx1 was evaluated. Positive staining for Pbx1 was observed in the superficial layer of 3 cases, in the basal layer of 6 cases, and in the monolayered epithelium of 4 cases (Figure 4a). On the other hand, no case showed positive staining in nuclei for Tlx1; only cytoplasmic staining in the superficial layer was detected in 2 cases. (Figure 4b, c, Table 3). Some of epithelial cells with cytoplasmic staining for Tlx1 showed negative result for Tlx1; however, other epithelial cells with cytoplasmic staining for Tlx1 showed positive result for immunohistochemical

analysis for Pbx1.

Pancreatic duct and acinic cells were positive for Pbx1 but negative for Tlx1 in all 6 cases. Stromal cells in the splenic tissue component showed positive staining for Pbx1 but were negative for Tlx1 in all 6 cases. In non-ECIPAS pancreatic tissue, positive

Table 3. The number and proportion of cases (%) that were immunohistochemically positive for each primary antibody and its anatomical position in ECIPAS.

Primary antibody	Multilayer		Monolayer
	Superficial	Basal	
CK7	6 (100%)	3 (50%)	6 (100%)
CK20	6 (100%)	0	2 (33%)
CK5/6	4 (67%)	6 (100%)	6 (100%)
p63	0	6 (100%)	3 (50%)
CA19-9	6 (100%)	0	6 (100%)
CEA	4 (67%)	0	3 (50%)
MUC1	4 (67%)	6 (100%)	6 (100%)
MUC4	6 (100%)	2 (33%)	6 (100%)
WT-1	0	0	0
D2-40	0	6 (100%) (weak)	0
c-kit	0	0	0
SOX9	3 (50%)	1 (17%)	3 (50%)
Adipophilin	2 (33%)	0	4 (67%)
Pbx1	3 (50%)	6 (100%)	4 (67%)
Tlx1	0	0	0

staining for Pbx1 was observed in the pancreatic duct and acini (Supplement Figure 2a). In contrast, positive staining for Tlx1 was observed in a few acinic cells, while the epithelial cells of

the pancreatic duct were negative (Supplementary Figure 2b). Similar results were obtained in the pancreatic head, body, and tail in all 3 cases.

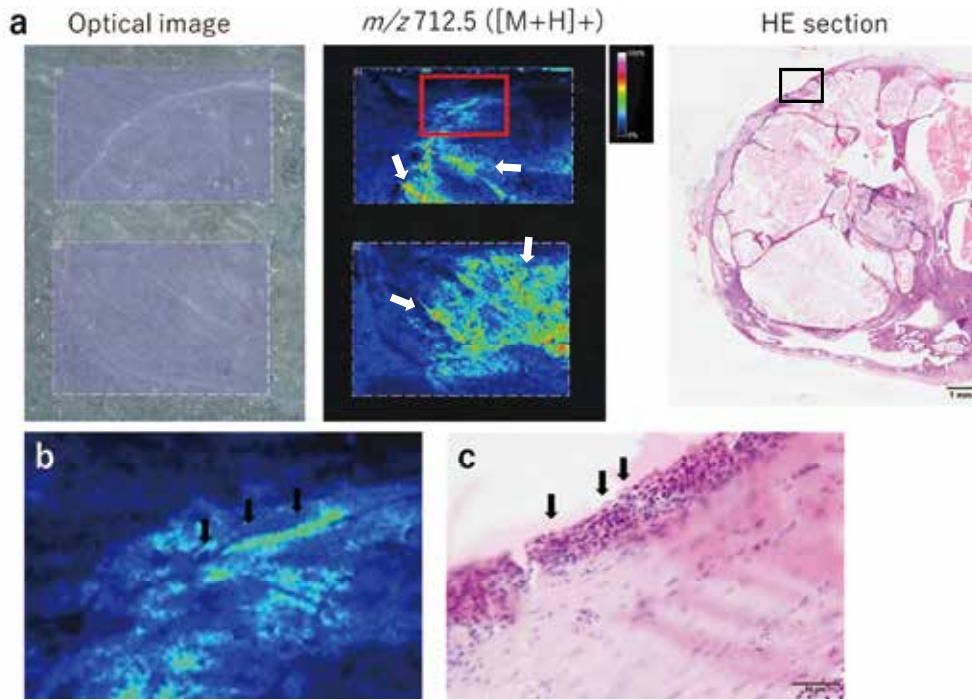


Fig 3. (a,b) Imaging mass spectrometry (IMS) of ECIPAS (case #2). (a) A protonated molecular ion at m/z 712.5 ($[M+H]^+$) was observed in the intracystic fluid, indicating the accumulation of lipids (arrows). Formalin-fixed and paraffin-embedded (FFPE) sections used for IMS were also subjected to hematoxylin-eosin (HE) staining. (b) High power view of the red box indicated in (a). A weak signal was detected in the cyst wall, implying lipid accumulation (arrow). (c) High power view of the box of HE section in (a); the area corresponding to (b) is shown. Epithelial cells with clear and abundant cytoplasm were detected in the cyst wall where a weak signal was detected. Since the paraffin section had been damaged during the imaging mass spectrometry procedure, the section is partially detached from glass slide.

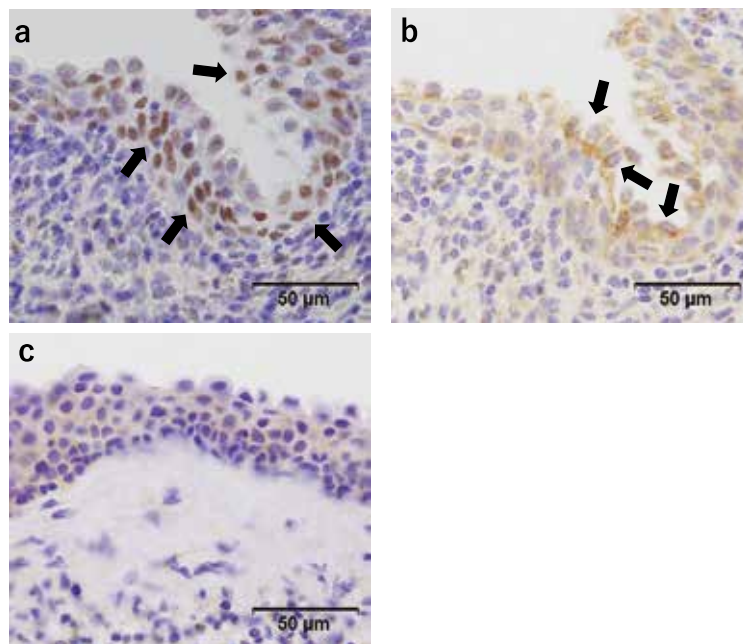


Fig 4. Immunohistochemistry for Pbx1 and Tlx1. (a) Immunohistochemistry for Pbx1 in multilayered epithelium in case #5. Pbx1 positive staining was observed in the superficial and basal layer (arrows). (b) Immunohistochemistry for Tlx1 in case #5. Tlx1 was partially positive only in the cytoplasm (arrow), but nuclear staining was not detected. (c) Immunohistochemistry for Tlx1 in case #6.

DISCUSSION

In this study, we analyzed the histological and immunohistopathological features of ECIPAS, as well as the functional features of the epithelia, using surgical specimens from a single institute. The patients' clinical features resembled those previously reported (13), though only case #4 exhibited elevated CA19-9 levels. Although the exact etiology underlying the elevation of CA19-9 in case #4 is unknown, a certain inflammatory change concerned with occasional back pain could have been related to the elevation of CA19-9.

Immunohistological analysis of the epithelial lining revealed that the superficial layer was positive for CK20, CA19-9, and CEA, whereas the basal layer was positive for p63 and D2-40. CK7, CK5/6, MUC1, MUC4 and SOX9 were positive in both the superficial and basal layers; expression of CK7, MUC4 and SOX9 was more frequent in the superficial layer than in the basal layer, whereas expression of CK5/6 and MUC1 was more frequent in the basal layer than the superficial layer. Our results are consistent with those of a previous report (12). CK7 and CK20 (low molecular weight forms of keratin), tend to be expressed in glandular epithelial cells, and SOX9 (a marker of pancreatic progenitor cells) are persistently expressed in adult pancreatic ductal cells (14, 15). CEA tend to be positive in some glandular epithelial cells and adenocarcinoma, but not in normal pancreatic ductal cells. In contrast, p63, CK5/6 and MUC4 tend to be expressed in squamous epithelial cells (16). D2-40 can also show positive staining in basal layer of squamous epithelium, although is well-known marker for mesothelial cells (17). Therefore, two different types of epithelia may coexist in ECIPAS. MUC1 is well known to be expressed in normal pancreatic duct cells; however, expression of MUC1 in squamous epithelium in esophagus has been reported (18). The fact that MUC1 can be expressed in both pancreatic duct cells and squamous epithelial cells may explain the expression of MUC1 in both superficial and basal layer of epithelium in ECIPAS. CA19-9 is known as an adenocarcinoma marker, but can also be positive in squamous cell carcinoma and even in a certain kind of normal squamous epithelial cell (19). Expression of WT-1, one of the mesothelial markers, and c-kit, one of the markers sometimes used as stem cell marker, was not seen in all cases.

Since the intracystic fluid contained cholesterol clefts, and adipophilin-positive epithelial cells were detected, the presence of lipid in intracystic fluid was expected. Imaging mass spectrometry revealed the distribution of a protonated molecular ion at m/z 712.5 ($[M+H]^+$) in the intracystic fluid. Since α -cyano-4-hydroxycinnamic acid/acetonitrile/trifluoro acetic acid was used as the matrix solution, the observed signals were likely to be lipids; moreover, the adipophilin-positive epithelial cells are expected to be capable of excreting lipids. Although further research is needed, this is the first report to show the presence of epithelial cells with function of excreting lipids in ECIPAS. Thus, it is questionable whether epithelium derived from the pancreatic duct, often containing pancreatic juice, is able to gain lipid-excretion function by metaplastic transformation.

Pbx1 and Tlx1 are essential factors for the organogenesis of the spleen. Pbx1 promotes spleen expansion via transactivation of Nkx2.5 and Tlx1, and repression of the CDK-inhibitor p15 (20). Tlx1-expressing cells have been suggested to be mesenchymal progenitor cells with the potential to function as lymphoid tissue organizer cells, and they have the potential to differentiate into mature stromal cells in the neonatal spleen and in severely damaged young adult spleens (21). Pbx1 is also thought to be a regulator of a marker of early splenic anlage (Wt1) through regulation of Tlx1 (22). Moreover, the absence of Pbx1 or Tlx1 causes asplenia (21-24), and Pbx1 is essential for pancreatic

development and function (25). Our results revealed that Pbx1 was present in the cystic epithelium and stromal cells in the splenic tissue component. The meaning of cytoplasmic positivity for Tlx1 in the superficial layer of cystic epithelium remained unclear. However, existence of epithelial cells showing cytoplasmic positivity by immunohistochemistry for Tlx1, but without expression of Pbx1 concerning with pancreatic development and function, was indicated. Given that the spleen starts to develop as the dorsal splenopancreatic mesenchyme, and that expression of Tlx1 in the stromal cells in adult spleen is decreased unless events such as massive death in splenic stromal cells is occurred (21), this result may be related to the development of splenic tissue in ECIPAS.

Several hypotheses regarding the histogenesis of the epithelium of splenic epidermoid cyst (SEC) have been proposed, such as inclusion of mesothelial cells (26), teratomatous origin, or inclusion of fetal squamous epithelium (27). Kadota *et al.* suggested that the origin of epithelial cells of ECIPAS and SEC are similar (28); however, Hirabayashi *et al.* suggested that the epithelia have different origins (12). Recently, increasing evidence has suggested that cyst epithelium of ECIPAS is derived from the pancreatic duct (12, 28-30). The present study detected various combinations of epithelial cells by marker expression: squamous cell, pancreatic duct cell, and sebaceous gland. Although sebaceous gland differentiation of squamous epithelium has been indicated in several reports of lymphoepithelial cyst in the pancreas (31, 32), the presence of variable epithelial cells in individual ECIPAS is not consistent with epithelial cells arising from metaplastic changes of pancreatic duct cells. Smooth muscle in the cyst wall was also detected; since a layer of smooth muscle does not surround the pancreatic duct, the origin of smooth muscle cannot be explained by invagination of the pancreatic duct. From these findings, we hypothesize that epithelial cells or other aberrant tissue may have invaginated into pancreatic tissue, the splenopancreatic mesenchyme, or intrapancreatic accessory spleen during the development stage. Our hypothesis does not disprove other hypotheses reported before, since multiple mechanisms may contribute to the formation of ECIPAS, a possibility that is supported by the variety of markers expressed in cyst epithelium. Further evaluations of embryonic or neonatal intrapancreatic accessory spleen or ECIPAS is needed to confirm these hypotheses. However, it is difficult to collect embryonic or neonatal cases, since all previously reported cases of ECIPAS have been detected in adults. Thus, the development of a new animal model of ECIPAS is required.

In conclusion, the findings of this study elucidated new aspects of ECIPAS and its epithelia: expression of Pbx1 was detected in the epithelial cells by immunohistochemistry, and accumulation of lipid in the intracystic fluid was revealed. As this study was limited to a few cases, these findings should be verified with more extensive studies that examine a larger number of cases. Genetic profiling is also required to confirm the hypotheses regarding the pathogenesis of ECIPAS.

CONFLICT OF INTERESTS

All authors declare no conflict of interest for this article.

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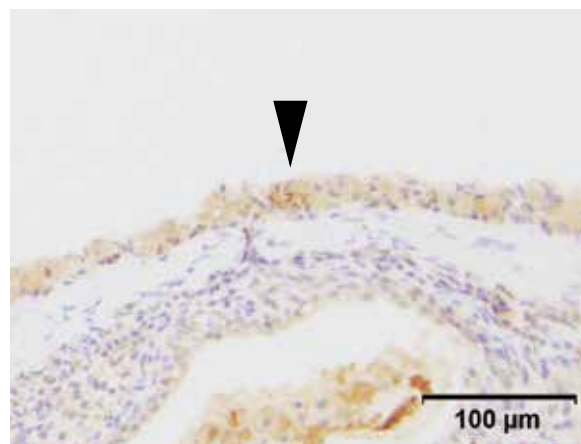
AUTHOR CONTRIBUTION

S.S. and K.T. designed the research ; M.I-S. and K.T. secured funding ; S.S., M.I-S., Y.M., T.K.1, T.K.2, and S.T performed the experiments ; Y.S., Y.B., H.U., and M.S. provided case samples ; S.S., M.I-S, M.M., H.O., T.O. and K.T. performed data validation and analysis, and S.S., M.I-S. and K.T. prepared the original draft of the manuscript.

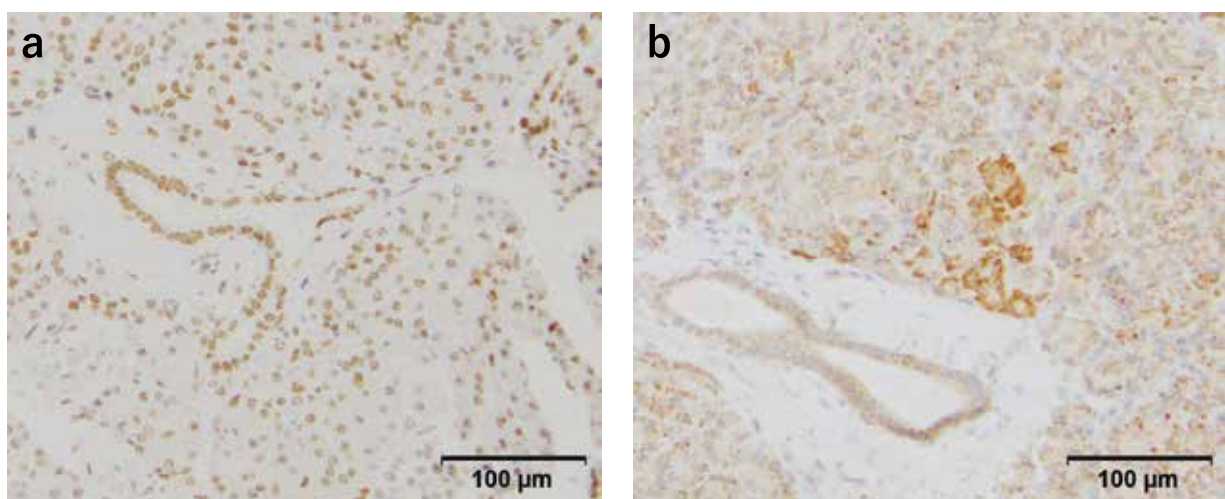
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Supplementary Fig 1. Immunohistochemistry for adipophilin in case #2. Positive staining was detected in epithelial cells with clear and abundant cytoplasm (arrow head); notably, adipophilin positive epithelial cells were less numerous than in case #4.



Supplementary Fig 2. Immunohistochemistry for Pbx1 and Tlx1 in non-ECIPAS pancreatic tissue (case #A1). (a) Expression of Pbx1 was detected in the pancreatic duct and acini. (b) Tlx1 was positive in a few acinic cells, however pancreatic duct epithelial cells were negative.

Supplementary Table 1. Details of the three non-ECIPAS autopsy cases.

Case	Age	Sex	Cause of death	Postmortem time	Changes in pancreatic duct
#A1	80-89	M	Systemic amyloidosis, cardiac failure	2 h 6 min	mucinous metaplasia
#A2	60-69	M	Sigmoid colon cancer, interstitial pneumonia	4 h 28 min	no remarkable change
#A3	80-89	M	Pulmonary thrombosis, acute pericarditis	2 h 6 min	no remarkable change

Supplementary Table 2. Twenty-nine candidate lipids with a molecular ion at m/z 712.5 observed by IMS.

Systematic name	Formula	Mass
1,2-dihexadecanoyl-sn-glycero-3-O-(N,N,N-trimethyl)-homoserine	C ₄₂ H ₈₁ NO ₇	711.6013
1-tridecanoyl-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phosphocholine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-tridecanoyl-glycero-3-phosphocholine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-dodecanoyl-2-(7Z,10Z,13Z,16Z-docosatetraenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(9Z-tetradecenoyl)-2-(8Z,11Z,14Z-eicosatrienoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(9Z-hexadecenoyl)-2-(6Z,9Z,12Z-octadecatrienoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(9Z-hexadecenoyl)-2-(9Z,12Z,15Z-octadecatrienoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(6Z,9Z,12Z-octadecatrienoyl)-2-(9Z-hexadecenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(9Z,12Z,15Z-octadecatrienoyl)-2-(9Z-hexadecenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-hexadecanoyl-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(8Z,11Z,14Z-eicosatrienoyl)-2-(9Z-tetradecenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-2-tetradecanoyl-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-(7Z,10Z,13Z,16Z-docosatetraenoyl)-2-dodecanoyl-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1,2-di-(9Z,12Z-heptadecadienoyl)-sn-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-hexadecanoyl-2-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
1-tetradecanoyl-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-glycero-3-phosphoethanolamine	C ₃₉ H ₇₀ NO ₈ P	711.4839
Pelargonidin 3-(2G-xylosylrutinoside)	C ₃₂ H ₃₉ O ₁₈	711.2136
Cyanidin 3-(4"-malonyl-2"-glucuronosylglucoside)	C ₃₀ H ₃₁ O ₂₀	711.1409
Cyanidin 3-(6"-malonyl-2"-glucuronosylglucoside)	C ₃₀ H ₃₁ O ₂₀	711.1409
-	C ₃₁ H ₃₅ O ₁₉	711.1773
Peonidin 3-(6'-malonylglucoside)-5-glucoside	C ₃₁ H ₃₅ O ₁₉	711.1773
Delphinidin 3,5-di(6-acetylglucoside)	C ₃₁ H ₃₅ O ₁₉	711.1773
2,3-bis-(3R-hydroxy-tetradecanoyl)-alphaD-glucosamine-1-phosphate	C ₃₄ H ₆₆ NO ₁₂ P	711.4323
N-(2-hydroxyhexacosanoyl)-4R-hydroxysphinganine	C ₄₄ H ₈₉ NO ₅	711.6741
N-(2-hydroxytetracosanoyl)-4R-hydroxyeicosasphinganine	C ₄₄ H ₈₉ NO ₅	711.6741
N-(hexadecanoyl)-1-alpha-glucosyl-9-methyl-4E,8E-sphingadienine	C ₄₁ H ₇₇ NO ₈	711.5649
N-(2R-hydroxy-3E-nonadecenoyl)-1-beta-D-glucopyranosyl-9-methyl-tetradecasphing-4E,8E-dienine	C ₄₀ H ₇₅ NO ₉	711.5285
N-(2R-hydroxy-3E-octadecenoyl)-1-beta-D-glucopyranosyl-hexadecasphing-4E,8Z-dienine	C ₄₀ H ₇₅ NO ₉	711.5285
N-(eicosanoyl)-1-beta-glucosyl-4E,6E-pentadecasphingadienine	C ₄₁ H ₇₇ NO ₈	711.5649