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Original Contribution

Histological and immunohistochemical analyses of splenic epidermoid cysts

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ABSTRACT

Splenic epidermoid cyst (SEC) is a rare condition. We aimed to evaluate the immunohistochemical profiles of the epithelial lining of SECs. A total of 7 SEC cases were analyzed: 2 cases involved a monolayered epithelial lining and 5 cases involved a multilayered epithelial lining. Among the multilayered SECs, the superficial/luminal layer showed mucin 4 (MUC4), cytokeratin 5/6 (CK5/6), and CK7 expression in 5 cases (100%); MUC1, carcinoembryonic antigen (CEA), CA19-9, and thrombomodulin expression in 4 cases (80%); Wilms' tumor-1 (WT-1) and Hector Battifora mesothelial-1 (HBME-1) expression in 2 cases (40%), but it did not express p63 or D2-40. The basal layer expressed MUC1, CK5/6, p63, and thrombomodulin in 5 cases (100%); CK7 and WT-1 in 4 cases (80%); D2-40 in 3 cases (60%); CA19-9 and HBME-1 in 2 cases (40%) and MUC4 in 1 case (20%) but it did not express CEA. The analysis showed that all cases of multilayered SECs were negative for MUC2, MUC5AC, MUC6, CK20, calretinin, uroplakin-II, and uroplakin-III. Both cases of monolayered SECs expressed CK5/6, CK7, HBME-1, WT-1, and thrombomodulin but not MUC2, MUC4, MUC5AC, MUC6, p63, CEA, CK20, CA19-9, D2-40, uroplakin-II, or uroplakin-III. One case of monolayered SEC expressed MUC1 and calretinin. Our findings indicate that monolayered SECs have mesothelial-like characteristics, whereas multilayered SECs have glandular and squamous-like characteristics besides mesothelial-like characteristics. Furthermore, monolayered SECs may develop from mesothelial inclusion and monolayered SECs develop squamous and glandular metaplasia, which results in multilayered SECs.

1. Introduction

Development of a splenic epidermoid cyst (SEC) is a rare condition. According to a review of 42,327 autopsy cases, the incidence rate of SECs was found to be 0.07% [1]. Histological features of the epithelial splenic cyst lining are variable and include mesothelial-like flat, cuboidal, columnar, transitional, or stratified squamous cells [2]. Because of these histological features, splenic cysts with an epithelial lining are not only known as epidermoid cysts but also as epithelial cysts or mesothelial cysts [1,3,4]. Epidermoid cysts can be observed not only in the spleen but also in accessory spleens. It has been previously reported that epidermoid cysts in accessory spleens can be observed only in intrapancreatic accessory spleens but not in extrapancreatic accessory spleens; moreover, the epithelial lining of intrapancreatic epidermoid cysts have mixed features, such as glandular, squamous, and mesothelial cells, upon immunohistochemical analysis [5]. In this study, we evaluated the histological features and immunohistochemical characteristics of the epithelial lining of the SECs to gain an understanding of the SEC origin. Furthermore, we compared the histological and immunohistochemical characteristics between the SECs and the intrapancreatic epidermoid cysts.

2. Materials and methods

2.1. Cases and sample preparation

A total of 7 SECs that were surgically resected between January 1991 and February 2016 at Tokai University Hospital were included for analysis. The tissue samples were fixed in formalin and embedded in paraffin. Then, 4-μm sections were cut and subjected to hematoxylin-

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Table 1
Antibodies used in the immunohistochemical analysis.

Antibody	Clone	Dilution	Source
CEA	CEM010	1:100	Takara
CK7	OV-TL	1:100	Dako
CK5/6	D5/16 B4	1:100	Zymed
CK20	Ks20.8	1:100	Dako
p63	4A4	Ready-to-use	Ventana
CA19-9	NS19-9	1:20	TFB
D2-40	D2-40	1:2	Nichirei
MUC1	Ma695	1:50	Leica Microsystems
MUC2	Ccp58	1:100	Leica Microsystems
MUC4	8G7	1:25	Cell Marque
MUC5AC	CLH2	1:100	Leica Microsystems
MUC6	CLH5	1:100	Leica Microsystems
Uroplakin-II	BC21	1:2	Nichirei
Uroplakin-III	AU1	Ready-to-use	Nichirei
Thrombomodulin	1009	1:20	Dako
HBME-1	HBME-1	1:50	Dako
WT-1	WT49	1:50	Leica Microsystems
Calretinin	4HCLC	1:02	Life Technologies

Takara, Shiga, Japan; Dako, Glostrup, Denmark; Zymed, Carlsbad, CA, USA; Ventana Medical Systems, Tucson, AZ, USA; TFB, Tokyo, Japan; Nichirei, Tokyo, Japan; Leica Microsystems, Newcastle upon Tyne, United Kingdom; Cell Marque, Rocklin, CA, USA; Life Technologies, Carlsbad, CA, USA.
CEA, carcinoembryonic antigen; CA, carbohydrate antigen; CK, cytokeratin; MUC, mucin; HBME-1, Hector Battifora mesothelial-1; WT-1, Wilms' tumor-1.

eosin (HE) staining. This study was approved by the Research Ethics Committee of Tokai University School of Medicine (No. 17R078).

2.2. Immunohistochemical analysis

The antibodies used for the immunohistochemical staining of the formalin-fixed, paraffin-embedded tissues are summarized in Table 1. Immunohistochemistry was performed using a previously described protocol [5]. Except for the staining for carcinoembryonic antigen (CEA) and thrombomodulin, the automated immunohistochemistry instruments were used according to the manufacturer's instructions. The BondMax system (Leica Microsystems, Newcastle upon Tyne, United Kingdom) was used for the detection of cytokeratin (CK)7, CK5/6, CK20, CA19-9, D2-40, mucin (MUC)1, MUC2, MUC4, MUC5AC, MUC6, uroplakin-II, uroplakin-III, Hector Battifora mesothelial-1 (HBME-1), Wilms' tumor-1 (WT-1), and calretinin. Ventana Benchmark XT (Ventana Medical Systems, Tucson, AZ, USA) was used for the staining of p63. For CEA and thrombomodulin, the deparaffinized sections were placed in a solution of 0.3% hydrogen peroxide/methanol for 30 min to block endogenous peroxidase activity after antigen retrieval for thrombomodulin by treatment with 0.1% trypsin for 15 min at room temperature. After washing with phosphate buffered solution, the sections were incubated with primary antibodies against CEA or thrombomodulin for 1 h at room temperature. Sections were then incubated with Histofine Simple Stain MAX-PO MULTI (Nichirei, Tokyo, Japan) for CEA and polyclonal goat anti-mouse immunoglobulin (Dako, Glostrup, Denmark) for thrombomodulin for 30 min at room temperature. 3,3′-diaminobenzidine was used as a chromogen.

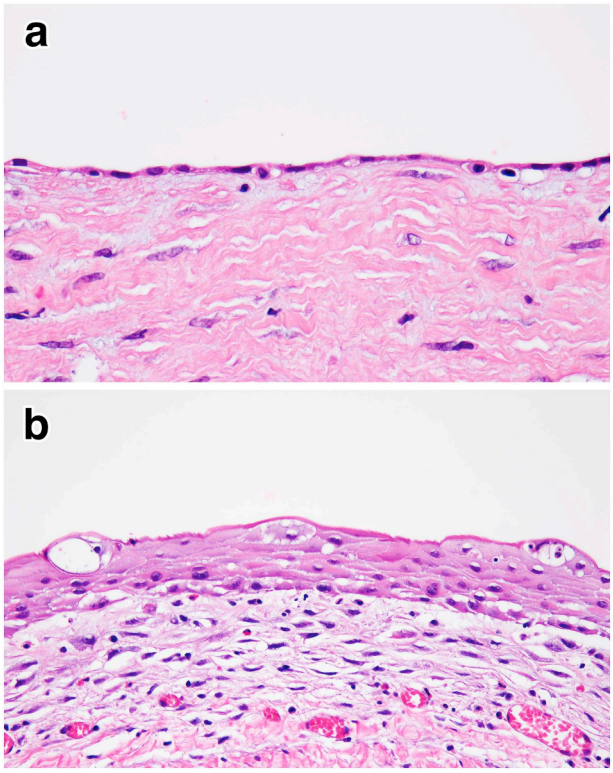

Fig. 1. Representative images of hematoxylin-eosin stains.
Monolayered splenic epidermoid cysts are lined by flat or low cuboidal epithelial cells possessing round nuclei, which resemble mesothelium (a).
Multilayered splenic epidermoid cysts are lined by stratified squamous-like epithelium, of which the surface layer shows a hobnail or wavy appearance (b).

3. Results

3.1. Clinicopathological findings

The median age of the patients with SEC was 24 years (range 14–42 years), among whom 3 were male (43%) and 4 were female (57%). The median size of the cysts was 100 mm (range 60–180 mm). Four patients complained of abdominal pain, while the others reported no pain. The serum carbohydrate (CA) 19-9 levels were above the normal range in 1 case, were within normal range in 4 cases, and were not recorded or not analyzed in 2 cases. Four patients underwent a splenectomy, two underwent marsupialization, and one underwent cystectomy. Five patients developed multilocular cysts and two developed unilocular cysts (Table 2).

Table 2
Summary of the splenic epidermoid cyst cases.

Case	Age (y)	Sex	Operation	Symptom	Serum CA19-9 (U/mL, normal range < 30 U/mL)	Size (mm)	Cyst	Epithelial lining
1	35	M	Splenectomy	None	13	60	Multilocular	Monolayer
2	26	F	Splenectomy	Abdominal pain	18	100	Unilocular	Multilayer
3	14	F	Cystectomy	Abdominal pain	n.a.	60	Multilocular	Multilayer
4	42	F	Splenectomy	Abdominal pain	4783.8	168	Multilocular	Multilayer
5	23	F	Splenectomy	Abdominal pain	n.a.	68	Unilocular	Multilayer
6	16	M	Marsupialization	None	13	100	Multilocular	Monolayer
7	24	M	Marsupialization	None	25.9	180	Multilocular	Multilayer

M, male; F, female; n.a., not available.

Table 3
Summary of the immunohistochemical analysis.

	Multilayer SEC (n = 5)			Monolayer SEC (n = 2)
	Superficial/luminal	Basal	Overall	
MUC1	4 (80%)	5 (100%)	5 (100%)	1 (50%)
MUC2	0	0	0	0
MUC4	5 (100%)	1 (20%)	5 (100%)	0
MUC5AC	0	0	0	0
MUC6	0	0	0	0
CK5/6	5 (100%)	5 (100%)	5 (100%)	2 (100%)
CK7	5 (100%)	4 (80%)	5 (100%)	2 (100%)
CK20	0	0	0	0
CA19-9	4 (80%)	2 (40%)	4 (80%)	0
CEA	4 (80%)	0	4 (80%)	0
p63	0	5 (100%)	5 (100%)	0
D2-40	0	3 (60%)	3 (60%)	0
Calretinin	0	0	0	1 (50%)
HBME-1	2 (40%)	2 (40%)	2 (40%)	2 (100%)
WT-1	2 (40%)	4 (80%)	4 (80%)	2 (100%)
Thrombomodulin	4 (80%)	5 (100%)	5 (100%)	2 (100%)
Uroplakin-II	0	0	0	0
Uroplakin-III	0	0	0	0

SEC, splenic epidermoid cyst; CEA, carcinoembryonic antigen; CA, carbohydrate antigen; CK, cytokeratin; MUC, mucin; HBME-1, Hector Battifora mesothelial-1; WT-1, Wilms' tumor-1.

3.2. Histological findings

Two cases demonstrated an epithelial lining composed of monolayered, flat, or low cuboidal cells possessing round nuclei, which resembles mesothelium (Fig. 1a). Five cases demonstrated a lining composed of multilayered, stratified squamous epithelium- or transitional epithelium-like epithelium, wherein the surface layer showed a hobnail or wavy appearance (Fig. 1b). No keratinization was observed in all cases (Table 3).

3.3. Immunohistochemical findings

The immunohistochemical analysis was conducted separately for multilayered SECs (n = 5) and monolayered SECs (n = 2). The immunohistochemical expression of multilayered SECs was evaluated in superficial/luminal and basal layers. Both the superficial/luminal and basal layers of the epithelial lining showed negative results for MUC2, MUC5AC, MUC6, CK20, calretinin, uroplakin-II, and uroplakin-III. The superficial/luminal layer showed positive results for MUC4, CK5/6, and CK7 in all 5 cases (100%); for MUC1, CEA, CA19-9, and thrombomodulin in 4 cases (80%) and for WT-1 and HBME-1 in 2 cases (40%). The superficial/luminal layer showed negative results for p63 and D2-40 in all 5 cases. The basal layer showed positive results for MUC1, CK5/6, p63, and thrombomodulin in all 5 cases (100%); for CK7 and WT-1 in 4 cases (80%); for D2-40 in 3 cases (60%); for CA19-9 and HBME-1 for 2 cases (40%); and for MUC4 in 1 case (20%). The basal layer showed negative results for CEA in all 5 cases (Fig. 2).

The 2 cases of monolayered SECs showed positive results for CK5/6, CK7, HBME-1, WT-1, and thrombomodulin but negative results for MUC2, MUC4, MUC5AC, MUC6, p63, CEA, CK20, CA19-9, D2-40, uroplakin-II, and uroplakin-III. One case of monolayered SEC showed positive results for MUC1 and calretinin (Fig. 3).

4. Discussion

This study analyzed the immunohistochemical profiles of the epithelial lining of SECs. The results showed differing immunohistochemical expression profiles between monolayered SECs and multilayered SECs. Both monolayered and multilayered SECs demonstrated positive MUC1, CK7, CK5/6, HBME-1, WT-1, and thrombomodulin expression but no MUC2, MUC5AC, MUC6, CK20, uroplakin-II, or uroplakin-III expression. MUC4, p63, CEA, CA19-9, and D2-40 expression was observed only in multilayered SECs, whereas calretinin expression was detected only in monolayered SECs.

Typically, HBME-1, WT-1, thrombomodulin, and calretinin expression can be observed in mesothelial cells [6]. D2-40 and CK5/6 are expressed not only in mesothelial cells but also squamous epithelial cells [6-9]. Moreover, p63, MUC1, and MUC4 have been shown to be expressed in squamous epithelium [7,8,10,11]. CEA and CA19-9 expression is observed in adenocarcinoma as well as benign glands [12]. The observed immunohistochemical expression patterns indicate that monolayered SECs have mesothelium-like characteristics, whereas multilayered SECs have glandular and squamous-like characteristics apart from mesothelium-like characteristics. In terms of the multilayered SECs, the surface/luminal layers expressed MUC4, CEA, CK7, and CA19-9 more strongly than the basal layers, whereas the basal layers expressed p63, D2-40, WT-1, MUC1, and thrombomodulin more strongly than the surface/luminal layers. Therefore, we presume that the surface/luminal layer of multilayered SECs has a more glandular character than the basal layer. In contrast, the basal layer demonstrates the characteristics of mesothelium and squamous epithelium more than glandular epithelium does.

Several hypotheses about the origin and histogenesis of SECs have been proposed such as embryonic inclusions of epithelial cells from adjacent structures, invagination of the capsular surface mesothelium during development, and a monodermal teratomatous nature [4,13,14]. Lifschitz-Mercer et al. [15] reported that SECs are either of teratomatous origin or develop from the inclusion of fetal squamous epithelium rather than squamous metaplasia of the mesothelium or inclusions of mature squamous epithelium because the cytokeratin (CK10, CK11, CK13, CK18, and CK19) expression pattern of SECs is similar to that of the stratified squamous epithelia of ovarian mature cystic teratoma, fetal epidermis, adult epidermis, and squamous metaplasia in peritoneal cysts. In contrast, Burrig and Arber et al. [14,16] suggested that the epithelial lining of SECs originates from mesothelial cells on the basis of transmission electron-microscopy and HBME-1 immunohistochemistry findings. In this study, both monolayered and multilayered SECs showed mesothelial characteristics such as expression of HBME-1, WT-1, and thrombomodulin. Multilayered SECs showed squamous and glandular characteristics besides mesothelial features. On the basis of these histological and immunohistochemical profiles, we speculate that monolayered and multilayered SECs have a common origin. Firstly, monolayered SECs may develop from invagination of the mesothelium, and secondly, metaplastic changes in squamous and glandular characteristics occur in monolayered SECs, resulting in multilayered epidermoid cysts.

The immunohistochemical profiles of 6 cases of epidermoid cysts in intrapancreatic accessory spleens have been described previously [5]. In this study, all the epidermoid cysts showed a multilayered epithelial lining. Immunohistochemical studies showed that the epidermoid cysts expressed MUC1, MUC4, p63, D2-40, CK5/6, CK7, CK20, CEA, CA19-9, and HBME-1 but yielded negative results for MUC2, MUC5AC, MUC6, WT-1, calretinin, thrombomodulin, uroplakin-II, and uroplakin-III. When we compared the epidermoid cysts in the intrapancreatic accessory spleen and multilayered SECs, we found that MUC1, MUC4, p63, D2-40, CK5/6, CK7, CEA, CA19-9, and HBME-1 were commonly expressed in both types, but CK20 expression was detected only in the epidermoid cysts of the intrapancreatic accessory spleen, whereas WT-1, and thrombomodulin expression was detected only in multilayered SECs. The multilayered SECs showed similar immunohistochemical profiles to that of the epidermoid cysts of the intrapancreatic accessory spleen. However, SECs have a more mesothelial character than epidermoid cysts of the intrapancreatic accessory spleen in that WT-1 and thrombomodulin expression is detectable only in multilayered SECs. Thus, we believe that the origin of SECs is different from that of epidermoid cysts of the intrapancreatic accessory spleen; the

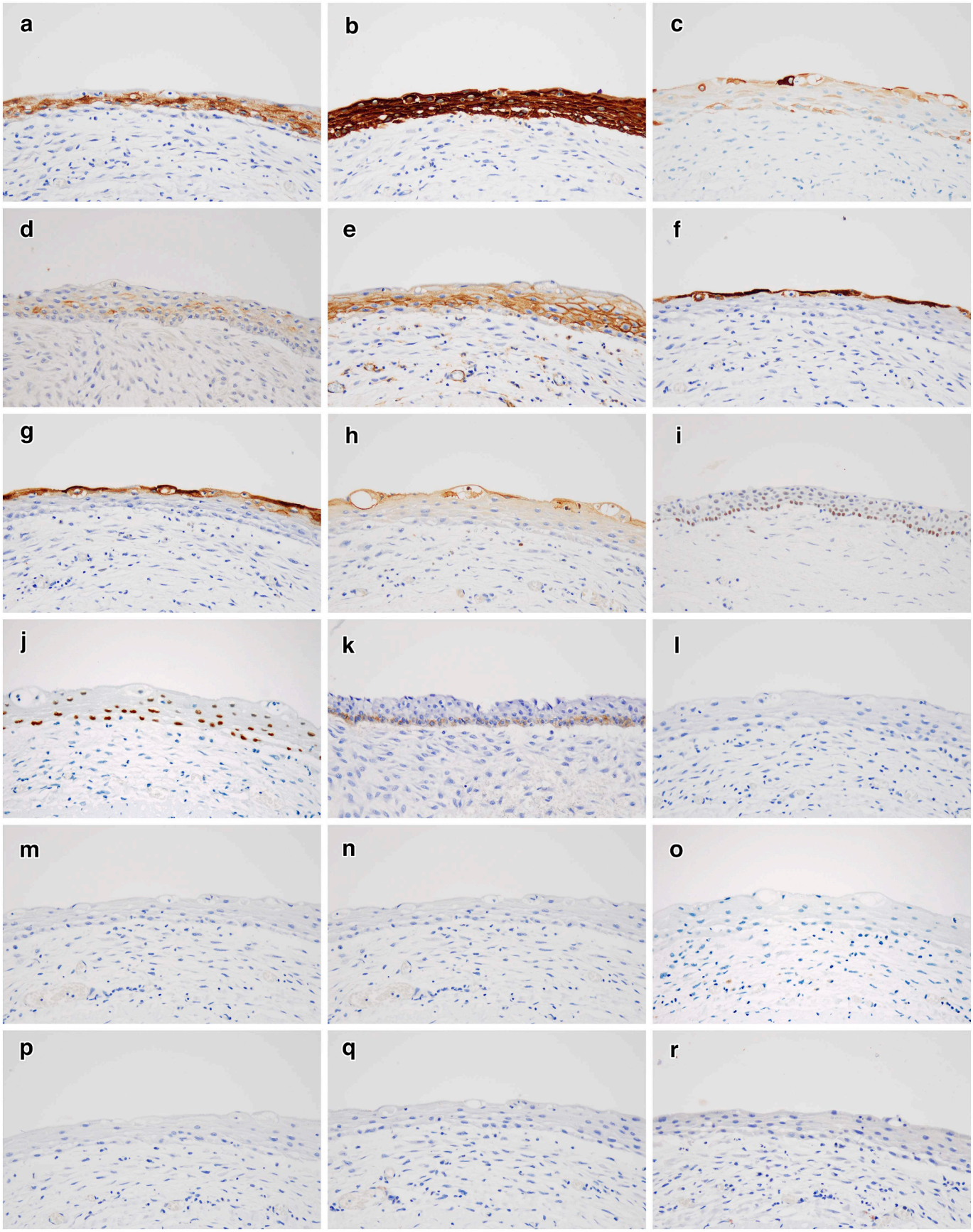

Fig. 2. Representative images of the immunohistochemical analysis of the epithelial lining of a multilayered splenic epidermoid cyst.
All layers expressed MUC1 (a), CK5/6 (b), CK7 (c), HBME-1 (d), and thrombomodulin (e). The superficial/luminal layer is mainly positive for expressed MUC4 (f), CA19-9 (g), and CEA (h). The basal layer is mainly positive for WT-1 (i), p63 (j), and D2-40 (k). MUC2 (l), MUC5AC (m), MUC6 (n), CK20 (o), calretinin (p), uroplakin-II (q), and uroplakin-III (r) were negative.

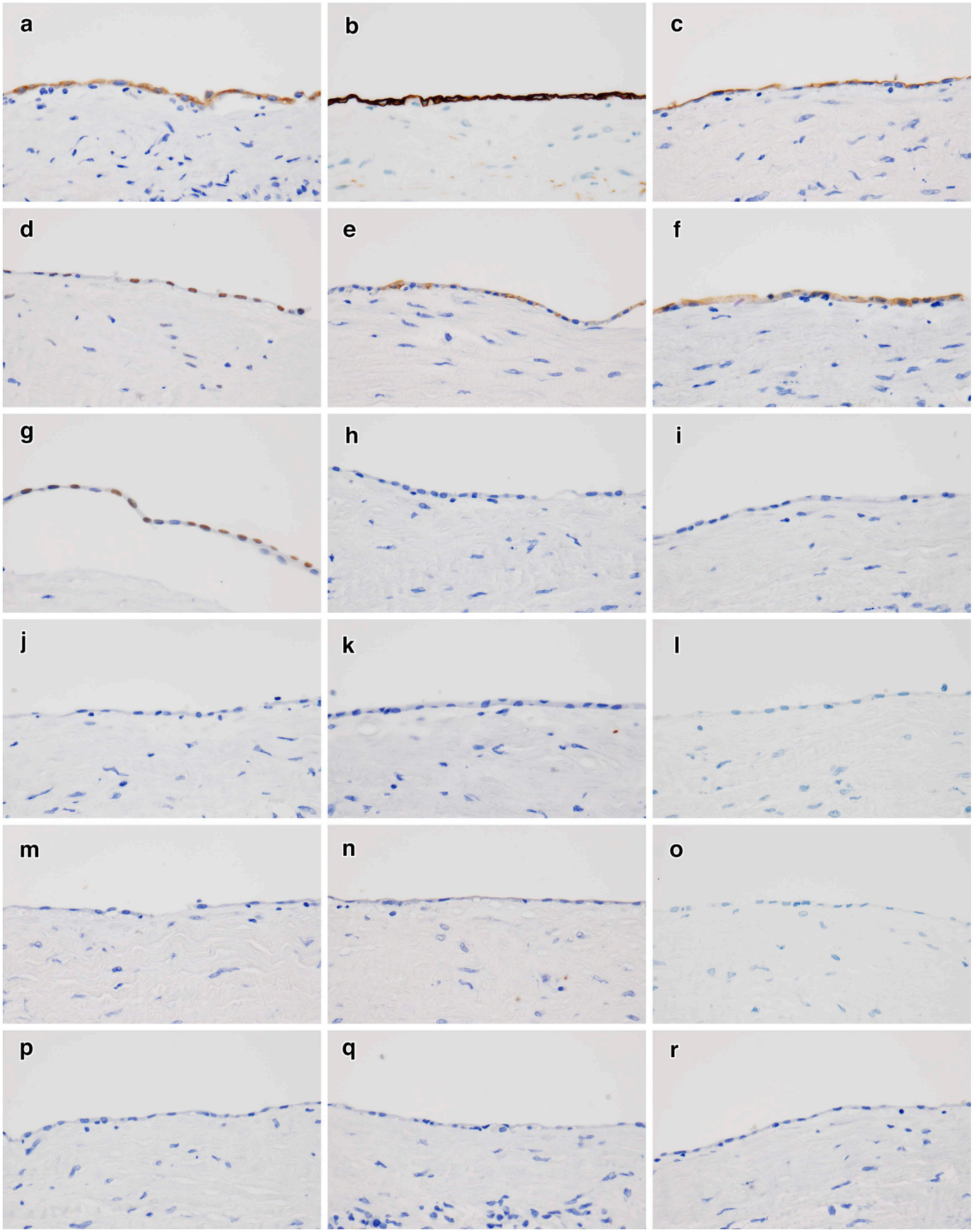

Fig. 3. Representative images of the immunohistochemical analysis of the epithelial lining of a monolayered splenic epidermoid cyst.
Both cases showed expression of CK5/6 (a), CK7 (b), HBME-1 (c), WT-1 (d), and thrombomodulin (e). One case showed expression of MUC1 (f) and calretinin (g). MUC2 (h), MUC4 (i), MUC5AC (j), MUC6 (k), CK20 (l), CA19-9 (m), CEA (n), p63 (o), D2-40 (p), uroplakin-II (q), and uroplakin-III (r) were negative.

intrapancreatic accessory spleen may have developed from the inclusion of the pancreatic duct with squamous metaplasia. However, SECs originate from mesothelial inclusion. Sasou et al. [3] also suggested that intrapancreatic accessory splenic cysts may be an embryonic inclusion of the pancreatic duct, while splenic cysts may be an inclusion of the mesothelium, based on the immunohistochemical patterns of a rare case of cysts simultaneously occurring in the intrapancreatic accessory spleen and spleen.

In conclusion, the findings of this study indicate two histological and immunohistochemical characteristics of SECs: monolayered SECs have mesothelial characteristics and multilayered SECs have a mixture of glandular, squamous, and mesothelial epithelium characteristics. On the basis of the immunohistochemical expression pattern, we speculate that firstly, monolayered SECs develop from mesothelial inclusion and monolayered SECs undergo squamous and glandular metaplasia, resulting in multilayered SECs. In addition, the immunohistochemical expression pattern of multilayered SECs was different from that of the intrapancreatic accessory spleen. Therefore, it is possible that SECs and epidermoid cysts of the intrapancreatic accessory spleen have a different origin. As this study was limited with few cases, our findings should be verified with larger studies that include more patients.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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None to disclose.

Research involving human participants

This study was approved by the Research Ethics Committee of Tokai University School of Medicine (No. 17R078).

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