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NOTE

Pathology

Pancreatic adenosquamous carcinoma with invasion to the spleen in a cat

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ABSTRACT. A four-and-a-half-year-old female Scottish Fold cat underwent partial pancreatectomy with en-bloc splenectomy. The resected specimen was a biphasic tumor that was diagnosed histologically and immunohistochemically as pancreatic adenosquamous carcinoma (ASC), a ductal carcinoma variant according to the WHO classification of tumors in humans. There was a gradual transition between the adenocarcinoma component and the squamous cell carcinoma component. The squamous cell carcinoma component comprised approximately 30–40% of the tumor. A pancreatic tumor infiltrated into the gastrosplenic ligament and spleen with regional lymph node and mesenteric metastases. Pancreatic ASC has not been reported in animals. This is a case report of feline pancreatic ASC with splenic involvement.

KEY WORDS: adenosquamous carcinoma (ASC), cat, pancreas

Feline exocrine pancreatic adenocarcinoma, a tumor with aggressive behavior, high metastatic rates, and poor prognoses [6, 8, 9], is the most common neoplastic condition of the exocrine pancreas. It commonly originates from the duct system, but may also originate from acinar tissue [13]. Conflicting data about the predominance of tubular carcinoma or acinar carcinoma in cats has been reported [14]. In domestic animals, the histogenesis of pancreatic adenocarcinoma has not been elucidated [6]; pancreatic adenocarcinomas are subtyped by the predominant arrangement of neoplastic cells [3, 6, 9]. It is uncertain whether the subtype of the pancreatic adenocarcinoma has prognostic significance [9].

Here, we report feline pancreatic adenosquamous carcinoma (ASC) presenting invasion to the gastrosplenic ligament and spleen with regional lymph node and mesenteric metastases. Pancreatic ASC is a rare pancreatic ductal adenocarcinoma variant with a worse prognosis in humans [4, 5] that has not yet been reported in animals. However, the exact lineage of the cell of origin of pancreatic ductal adenocarcinoma remains unclear in humans [15]. This report describes the pathological findings regarding pancreatic ASC in a cat.

A four-and-a-half-year-old female Scottish Fold cat was presented to a private veterinary practice. The main complaints as reported by the owner were anorexia and weight loss. Mild anemia was revealed at initial presentation. About one month after initial presentation, an abdominal mass was palpated. Abdominal ultrasonography revealed soft tissue masses in the abdomen. A partial pancreatectomy with en-bloc splenectomy was performed. A formalin fixed sample was submitted to Azabu University Veterinary Teaching Hospital for histopathological examination. The cat recovered from anesthesia, but died one day after the operation. Necropsy was not performed.

Paraffin-embedded tissue samples were processed routinely for histopathological examination. Four-micrometer-thick sections were stained with hematoxylin and eosin (HE), and selected sections were stained with azan trichrome, periodic acid-Schiff (PAS) reaction, Alcian blue (AB, pH 2.5) stain and Watanabe's reticulin impregnation.

Additional sections were also subjected to immunohistochemistry. Primary antibodies used are summarized in Table 1. Labeled antigens were detected using a Histofine Simple Stain MAX PO (MULTI) kit (Nichirei Biosciences: Tokyo, Japan). Each antibody was visualized using 3,3′-diaminobenzidine (DAB; Nichirei Biosciences) and slides were counterstained with Mayer's hematoxylin.

The resected specimen consisted of the spleen, a large mass (approximately 6 × 5 × 3 cm) which adhered to the splenic hilum, and smaller disseminated masses on the gastrosplenic ligament. On the diaphragmatic surface of the spleen, there were also some ill-defined slightly raised whitish plaques (Fig. 1a). On the cut surfaces, a large mass showed a whitish fibrotic appearance with tiny scattered translucent areas and a few cystic spaces of up to 20 mm in diameter with yellowish creamy debris. A large mass involving a part of the pancreatic left (transverse) limb on its far side margin invaded and replaced splenic parenchyma (Fig. 1b).

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Table 1. List of primary antibodies used in the present case

Antibody	Clone	Source	Dilution
AE1/AE3	AE1, AE3	Nichirei (Tokyo, Japan)	Prediluted
CAM 5.2	CAM 5.2	Becton Dickinson (Franklin Lakes, NJ, USA)	1:100
CK5/6	D5/16 B4	Nichirei	Prediluted
CK7	OV-TL 12/30	Zymed (San Francisco, CA, USA)	Prediluted
CK14	LL002	Serotec (Kidlington, UK)	1:200
CK17	E3	Dako (Glostrup, Denmark)	1:20
CK19	RCK108	Dako	1:100
Sox-9	Rabbit polyclonal	Santa Cruz (Santa Cruz, CA, USA)	1:100
PCNA	PC 10	Dako	Prediluted
Ki-67	MIB-1	Dako	Prediluted

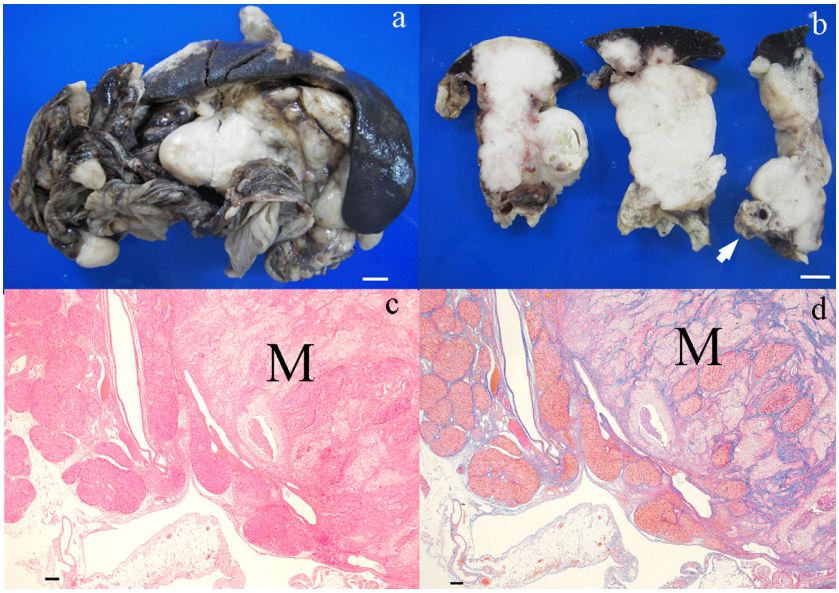

Fig. 1. a) Resected specimen. The spleen, a large mass adhered to the splenic hilum, and smaller masses on the gastrosplenic ligament. Scale bar=1 cm. b) Three cut surfaces of the resected specimen. A large mass involving a part of the pancreatic left (transverse) limb (arrow), invaded, and replaced splenic parenchyma. Scale bar=1 cm. c) and d) Low power microscopic view of the region shown by the arrows in b. A large tumor mass (M) in the pancreas shows invasive growth. Hematoxylin and eosin (HE) (c) and Azan trichrome staining (d). Scale bar=200 μm.

Histologically, a biphasic tumor associated with marked desmoplastic reaction was identified, consisting of adenocarcinoma and squamous cell carcinoma (Fig. 2). The mitotic count of tumor cells within a high-power field (×400) was 5–6 and there were also a number of atypical mitotic figures. The adenocarcinoma component was characterized by glandular and duct-like structures of neoplastic columnar to cuboidal cells without intracytoplasmic zymogen granules, and contained extracellular mucin stained with PAS and AB (Fig. 3a–c). Cellular atypia (anisocytosis and anisokaryosis) was marked. The squamous cell carcinoma component was characterized by irregular nests of neoplastic polygonal cells with distinct cellular borders, pleomorphic anisokaryotic nuclei, vacuolar to eosinophilic cytoplasm, and varying degrees of individual keratinization. In larger nests, a cystic space was formed with cell debris (Fig. 4). There was a gradual transition between the two components and they occupied the same area with various proportions (Fig. 2). The squamous cell carcinoma component comprised approximately 30–40% of the tumor.

The tumor spread beyond the pancreatic parenchyma and invaded the splenic parenchyma. The borders of the tumor blended into the spleen and remnants of the pancreatic lobe (Fig. 1c and 1d). Vascular and lymphatic invasions with neoplastic emboli, regional lymph node metastases, and mesenteric metastases were also observed. Metastatic foci contained both adenocarcinoma and squamous cell carcinoma components.

Immunohistochemical results are shown in Table 2. Both adenocarcinoma and squamous cell carcinoma components were positive for AE1/AE3 and negative for CK7, CK 17 and CK19. The adenocarcinoma component was positive for CAM 5.2 (Fig. 5c) and Sox-9 (Fig. 3a Inset). The squamous cell carcinoma component was positive for cytokeratin 5/6 (CK 5/6) (Fig. 5a), CK14 (Fig. 5b), and partly positive for CAM 5.2 (Fig. 5c).

According to the WHO histological classification of tumors of domestic animals, malignant tumors of the exocrine pancreas are classified into ductal (tubular) adenocarcinoma, acinar cell carcinoma, and undifferentiated (anaplastic) carcinoma [3].

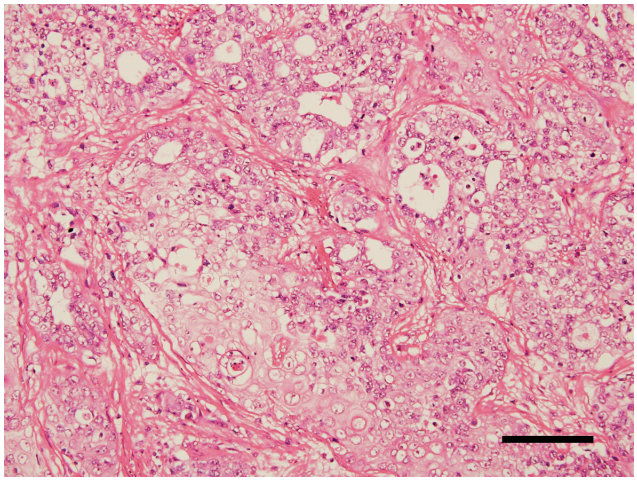

Fig. 2. Adenosquamous carcinoma. Admixture of adenocarcinoma and squamous cell carcinoma. Hematoxylin and eosin (HE). Scale bar=100 μm.

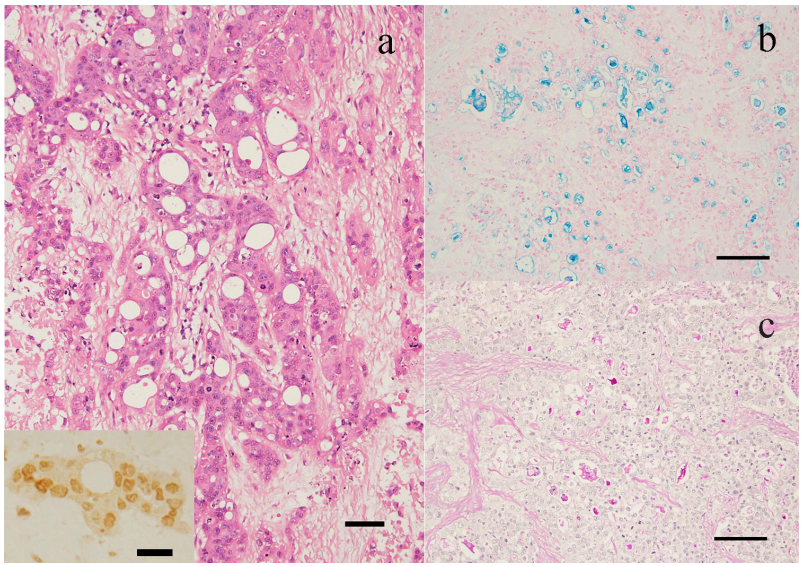

Fig. 3. a) The adenocarcinoma characterized by glandular neoplastic cell components. Hematoxylin and eosin (HE). Scale bar=50 μm. Inset. Immunoreactivity with Sox-9. Scale bar=20 μm b) and c) Glandular structures contained extracellular mucin positive for AB (b) and periodic acid-Schiff (PAS) (c). Scale bar=100 μm.

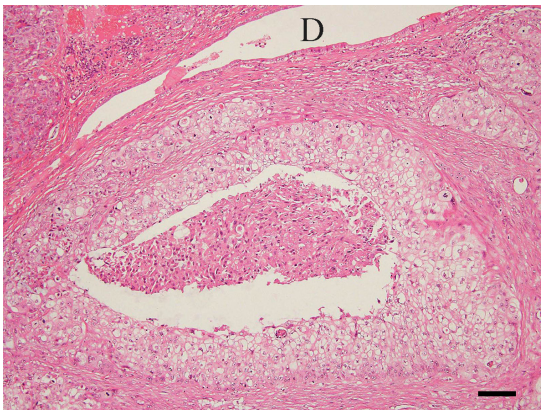

Fig. 4. The squamous cell carcinoma characterized by nests of squamous neoplastic cell components. A central cystic space with necrotic tissue debris. A dilated interlobular duct (D). Hematoxylin and eosin (HE). Scale bar=100 μm.

According to the WHO classification of tumors in humans, a malignant epithelial neoplasm consists of both glandular and squamous differentiation; if the squamous component comprises at least 30% of the neoplasm, it is classified as pancreatic ASC [4, 5]. ASC is a histological variant of ductal adenocarcinoma and was previously designated as adenoacanthoma, mixed squamous and adenocarcinoma, and mucoepidermoid carcinoma [4, 5].

Immunohistochemically, the labeling pattern of ASC is similar to that of ductal adenocarcinoma in humans [5]. Most express cytokeratins (AE1/AE3, CAM 5.2, CK7, CK8, CK18, CK19) [4, 5]. While the squamous cell carcinoma component predominantly expresses CK5/6 [5, 7] and CK14 [1], the expression of CK7 [5] and Sox-9 is often restricted to the adenocarcinoma component [5, 12]. Additionally, acinar cell carcinomas in humans are positive for CK8, CK18, CAM 5.2, and AE1/AE3 [5]. In cats, antibodies to CK7 [2, 9, 10], CK19 [10], CK20 [2, 9, 10], and carcinoembryonic antigens [10] for ductal cells, are useful for the characterization of pancreatic neoplasms.

In the present feline case, Sox-9 was a staining marker for the adenocarcinoma component, and CK 5/6 and CK14 were staining markers for the squamous cell carcinoma component. AE1/AE3 was a staining marker for both adenocarcinoma and squamous cell carcinoma components. CAM 5.2 was a staining marker of limited value used to distinguish the adenocarcinoma component from the squamous cell carcinoma component.

Table 2. Immunohistochemical results of the tumor and pancreas

	Adenosquamous carcinoma		Pancreas		
	Adenocarcinoma	Squamous cell carcinoma	Ductal cells	Acinar cells	Islet cells
AE1/AE3	+	+	+	±	−
CAM5.2	+	±	+	−	−
CK5/6	−	+	−	−	−
CK7	−	−	±	−	−
CK14	−	+	−	−	−
CK17	−	−	−	−	−
CK19	−	−	−	−	−
Sox-9	+	−	+	±	−
PCNA	11.3%	<1%	NE	NE	NE
Ki-67	1.6%	<1%	NE	NE	NE

Grade symbols −, ± and + represent negative, slightly or partly positive, and positive, respectively. NE: Not examined.

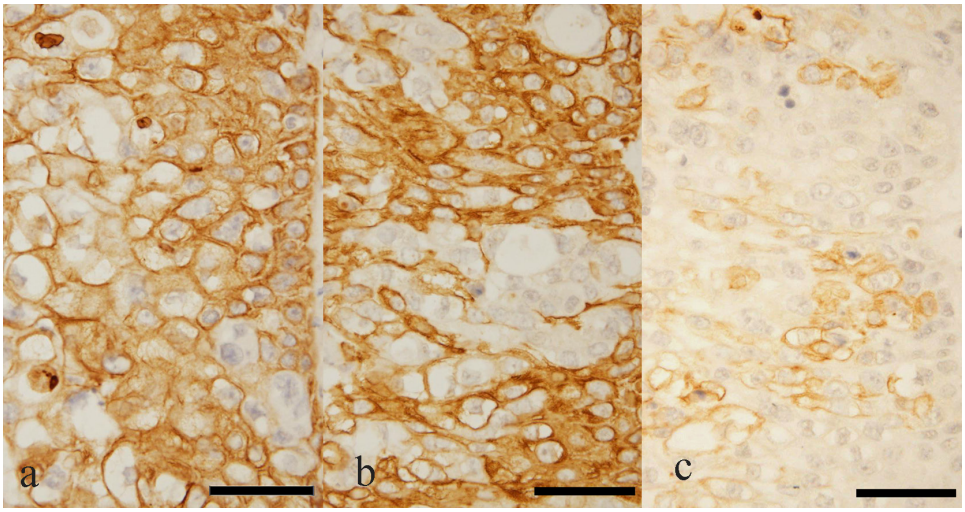

Fig. 5. Immunohistochemistry. An area of the squamous neoplastic cell component along with the focal glandular neoplastic cell component. The squamous neoplastic cell component was positive for CK 5/6 (a) and CK14 (b). The glandular neoplastic cell component was positive for CAM 5.2 (c). Scale bar=50 μm.

Histologically and immunohistochemically, the present tumor was diagnosed as a pancreatic ASC, according to the WHO classification of tumors in humans. However, the histogenesis of feline pancreatic ASC is yet to be elucidated.

Feline pancreatic adenocarcinoma is an aggressive tumor with poor prognosis, irrespective of histological subtype [9]. Metastatic lesions are present in up to 80% of pancreatic adenocarcinoma cases [8, 9, 11]. Extension into the small intestine and distant metastases to the liver are most frequent [8, 9, 11]. The present pancreatic ASC was also aggressive and had a poor prognosis. The tumor infiltrated into the spleen with regional lymph node and mesenteric metastases. Further case studies are needed to establish biological behavior and prognosis of feline pancreatic ASC.

In conclusion, this is a case report, which describes the pathology of feline pancreatic ASC.

CONFLICT OF INTEREST. The authors declare no conflict of interest with respect to the publication of this manuscript.

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