A histopathological study on adrenal cysts in ferrets

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Abstract: Adrenal disorders are common in ferrets, but there are few studies on cystic lesions of the adrenal gland. The present study describes pathological and immunohistochemical features of adrenal cysts in eleven ferrets and discusses their histogenesis. In nine of eleven cases examined, which included seven, one, and one right, left, and bilateral cases, respectively, cysts were in the adrenal cortex and lined with epithelial cells. These epithelial cells contained an Alcian blue-negative/PAS-positive material and were positive for cytokeratin (CK) 7. The staining pattern was similar to that of biliary epithelial cells in the ferret. In five of the cases, there were small ducts adjacent to the cysts that were positive for CK7 and CK20 and negative for CK19. Based on the anatomical proximity between the right adrenal and liver, the immunohistochemical features of the small duct cells were comparable to those of hepatic oval cells. These results indicate the possibility that these adrenocortical cysts in the ferret originated from the biliary system. In the other two cases, the cysts lacked an epithelial cell lining, and there were dilated lymphoid vessels around the cysts. These cysts were assumed to have developed in the adrenal medulla, because the cyst wall was positive for glial fibrillary acidic protein and there were adrenal medullary cells positive for synaptophysin in the cyst wall. Therefore, the medullary cysts may have been associated with dilated vasculatures.

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Key words: ferret, adrenal cyst, biliary duct, lymphoid vessel

Introduction

The ferret (Mustela putorius), a small carnivore species that belongs to the family Mustelidae, is widely bred as an experimental animal or pet. It is thought to be suitable as an alternative to dogs and nonhuman primates in biomedical research. In toxicological disciplines, ferrets are mainly used as a non-rodent animal model for repeated-dose safety assessment testing, particularly for vaccine research. Recently, ferrets have also been known for their high incidence rate of endocrinopathy including adrenal diseases. Hyperplastic or neoplastic lesions are common in the ferret adrenal gland. Unlike other animals, ferrets produce sex hormones rather than cortisols in the adrenal gland that cause unique symptoms, such as vulva swelling and prostatic cysts.

Meanwhile, cystic lesions are incidentally found in the normal and affected adrenal glands of ferrets. Previous study reported that adrenal cysts may originate from the biliary system, but their pathological conditions have not been clarified. In the present study, we described clinical and histopathologic findings of adrenal cysts in ferrets and discussed the pathological significance of this lesion. Furthermore, we discussed the mechanism of adrenal cyst development along with a comparison with adrenal cystic lesion in humans.

Materials and Methods

Samples

Adrenal cysts from 11 ferrets were examined. All tissue samples were surgically removed at Miwa Exotic Animal Hospital between 2010 and 2017.

Histological analysis

Samples were fixed in 10% neutral buffered-formalin and embedded in paraffin wax by a routine procedure. Paraffin sections were cut to a thickness of 4 µm and stained with hematoxylin and eosin (HE) or Alcian blue and periodic acid-Schiff (AB-PAS). Normal right adrenal and liver tissues of ferrets were also stained with AB-PAS for comparison.

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Immunohistochemical analysis

Consecutive sections were labelled immunohistochemically to confirm the nature of the cells composing the cyst wall. Endogenous peroxidase was blocked by treatment with 3% hydrogen peroxide in methanol for 5 min. To unmask antigens, sections were then treated with citrate buffer (pH 6.0) or Target Retrieval Solution (pH 9.0; Dako, Carpinteria, CA, USA) by heating in an autoclave (121°C) for 10 min or incubated with 125 µg/ml proteinase K (Wako, Tokyo, Japan) solution at room temperature for 15 min. After washing with Tris-buffered saline (TBS), the slides were treated with 8% skimmed milk in TBS. Primary antibodies used are listed in Table 1. After incubation with each primary antibody at 4°C overnight, immunolabeled antigens were visualized using the Dako Envision+ System (Dako) and reacted with 0.05% 3’3-diaminobenzidine plus 0.03% hydrogen peroxide in Tris-hydrochloric acid buffer, and then the sections were counterstained with hematoxylin. Normal right adrenal and liver tissues of ferrets were used for positive controls, and the localization of positive cells in the controls is described in Table 1.

Results

Clinical signs and macroscopic findings

The ferrets from which the archived samples were obtained consisted of five spayed females and six castrated males, aged 2–7 years (average: 4.9 years) (Table 2). Eight cases (cases 1–7 and 11) presented symptoms of endocrinopathy. All the cases showed adrenal cysts, and two cases (cases 2 and 7) displayed adrenocortical hyperplasia on the non-cystic side. Adrenal cysts were unilocular or multilocular and were of various sizes (Fig. 1a, b). The adrenal cysts were found on the right, left, and both (bilateral) sides in eight cases, two cases, and one case, respectively. Macroscopically, most of the adrenal parenchyma was replaced by cysts in all cases (Fig. 1a, b). Bile-like viscous green contents were observed in the cysts in some cases (Fig. 1a). Improvement of endocrinopathy symptoms was observed after surgery in six cases (cases 1, 2, 5–7, and 11); however, alopecia recurred in two of these cases (cases 1 and 6). In case 4, the effect of adrenalectomy on strangury was unclear because the ferret was treated with urinary catheterization. The clinical outcomes of cases 3 and 10 were not available.

Table 1. Antibodies Used for Immunohistochemistry

| Antibody to | Supplier       | Dilution | Pretreatment | Positive cells in adrenal gland | Positive cells in liver |
|-------------|----------------|----------|--------------|---------------------------------|------------------------|
| CD 31       | Thermo Scientific | Ready to use | Cit          | Endothelial cells               | Endothelial cells and bile duct epithelial cells |
| CK 7        | Dako           | 1:100    | ProK         | None                            | Bile duct epithelial cells |
| CK 19       | Leica          | Ready to use | ProK       | None                            | Bile duct epithelial cells |
| CK 20       | Dako           | 1:50     | ProK         | None                            | Oval cell and bile duct epithelial cells |
| GFAP        | Dako           | 1:1000   | None         | Sustentacular cells in adrenal medulla | None |
| Neurofilament | CELLMARQUE    | 1:250    | Cit          | Medullary cells                  | None |
| S100        | Dako           | 1:500    | None         | Sustentacular cells in adrenal medulla | None |
| Synaptophysin | Dako          | 1:50     | TRS          | Medullary cells                  | None |

Cit, heating in sodium citrate buffer (pH6); ProK, proteinase K; TRS, heating in target retrieval solution (pH9).

Table 2. Ferret Cases of Adrenal Cysts

| Case | Sex | Age | Side of lesion | Symptoms of endocrinopathy | Note                      |
|------|-----|-----|----------------|----------------------------|---------------------------|
| 1    | CM  | 2y8m | Right           | Alopecia, vulva swelling   | Left: adrenocortical hyperplasia |
| 2    | SF  | 4y   | Right           | Alopecia                   |                           |
| 3    | SF  | 5y   | Right           | Vulva swelling             |                           |
| 4    | CM  | 5y1m | Right           | Strangury                  |                           |
| 5    | SF  | 5y6m | Right           | Alopecia                   |                           |
| 6    | CM  | 5y6m | Right           | Alopecia, vulva swelling   | Left: adrenocortical adenoma |
| 7    | SF  | 5y7m | Right           | Alopecia, vulva swelling   |                           |
| 8    | SF  | 6y   | Both            | ND                         |                           |
| 9    | CM  | 7y10m | Left           | ND                         |                           |
| 10   | CM  | 2y6m | Left            | ND                         | Ultrasonography: intraperitoneal cyst |
| 11   | CM  | 4y8m | Right           | Alopecia                   |                           |

CM, castrated male; SF, spayed female; ND, not detected.
Histopathological findings

Histopathologically, there were multiple cysts lined with a cuboidal to flat epithelium in the adrenal cortex in cases 1–9 (Fig. 2a). The cysts often contained an eosinophilic amorphous material. In five of the cases (cases 2–5 and 7), small ducts were observed around these cysts (Fig. 2b). In three of the cases (cases 2, 4, and 8), calcification and osseous metaplasia were observed in the cyst wall. However,

Fig. 1. Gross pictures of the adrenal cyst in ferrets. a) Case 4. An adrenal cyst in the right adrenal with viscous greenish fluid (arrow) is found very close to the liver. b) Case 10. A large cyst in left adrenal is filled with clear fluid.

Fig. 2. Histological findings of the adrenal cyst (HE stain). a) Case 3. A multilocular cyst is found in the adrenal cortex. The cyst is lined with cuboidal to flat epithelial cells and contains eosinophilic hyalinized materials. Scale bar = 200 µm. b) Case 7. Small ducts with cuboidal epithelia (arrows) adjacent to a cyst (asterisk). Scale bar = 50 µm. c) Case 10. The luminal surface of the cyst lacks epithelial lining, and a loose connective tissue is observed on the luminal surface (arrowheads). Adrenal cortical cells are observed beneath the connective tissue (asterisk). Scale bar = 200 µm. d) Case 10. A dilated lymphatic vessel (asterisk) is found around the cyst. Scale bar = 200 µm.
in cases 10 and 11, the cysts lacked an epithelial lining, and loose connective tissues covered the luminal surface of the cyst (Fig. 2c). Beneath the cyst wall, there were adrenal cortical cells which formed a layered structure like the normal adrenal cortex (Fig. 2c). Also, there were dilated lymphatic vessels around the cysts (Fig. 2d). Mesentery lymph nodes in case 11 were pathologically diagnosed as dilatation of the lymphoid sinus.

The normal bile duct epithelium of the ferret was AB negative and PAS positive (Fig. 3a), but the normal adrenal tissues were negative for both AB and PAS. In cases 1–9, the epithelial cells lining the cyst wall and the materials within the cysts were AB negative and PAS positive (Fig. 3b). The epithelial cells that composed the small ducts around the cysts were also AB negative and PAS positive (Fig. 3c).

**Immunohistochemical findings**

In cases 1-9, epithelial cells of the cyst wall were weakly positive for cytokeratin (CK) 7, and in cases 3–5 and 7, the epithelial cells were also positive for CK19 (Table 3, Fig. 4a, b). The epithelial cells of the cyst wall were negative for CK20, glial fibrillary acidic protein (GFAP), neurofilament (NF), S100, and synaptophysin (SYP) in the nine cases (Table 3, Fig. 4c). In case 2, the epithelial cells were also positive for CD31 (Fig. 4d). On the other hand, epithelial cells constituting the small ducts around cysts were

![Fig. 3. Histochemical findings of the normal bile duct and adrenal cysts (AB-PAS stain). a) Intrahepatic bile duct epithelial cells contain an AB negative/PAS positive material. b) Case 2. Material inside the adrenal cysts and epithelial cells lining the cyst is AB negative/PAS positive. c) Case 2. Some epithelial cells of the small ducts contain AB negative/PAS positive material. Scale bar = 50 µm.](image)

| Table 3. Results of Immunohistochemical Analysis of the Adrenal Cysts | Immunohistochemistry |
|---------------------------------------------------------------|-------------------|
| Case No. Component                                            | CD31  | CK7 | CK19 | CK20 | GFAP | NF | S100 | SYP |
| 1 Cyst-lining E.                                               | −     | +   | −    | −    | −    | −  | −    | −   |
| 2 Cyst-lining E.                                               | +     | +   | −    | −    | −    | −  | −    | −   |
| E. of small duct                                              | +     | +   | −    | +    | −    | −  | −    | −   |
| 3 Cyst-lining E.                                               | −     | +   | +    | −    | −    | −  | −    | −   |
| E. of small duct                                              | −     | +   | −    | +    | −    | −  | −    | −   |
| 4 Cyst-lining E.                                               | −     | +   | +    | −    | −    | −  | −    | −   |
| E. of small duct                                              | +     | +   | −    | +    | −    | −  | −    | −   |
| 5 Cyst-lining E.                                               | −     | +   | −    | −    | −    | −  | −    | −   |
| E. of small duct                                              | −     | +   | −    | +    | −    | −  | −    | −   |
| 6 Cyst-lining E.                                               | −     | +   | −    | −    | −    | −  | −    | −   |
| E. of small duct                                              | −     | +   | −    | +    | −    | −  | −    | −   |
| 7 Cyst-lining E.                                               | −     | +   | +    | −    | −    | −  | −    | −   |
| E. of small duct                                              | −     | +   | +    | −    | −    | −  | −    | −   |
| 8(L) Cyst-lining E.                                            | −     | +   | +    | −    | −    | −  | −    | −   |
| 8(R) Cyst-lining E.                                            | −     | +   | −    | −    | −    | −  | −    | −   |
| 9 Cyst-lining E.                                               | −     | −   | −    | −    | −    | −  | −    | −   |
| 10 Cystwall cells                                              | −     | −   | −    | −    | −    | −  | −    | −   |
| 11 Cystwall cells                                              | −     | −   | −    | −    | −    | −  | −    | +   |

E, epithelial cells; +, positive; −, negative.
positive for CK7 and CK20, but negative for CK19 (Table 3, Fig. 5a–c). Epithelial cells of some small ducts were also positive for CD31 (Fig. 5d).

In contrast, in cases 10 and 11, the luminal surface of the cyst wall was positive for GFAP, and there were SYP-positive cells in the cyst wall (Table 3, Fig. 6a, b). The connective tissue of the cyst wall was positive also for NF and S100 (Fig. 6c, d) in case 10. There were no cells positive for CD31, CK7, CK19, or CK20 (Fig. 6e–h).

Discussion

In cases of hyperadrenocorticism in ferrets, the left adrenal is more affected than the right. However, adrenal cysts in ferrets tend to occur on the right side rather than the left, as reported in the present study. Meanwhile, there is no association of the occurrence with sex or age. In the present study, six out of eleven cases showed symptoms of adrenal endocrinopathy without other adrenal lesion, and four of these cases displayed temporary or long-term improvement of symptoms after adrenalectomy. This result indicates that adrenal cysts in ferrets may be associated with hyperadrenocorticism. However, the mechanism of how cystic lesions cause endocrinopathy was not clarified in our investigation.

We observed adrenal cysts in the present ferret cases either in the cortex (cases 1–9) or in the medulla (cases 10 and 11), and their histological characteristics were different. Cortical cysts were lined with epithelial cells, and there were small ducts around the cysts. These epithelial cells lining the cysts and small ducts contained an AB-negative/PAS-positive material with similar characteristics to those of the gall bladder mucosa. Conversely, medullary cysts were capsulated with connective tissue and lacked the epithelial cell lining. There were also dilated lymphatic vessels around the medullary cysts. Case 11 showed lymphoid sinus dilation in the mesentery lymph nodes. These findings sug-

![Fig. 4](image_url) Immunohistochemical findings of epithelial cells lining the adrenal cysts. a) Epithelial cells are weakly positive for CK7. Case 2. b) The cells are membranous positive for CK19. Case 4. c) The cells are negative for CK20. Case 2. d) The cells are strongly positive for CD31. Case 2. Scale bar = 50 µm.

![Fig. 5](image_url) Immunohistochemical findings of epithelial cells of the small ducts. Case 2. a) Epithelial cells are positive for CK7. b) The cells are negative for CK19. c-d) The cells are strongly positive for CK20 (c) and CD31 (d). Scale bar = 50 µm.
suggest that the medullary cysts may have arisen from ectatic lymphatic vessels.

In humans, adrenal cysts are histopathologically categorized into four types: epithelial, endothelial, pseudocyst, and parasitic. Based on this classification, the cortical cysts in the ferret may correspond to epithelial cysts, and the medullary cysts may correspond to the pseudocysts. The endothelial cyst and pseudocyst are the most common adrenal cysts in humans and they are considered variants of adrenal vascular cysts. The histological characteristics of the medullary cysts in the ferret were similar to those of human vascular cysts; thus they may share a similar pathogenesis during their development. In contrast, the epithelial cysts in humans are very rare, and the origin of the epithelium is still under discussion. Our present result indicating that the epithelial cysts were most common in the ferrets suggests that the adrenal epithelial cysts in the ferret may be a species-specific condition.

In humans, dogs, and cats, the biliary epithelium is positive for CK7, CK19, and CK20, and in rodents, hepatic oval cells are positive for CK20. The present findings revealed that biliary epithelial cells of the ferret are partially positive for these three markers. Therefore, epithelial cells of the liver of the ferret have similar immunohistochemical profiles to those of other mammalian species.

In the present study, immunohistochemical analysis revealed that the epithelial cells composing the cortical cyst wall displayed similar staining patterns to biliary epithelial cells in the ferret. Interestingly, the immunoreactivity for CK20 and CD31 of the small ducts around the cortical cysts was consistent with that of oval cells rather than that of biliary epithelial cells. This may suggest that the cells forming the small ducts are more immature than the cyst-lining epithelial cells. Together with the results of AB-PAS staining, this suggests that the immature ductal cells may have differentiated into biliary mucus-secreting cells and that the lumen was then dilated by the accumulation of mucus and formed the cystic lesions. This theory is consistent with a recent study suggesting that the adrenal cysts in the ferret are of biliary origin.

The right adrenal gland is located at a more cranial position than the left and is in a close contact with the caudal vena cava and the caudate lobe of the liver. This anatomical location of the right adrenal gland may explain the predilection for occurrence of adrenal cyst on the right side. During embryonic development, hepatoblasts derived from the foregut endoderm migrates to the adjacent septum transversum mesenchyme to form the liver bud. Aberrant migration of hepatoblasts results in ectopic liver tissues in various organs such as the spleen, testis, and adrenal. We therefore pos-

**Fig. 6.** Immunohistochemical findings of the cyst wall. Case 10. a) The connective tissue around the cyst is strongly positive for GFAP, and GFAP-positive cells are found also beneath the cyst wall (arrow). b–d) The connective tissue around the cyst is positive for SNP (b), NF (c), and S100 (d). e) Endothelial cells of the dilated lymphatic vessel (asterisk) are positive for CD31, but the luminal surface of the cyst (double asterisk) is negative. f–h) The wall of the cyst (double asterisk) is negative for CK7 (f), CK19 (g), and CK20 (h). Scale bar = 100 µm.
tulate that adrenal cysts of the ferret develop from aberrant hepatoblasts migrating into the adrenal gland. However, no hepatocytes were found in the affected adrenal glands of ferrets in the present study.

In humans, cases of adrenal cysts combined with lymphangiectasis in other parts of body22, 23 or lymphangioma22, 23 have been reported. This type of cyst frequently occurs in women, and the right adrenal is more often affected. A previous study also reported that the cyst wall was composed of fibrous tissues with an endothelial lining23. However, in the present ferret cases, the wall of the adrenal medullary cyst lacked an endothelial lining. In addition, adrenal parenchymal hemorrhage is regarded as a cause of adrenal pseudocysts in humans8. Therefore, extravasated fluid from ruptured lymphatic vessels may also cause adrenal pseudocysts. Thus, it is suggested that a rupture of dilated lymphatic vessels in the adrenal may induce medullary cysts in the ferret.

In conclusion, the present study revealed histological and immunohistochemical features of adrenal cysts in ferrets. Our results show that the adrenal cysts in the ferret develop either in the cortex or in the medulla. Cortical cysts tended to occur in the right adrenal, and the epithelial cells lining the cysts were immunohistochemically similar to biliary epithelial cells. On the other hand, medullary cysts lacked the epithelial lining and may be related to lymphangiectasis.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

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