



## NOTE

Pathology

# Ovarian mixed germ-cell tumor comprising mature teratoma and embryonal carcinoma in a four-toed hedgehog (*Atelerix albiventris*)

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**ABSTRACT.** This report describes the clinical and histopathological characteristics of a rare mixed germ-cell tumor comprising teratoma and embryonal carcinoma in the left ovary of a 10-month-old four-toed hedgehog, with chief complaints of loss of appetite and lethargy. Laparotomy revealed a swollen left ovary with small disseminated peritoneal nodules, and bilateral ovariectomy was performed. The left ovary had a mature teratoma with well-differentiated fat, bone, cartilage, salivary gland, trachea, keratin cyst, and nervous tissues, and an embryonal carcinoma consisting of poorly-differentiated epithelial cells arranged in tubular, alveolar, or solid patterns. Immunohistochemically, the embryonal carcinoma cells were positive for placental alkaline phosphatase and c-KIT. This is the first case of mature teratoma with embryonal carcinoma in the ovary of a hedgehog.

**KEY WORDS:** embryonal carcinoma, four-toed hedgehog (*Atelerix albiventris*), mature teratoma, mixed germ-cell tumor, placental alkaline phosphatase

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The incidence of tumors in four-toed hedgehogs is reported to be extremely high (29–69%), regardless of whether they live in captivity, in zoos, or in home environments [5, 7, 10, 11, 13]. There have been two reported cases of ovarian germ-cell tumors—an anaplastic germinoma and a malignant (immature) teratoma [10, 14]. The term mixed germ-cell tumor is used to describe tumors with more than two types of germ-cell components [12, 15]. In this case report, we clinically, pathologically, and immunohistochemically analyzed a mixed germ-cell tumor, wherein a benign (mature) teratoma and embryonal carcinoma coexisted in the left ovary.

A 10-month-old unsterilized four-toed hedgehog was brought to the clinic with chief complaints of loss of appetite and lethargy. Examination under isoflurane anesthesia revealed an abdominal swelling. Radiographs revealed a mass with scattered radiopaque calcified lesions and ascites in the region posterior to the left kidney in the abdominal cavity (Fig. 1). Abdominal paracentesis collected 160 ml of fluid, which was exudative, and showed negative bacterial culture, and had a specific gravity of 1.026, which is similar to that of urine. A 5 × 5 × 5 cm mass was confirmed on abdominal ultrasonography, and mixed-echo presentation was indicative of a polycystic lesion. The results of a hematological examination were normal except for a slightly elevated blood urea nitrogen level. Based on these results, we performed a laparotomy to excise the tumor mass and to better examine the abdominal cavity.

During the surgery, an enlarged left ovary and small disseminated peritoneal nodules in the peritoneum were found. Ovariectomy was performed to excise the tumor mass in the left ovary; however, we were unable to excise the disseminated nodules in the peritoneum. The animal died 2 days after the surgery; necropsy was not performed.

The resected specimen was fixed in 10% neutral buffered formalin and embedded in paraffin wax. Subsequently, 4 μm sections were stained with hematoxylin and eosin. Labeled-polymer immunohistochemistry was performed using N-Histofine MAX PO (M or R; Nichirei Biosciences, Tokyo, Japan), with anti-cytokeratin AE1/AE3 (1:400; mouse monoclonal antibody; DAKO, Glostrup, Denmark), anti-vimentin (1:1,000; mouse monoclonal antibody; Abcam, Cambridge, UK), anti-placental alkaline phosphatase (PLAP) (1:50; rabbit polyclonal antibody; DB Biotech, Kosice, Slovak Republic), anti-c-Kit (1:50; rabbit polyclonal antibody; DAKO), anti-PGP9.5 (1:200; rabbit polyclonal antibody; DAKO), and anti-WT1 (1:50; rabbit polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as primary antibodies [4]. The normal ovary and placental tissue of the hedgehog were used as positive controls. The placental trophoblast, granulosa, and ovarian superficial epithelial cells stained positive for PLAP; oocytes for PGP9.5; oocytes, granulosa and theca cells for c-Kit; and the granulosa, theca, and ovarian superficial epithelial cells for WT-1 (Table 1). As a negative control, mouse or rabbit isotopic immunoglobulin diluted to the same concentration was used as

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**Fig. 1.** Radiograph showing a mass with scattered calcification found in the abdomen.

**Table 1.** Immunoreactivity in tumor, normal placenta and normal ovary of hedgehog

	Tumor		Normal placenta	Normal ovary			
	Epithelial cells of the salivary gland and gastrointestinal-tract in teratoma	Poorly differentiated tumor cell in embryonal carcinoma	Placental trophoblast	Granulosa cell	Superficial epithelial cell	Oocyte	Theca cell
AE1/AE3	+	+	+/-	-	-	-	-
PLAP	-	+	+	+	+	-	-
c-KIT	-	+	-	+	+	+	+
PGP9.5	-	-	-	-	-	+	-
WT-1	-	-	-	+	+	-	+
Vimentin	-	-	-	+	+	-	+

PLAP: placental alkaline phosphatase. Grade: -, negative; +/-, partially positive; +, positive.

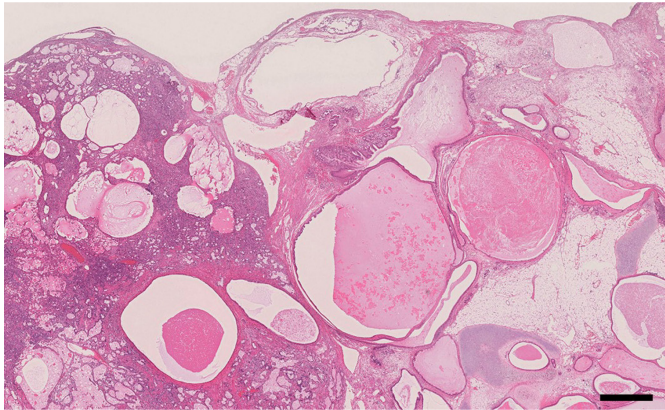
the primary antibody.

Gross pathology revealed a mixed soft white and pink mass 5 × 5 × 5 cm in size, and a partially green-colored abscess. The cut surface was solid, and multiple large and small cysts were found.

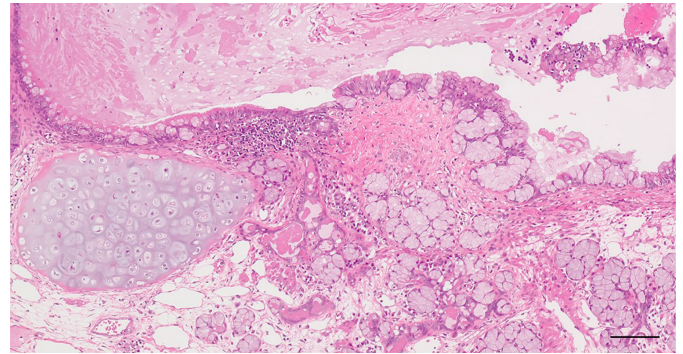
Histopathologically, the left ovary had been largely replaced by tumor tissue, and only a small amount of ovarian tissue remained in the marginal area. Approximately 60% of the tumor tissue was a mature teratoma, comprising mature fat, bone, cartilage, tooth, salivary gland, digestive tract, trachea, keratin cyst, and nervous tissues (Fig. 2). Microscopic observations confirmed that the cysts comprised dilated salivary glands and a digestive tract filled with secreted fluids (Fig. 3). Poorly-differentiated epithelial tumor was observed in the mature salivary and digestive glandular tissue of the teratoma (Fig. 2). The poorly-differentiated tumor cells had proliferated into a tubular and alveolar pattern, with areas of solid growth (Figs. 4 and 5). Part of the tumor had infiltrated the ovarian capsule and invaded the surrounding adipose tissue. The poorly-differentiated tumor cells had large, lightly dark, and vacuolated nuclei, comprising fine granular chromatin and distinct nucleoli, with a moderate quantity of occasionally vacuolated cytoplasm (Fig. 4). Mitotic index was 13 per 10 high power fields of view.

Immunohistochemically, the epithelial cells of the salivary gland and gastrointestinal tract in the mature teratoma were positive for AE1/AE3 and negative for PLAP, c-Kit, PGP9.5, WT-1, and vimentin. Poorly-differentiated tumor cells were positive for AE1/AE3 (Fig. 6), PLAP (Fig. 7), and c-Kit (Fig. 8) and negative for WT-1, PGP9.5, and vimentin.

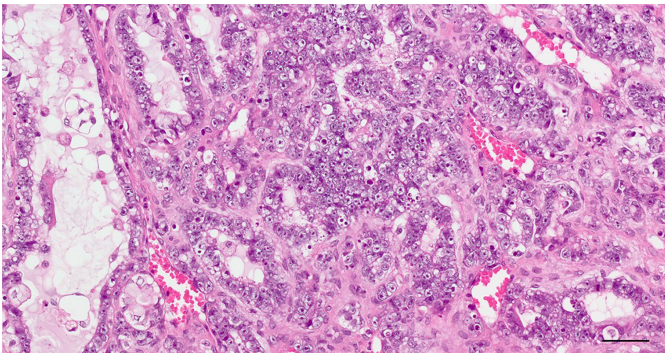
After performing ovariectomy, including the mass that had formed in the left ovary, we found that approximately half of the resected mass comprised a mature benign teratoma comprising well-differentiated tissues from three germ layers. In domestic animals, teratomas are classified either as mature (benign) or immature (malignant) [1]. Although benign teratomas are rare, they have been reported in horses, pigs, cows, dogs, cats, rodents, monkeys, woodchucks, and squirrels [1]. Malignant teratomas are very rare and have only been reported in dogs, horses, and mice [1–3, 8]. To our knowledge, there have been no reports of benign teratomas in the ovaries of hedgehogs, and only a single case of bilateral malignant teratoma with peritoneal dissemination has been reported [14]. In that report, the animal was diagnosed with malignant teratoma because part of the benign teratoma showed malignant mesenchymal cell proliferation and had metastasized to the abdominal cavity [14]. Similarly, in the present case, the animal had a benign teratoma with a malignant tumor that had formed adjacent to it. The authors speculate that the benign teratoma had formed first and that a malignant transformation had led to the formation of the malignant tumor.



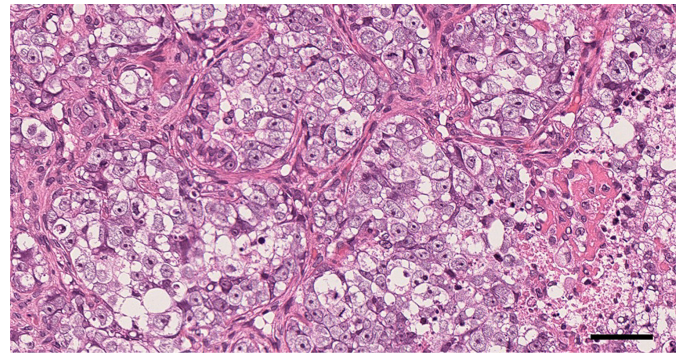
**Fig. 2.** On the right side, large cysts form between the adipose tissue, leading to a region with mature teratoma. On the left side, tumor growth shows a tubular and solid pattern with small to large cysts. Hematoxylin and eosin staining. Bar, 1 mm.



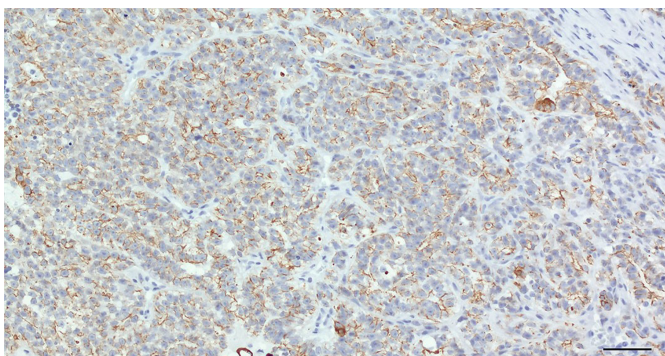
**Fig. 3.** Bronchi consisting of mature ciliated epithelium; salivary glands mainly composed of mucous glands, and differentiated cartilage. Hematoxylin and eosin staining. Bar, 100  $\mu$ m.



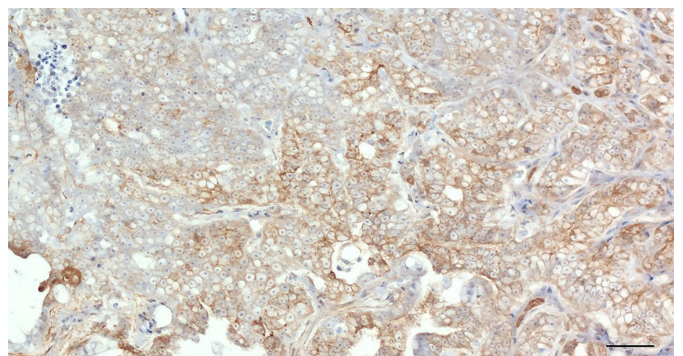
**Fig. 4.** Poorly-differentiated area shows tubular, alveolar, and solid pattern. Hematoxylin and eosin staining. Bar, 50  $\mu$ m.



**Fig. 5.** Poorly-differentiated cells show large, lightly dark, and vacuolated nuclei; distinct nucleoli; and moderate to abundant cytoplasm. Hematoxylin and eosin staining. Bar, 50  $\mu$ m.

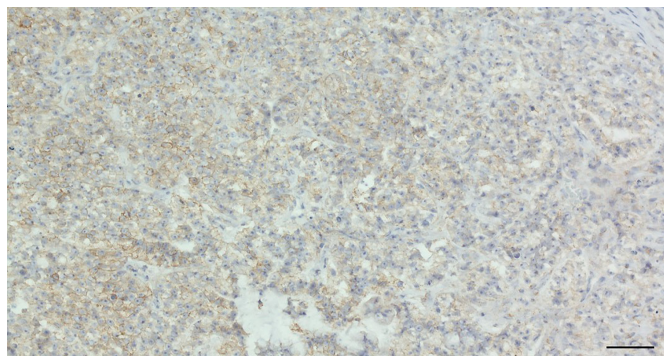


**Fig. 6.** The cytoplasm of the poorly-differentiated cells stains positive. AE1/AE3 immunostaining. Bar, 50  $\mu$ m.



**Fig. 7.** The cell membrane of the poorly-differentiated cells stains weakly to strongly positive. Placental alkaline phosphatase immunostaining. Bar, 50  $\mu$ m.

Germ-cell tumors can be divided into two categories. One shows a single histologic pattern, while the other has a mixture of two or more histologic patterns [12, 15]. In the present case, the tubular pattern resembled an embryonal carcinoma or yolk sac carcinoma, and the alveolar and solid pattern was similar to a dysgerminoma. However, yolk sac carcinoma was excluded from diagnosis because the present case lacked the characteristic reticular pattern and Schiller-Duval body [12]. It was important to perform immunostaining for a detailed differential diagnosis between embryonal carcinoma and dysgerminoma. However, since



**Fig. 8.** The cell membrane of the poorly-differentiated cells stains weakly positive. c-Kit immunostaining. Bar, 50  $\mu$ m.

there are no available reports of germ-cell markers in hedgehogs, we tested various markers for the ovary and placenta and found that c-Kit, PLAP, and PGP9.5 could be used as germ-cell markers. In the present case, poorly-differentiated tumor cells in a tubular pattern were positive for PLAP and c-Kit in addition to pan-cytokeratin antibody AE1/AE3. These staining findings were similar to those of embryonal carcinoma in humans; however, since it is c-Kit-negative in humans, we did not obtain identical results [9, 12]. The tumor cells in the alveolar and solid pattern were differentiated from dysgerminoma because they were negative for PGP9.5, which is positive in dysgerminoma of dogs, and oocytes in hedgehogs, and were positive for AE1/AE3, which is negative for oocytes [6]. Consequently, we determined that the area with the alveolar and solid pattern was a part of the embryonal carcinoma. Based on these results, we diagnosed this tumor as an ovarian mixed germ-cell tumor comprising mature teratoma and embryonal carcinoma. Due to the high mitotic index of the germ-cell tumor, invasion into the surrounding tissue beyond the ovarian capsule, and nodule formation suggesting peritoneal dissemination, we concluded that the tumor was histologically highly malignant.

From this case, we were able to elucidate that three types of germ-cell markers could be used and that a detailed diagnosis can be achieved by differentiating the germ-cell tumors of the ovaries in hedgehogs.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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