



NOTE

Pathology

Histological and immunohistochemical features of cutaneous mast cell tumor in six captive four-toed hedgehogs (*Atelerix albiventris*)

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ABSTRACT. This report described the histopathological and immunohistochemical features of cutaneous mast cell tumor (MCT) in six hedgehogs. The hedgehogs presented single cutaneous mass with ulcer and crusting. Histologically, the neoplastic lesions were characterized by the proliferation of well-differentiated mast cells (3 cases), and atypical mast cells (3 cases) with one atypical histiocytic morphology. Immunohistochemically, tumor cells were positive for KIT and mast cell tryptase, and were negative for Iba-1. In well-differentiated MCT, all patients were clinically improved and survived more than 365 days after surgical excision, whereas an atypical histiocytic MCT showed aggressive behavior with re-recurrence, and the animal died 115 days after surgery. These findings suggest that, compatible with other animals, well-differentiated MCT has a better prognosis in hedgehogs.

KEY WORDS: cutaneous mast cell tumor, four-toed hedgehog (*Atelerix albiventris*), histopathology, immunohistochemistry

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Mast cell tumor (MCT) is relatively common not only in dogs and cats but also in ferrets [20], and infrequently described in other species [3, 13]. In dogs and cats, MCT forms focal or multicentric nodular lesions in the skin, and may also affect visceral organs such as spleen and intestine. In the skin, MCT is one of the most frequently occurring tumors in dogs, accounting for up to 21% of canine skin tumors [5]. The 2-tier histologic grading of canine cutaneous MCT was primarily based on the number of mitoses, multinucleated cells, bizarre nuclei in 10 high-power fields (HPF) [6]. In cats, cutaneous MCT is histologically classified into well-differentiated MCT, pleomorphic MCT and less common atypical, poorly granulated MCT (or histiocytic MCT) [14]. A 2-tier histologic grading system has been suggested for feline cutaneous MCT in one study [15].

The captive four-toed hedgehog (*Atelerix albiventris*) is prone to developing neoplasm with a high incidence rate [1, 4, 10, 16]. The major body systems in which the tumors are found are integumentary, hemolymphatic and alimentary [4, 10]. In the skin, the most commonly diagnosed tumors are soft tissue sarcoma and mammary gland tumors [10, 16], round cell tumors have been occasionally reported. Case series of cutaneous histiocytic sarcoma have been reported [8, 17], whereas sporadic case reports of cutaneous MCT cases have been reported [9, 12, 13]. Furthermore, histopathological diagnostic criteria for MCT have not been established, and immunohistochemical evaluation of KIT and mast cell tryptase expression in normal mast cells and MCT are lacking in hedgehogs. The goals of this study were to investigate the histopathological and immunohistochemical features, as well as describe the clinical outcomes of a case series of cutaneous MCT in the four-toed hedgehog.

Tissue samples from 6 biopsy hedgehog cases were submitted to our laboratory between 2010 and 2020 for histopathological examination, and used in the present study (the total number of hedgehog biopsy cases during that period was 412). The clinical data including age, sex, tumor location, treatment and outcome of these cases are summarized in Table 1. All the samples were readily fixed in 10% neutral buffered formalin and embedded in paraffin wax. Four-µm-thick serial sections were stained with hematoxylin and eosin (HE) and Toluidine blue (TB). To evaluate the mitotic count, the number of mitosis per 10 HPF (400×) was counted under a light microscope in each case.

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Table 1. Case information of hedgehogs with cutaneous mast cell tumor in the present and previous studies

Case	Sex	Age	Tumor location	Histological finding	Mitotic count	Treatment	Outcome
Present							
Case 1	Sprayed F	21 m	Subcutaneous mass (0.1 × 0.3 cm), right ventral abdomen	Well-differentiated	1/10 HPF	Surgical excision	Died on day 533, necropsy examination confirm no recurrence, no metastasis; tumour not related
Case 2	M	42 m	Subcutaneous mass (<1 cm), right forelimb	Well-differentiated	2/10 HPF	Surgical excision; prednisolone (2 mg/kg)	No recurrence; alive after follow-up at 365 days
Case 3	F	11 m	Cutaneous mass (2.5 × 1.7 cm), left dorsal; ulcerated with crust	Well-differentiated	1/10 HPF	Surgical excision; prednisolone (2 mg/kg)	No recurrence; alive after follow-up at 365 days
Case 4	M	24 m	Cutaneous mass (1.5 × 0.7 cm), facial mass	Atypical	1/10 HPF	Surgical excision	Lost to follow-up
Case 5	F	24 m	Cutaneous mass (2.3 × 2.5 cm), central abdomen; crusted and bleeding surface	Atypical	2/10 HPF	Surgical excision	Lost to follow-up
Case 6	F	30 m	Cutaneous mass (3.4 × 2.2 cm), right ventral abdomen	Atypical (histiocytic)	16/10 HPF	Surgical excision; prednisolone (2 mg/kg)	Recurrence after 2 months, and after 3 weeks; died on day 115
Previous							
Raymond <i>et al.</i> [12]	F	~12 m	Subcutaneous mass (4.0 cm) central neck, metastasis to submandibular lymph node	Atypical	ND	-	Euthanized
Raymond <i>et al.</i> [11]	ND	36 m	Cutaneous mass, metastasis to axillary lymph node	Atypical	ND	ND	ND
	F	>12 m	Cutaneous mass concurrent haemangiosarcoma	Well-differentiated	ND	ND	ND
	ND	>12 m	Cutaneous mass	Well-differentiated	ND	ND	ND

F, female; HPF, high power fields; M, male; m, month; ND, no data.

Immunophenotypic characterization of neoplastic cells was performed using immunohistochemistry (IHC). Primary antibodies for KIT (CD117; 1 in 100 dilution; rabbit polyclonal antibody; Neomarkers, Fremont, CA, USA), mast cell tryptase (1 in 500 dilution; mouse monoclonal antibody; GeneTex, Irvine, CA, USA), and ionized calcium-binding adapter molecule 1 (Iba-1; 1 in 250 dilution; rabbit polyclonal antibody; Wako Pure Chemical Industries, Osaka, Japan) were used in the study. Sections from the normal skin containing some resident mast cells, intestine with interstitial cells of Cajal and lymph node of the hedgehog obtained from routine necropsy were used for positive control for KIT, mast cell tryptase and Iba-1 immunohistochemistry, respectively. For negative control, the primary antibodies were replaced by Tris-buffered saline [11]. Antigen retrieval was performed by incubating the sections with citrate buffer at 121°C for 10 min for KIT, mast cell tryptase and Iba-1. All markers were labeled using the EnVision™ system rabbit/mouse reagent conjugated to horseradish peroxidase (Dako, Tokyo, Japan), and labeling was visualized with 3-3'-diaminobenzidine solution (Dojindo, Tokyo, Japan) containing 0.03% H₂O₂ in a Tris-hydrochloric acid buffer. All sections were counterstained with Mayer's hematoxylin (Muto Pure Chemicals, Tokyo, Japan).

The six hedgehogs included four females (three entire and one neutered) and two entire males. The affected hedgehogs ranged from 11 to 42 months of age (median 25.3 months). On gross examination, a solid cutaneous nodule was found in all cases, ranging from 0.1 × 0.3 cm to 3.4 × 2.2 cm in diameter. Tumors were randomly located on the skin of hedgehogs, and cutaneous lesions were ulcerated with crusts (Fig. 1A).

Histologically, 3 cases were well-differentiated MCT (Cases 1–3). A non-encapsulated, well-demarcated neoplasm was located in the dermis. The neoplasm consisted of uniform round cells with abundant cytoplasm. These cells had fine basophilic cytoplasmic granules that were metachromatic staining by TB. Nuclei were round and central with finely stippled to coarse chromatin (Fig. 1B). Nuclear pleomorphism was mild.

Three other cases were atypical MCT (Cases 4–6). Neoplastic cells extended from the superficial dermis into the subcutaneous adipose tissue. The neoplasm was non-encapsulated, expansile and infiltrative. In case 4 and case 5, the neoplastic cells were poorly-differentiated with pleomorphism. Nuclei were variable in size, eccentric in location, with moderately coarse, clumped chromatin. However, basophilic granules may be scarce to absent in the cytoplasm (Fig. 1C). In case 6, neoplastic cells were round- to oval- or pleomorphic-shaped with abundant cytoplasm that resembled histiocytes (Fig. 1D). Nuclei were oval or indented, with marginated chromatin and one or two nucleoli were observed. Binucleated cells were often found. This case was considered as an atypical, poorly granulated (histiocytic) subtype.

Infiltrating eosinophils were frequently observed in 3 well-differentiated and 2 atypical MCT cases, whereas eosinophil infiltration was rarely seen in atypical histiocytic MCT case (Case 6) (Fig. 1B–D). Mitotic activity was low in 5 cases (Cases 1–5) with 1 to 2 mitoses/ 10 HPF, whereas there was 16 mitoses/ 10 HPF in case 6. With TB staining, metachromatic granules were confirmed in 5 cases (Cases 1–5) with moderately to strong staining in the cytoplasm (Fig. 2A). In contrast, neoplastic cells in atypical histiocytic MCT case had faint staining cytoplasmic granules (Fig. 2B).

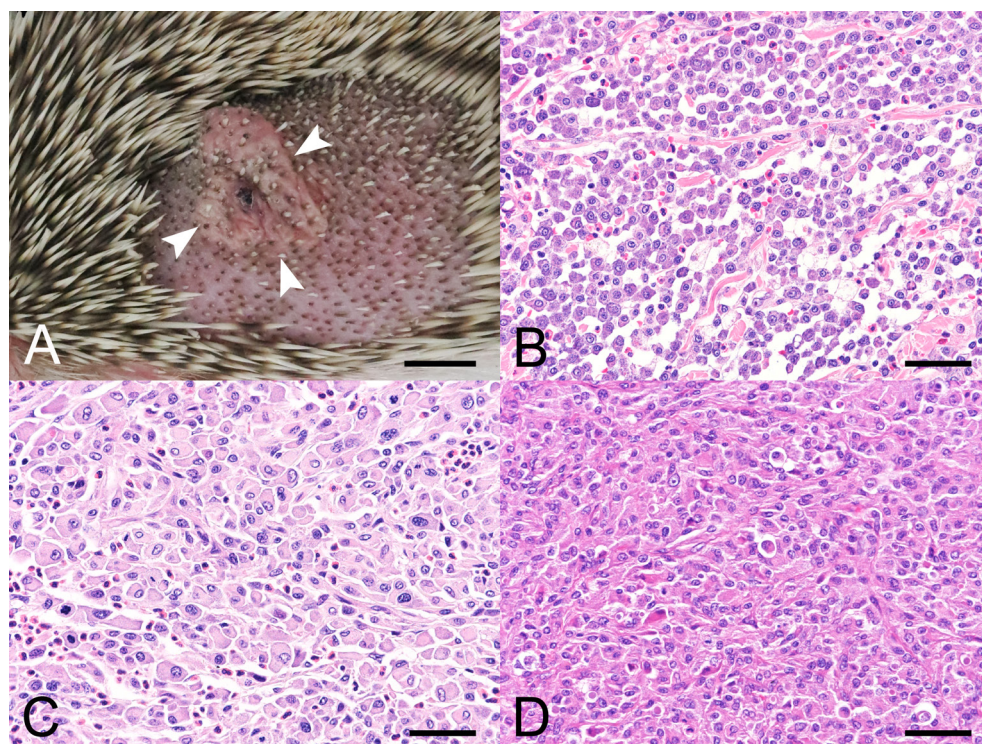


Fig. 1. Pathological findings of cutaneous mast cell tumor (MCT) in hedgehogs. (A) Case 3. Tumor associated with surface ulceration on the back (arrowheads) after shaving. Bar, 1 cm. (B) Case 3. Well-differentiated MCT. Neoplastic cells are uniform round with abundant cytoplasm. Hematoxylin and eosin (HE). Bar, 50 μ m. (C) Case 5. Atypical MCT. Neoplastic cells are poorly-differentiated with pleomorphism. HE. Bar, 50 μ m. (D) Case 6. Atypical histiocytic MCT. Neoplastic cells are polygonal with pale eosinophilic cytoplasm. HE. Bar, 50 μ m.

Immunohistochemically, interstitial cells of Cajal in the intestine were positive for KIT, and normal mast cells in the skin were positive for KIT and mast cell tryptase. The membrane of neoplastic cells was diffusely positive for KIT in all 6 cases (Fig. 3A). The cytoplasm of neoplastic cells was variably positive for mast cell tryptase in all 6 cases (Fig. 3B). Neoplastic cells were negative for Iba-1; however, scattered individual Iba-1 positive cells were compatible with infiltrated macrophages. Therefore, the immunohistochemistry results further supported the final diagnosis of cutaneous MCT in all 6 cases.

Clinical follow-up information was available in four cases (Cases 1–3 and 6). In three well-differentiated MCT cases (Cases 1–3), all patients were clinically improved and survived more than 365 days after surgical excision. Three hedgehogs (Cases 2, 3 and 6) were treated with oral administration of prednisolone (2 mg/kg). Case 1 died 533 days after surgical excision. The post-mortem examination was subsequently performed, but there was no tumor recurrence and no evidence of metastases. Recurrence of tumor was observed twice in atypical (histiocytic) case (Case 6) at 60 and 81 days after surgical excisions. The first recurrence was observed at near the original tumor, and the second recurrence was located at the same site of the original excision. Subsequently, conservative therapy with prednisolone was also used in this hedgehog. The patient died at 115 days, but necropsy was not available.

The current report describes a case series of cutaneous mast cell tumor in six captive four-toed hedgehogs, confirmed by histopathology and immunohistochemistry. The incidence of MCT in captive four-toed hedgehogs is reported to be low, from 1% (1 in 100) [10] to 4.5% (3 in 66) [12] in spontaneous arising neoplasm. Cutaneous MCT has been reported in adult hedgehogs with a range of 12 to 36 months [12, 13] (Table 1). In this study, the more comprehensive age range indicated that cutaneous MCT could develop in hedgehogs younger than 12 months. All previous cases of MCT in hedgehogs were female [9, 12, 13]. In the present study, two males have been recorded, even then there was a slightly higher percentage of female hedgehogs (the female to male ratio of 1:2 albeit with a small sample size). In the dog, cat and ferret, cutaneous MCT present most commonly as a solitary lesion, although MCT cases with multiple lesions have been reported [7, 14, 20]. All of the cases in this study developed single tumor lesion, similar to those recorded in the literature [9, 12, 13].

Microscopically, cutaneous MCT in hedgehogs may manifest in well-differentiated or atypical forms. Neoplastic cells in well-differentiated MCT cases (Cases 1–3) resembled normal mast cells with no or little pleomorphism [12]. In contrast, neoplastic cells in atypical MCT cases (Cases 4–6) were pleomorphic, with eccentric nuclei, megalokaryosis and low or moderate cytoplasmic granularity. One atypical MCT case (Case 6) shared many features with feline atypical MCT [14]. Eosinophilic infiltrations were easily found except for Case 6. Moreover, the morphological features and lack of eosinophilic infiltration in Case 6 resembled cutaneous histiocytic sarcoma in hedgehogs [17]. To the author's knowledge, atypical histiocytic MCT has not been previously reported in hedgehogs. Commercially available antibodies are widely used in domestic animals, including KIT, mast cell tryptase

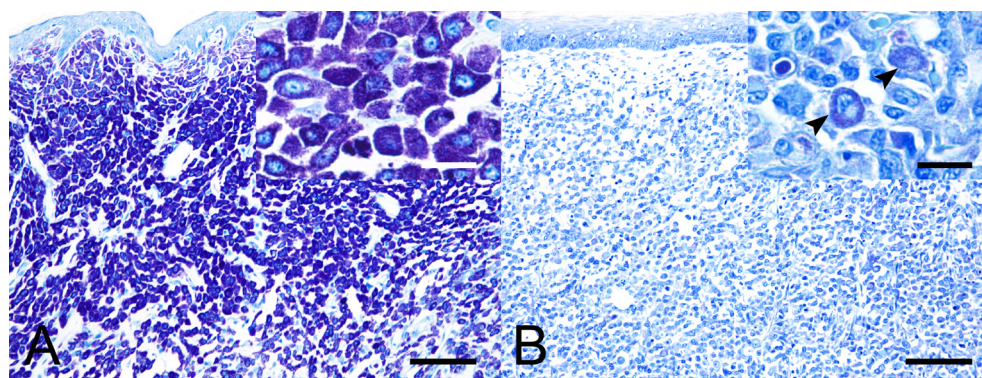


Fig. 2. Toluidine blue (TB) stained sections of cutaneous mast cell tumor (MCT) in hedgehogs. **(A)** Case 3. Well-differentiated MCT. Neoplastic cells exhibit strong staining cytoplasmic granules. Bar, 100 µm. **(B)** Case 6. Atypical histiocytic MCT. Neoplastic cells exhibit faint staining cytoplasmic granules. Bar, 100 µm. Inset: higher magnification showing cells with TB-positive cytoplasmic granules (arrowheads). Bar, 20 µm.

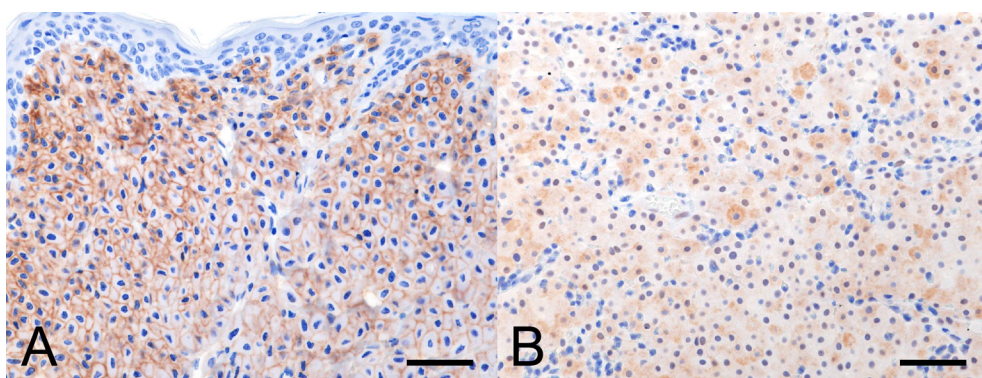


Fig. 3. Immunohistochemical features of cutaneous mast cell tumor in hedgehogs. **(A)** Case 3. Neoplastic cells are membranous positive for KIT. Bar, 50 µm. **(B)** Case 2. Neoplastic cells are positive for mast cell tryptase. Bar, 50 µm.

and Iba-1, to confirm the immunophenotype of neoplastic cells. The immunohistochemical findings were similar to those reported in previous studies of canine and feline MCT [7, 14], with the expression of KIT and mast cell tryptase by neoplastic cells. The KIT and mast cell tryptase immunohistochemistry in hedgehog cutaneous MCT were demonstrated for the first time, and supported the diagnosis. In hedgehogs, KIT was expressed not only in normal mast cells, and interstitial cells of Cajal in control tissues, but also in a case of subcutaneous MCT [9] and a case of ovarian mixed germ-cell tumor comprising mature teratoma and embryonal carcinoma [19]. In canine and feline cutaneous MCT, KIT expression patterns can be associated with tumor recurrence and survival time [7, 14]. In the present study, all six cases showed membrane KIT expression, which matched to pattern I labeling with a good prognosis in canine MCT [7]. Oral administration of prednisolone has been associated with prolonged survival in canine cutaneous MCT [2, 18]. In the present study, two well-differentiated MCT cases (Cases 2 and 3) were treated with oral prednisolone after surgical excision, and responded well to treatment without recurrence and/or metastasis. No response was seen in an atypical case (Case 6) with tumor re-recurrence treated with prednisolone. Therefore, further studies are needed to evaluate the usefulness of anti-inflammatory and immunosuppressant medication. Three cases with well-differentiated MCT survived over one year suggesting that cutaneous MCT may have a benign clinical course. In contrast, only one of three atypical MCT cases had clinical follow-up information with re-recurrence and without distant metastasis. In addition, metastasis beyond the regional lymph node was previously observed in two atypical MCT cases of hedgehogs [12, 13]. Therefore, local recurrence or metastases might be considered an unfavorable outcome in atypical MCT, however, only a few cases with follow-up data have been published.

In summary, the current report describes histopathological and immunohistochemical findings of cutaneous MCT in hedgehogs. The histopathological features of MCT in hedgehogs include various patterns, compatible with its tumor in other animals. IHC for KIT and mast cell tryptase can aid the diagnosis of atypical histiocytic MCT that morphologically resemble cutaneous histiocytic sarcoma in this species. Further studies with more cases are required in order to verify the clinical behavior and the effectiveness of chemotherapy in hedgehog cutaneous MCT.

CONFLICT OF INTEREST. The authors declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

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