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Case Report

Cutaneous mastocytosis with a mutation in the juxtamembrane domain of *c-kit* in a young laboratory beagle dog

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Abstract: Cutaneous mastocytosis, which resembles a subset of urticaria pigmentosa in humans, is rare in dogs. We herein report unrepresentative neoplastic proliferation of mast cells in ventral skin removed routinely from a nine-month-old female laboratory beagle dog at necropsy. A histological examination revealed diffuse extensive cellular infiltration from the superficial to deep dermis in most parts of the skin around the fourth and fifth mammary papilla without nodule formation. Tumor cells were fairly monomorphic, well-differentiated mast cells with round nuclei of small distinct nucleoli and moderate to abundant, slightly eosinophilic and granular cytoplasm. A perivascular arrangement of mast cells was noted at the margin of the lesions. Infiltration of eosinophils and degeneration of collagen were not observed in the dermis. Cutaneous mastocytosis was diagnosed based on these features. A sequence analysis of lesions revealed the deletion of Gln_{555} to Ile_{570} within the juxtamembrane domain of c-*kit* (exon 11). (DOI: 10.1293/tox.2015-0038; J Toxicol Pathol 2016; 29: 49–52)

Key words: cutaneous mastocytosis, dog, juxtamembrane domain, KIT, mutation, urticarial pigmentosa

Cutaneous mastocytosis (CM) is a rare disease in dogs. Some dogs have been diagnosed with primary CM resembling urticarial pigmentosa (UP) in humans[1, 2]. We herein report unrepresentative neoplastic proliferation of mast cells in the skin of a young laboratory beagle dog. To the best of our knowledge, this is the first report of a sequence analysis of c-*kit* from canine CM and we also revealed a c-*kit* mutation in the juxtamembrane domain (exon 11).

A nine-month-old female Marshall beagle dog (Marshall BioResources, North Rose, NY, USA), which was assigned to a test-article treatment group, was sacrificed at the end of a three-month repeated-dose oral toxicity study. Cutaneous lesions were considered to have developed spontaneously because no similar lesions were found in the other dogs given the compound and no pharmacological or biological activity was found for mast cells by a panel assay. The dog had been housed in a commodious cage in an environmentally controlled room (room temperature, 23 ± 3°C; relative humidity, 30–70%; lighting cycle, 12-h light/dark) and supplied expanded food once daily and tap water *ad libitum*. All experimental procedures were conducted after approval for the study was obtained from the Animal Care and Use Committee of Shionogi Research Laboratories.

Macroscopic changes were not detected in any organs including the skin. The skin on the right side around the fourth to fifth mammary papilla of the dog was routinely fixed with 10% neutral buffered formalin, processed, and embedded in paraffin. Paraffin-embedded sections were then stained with hematoxylin and eosin (HE) or toluidine blue stain. A CD117 antibody (1:50; Dako, Glostrup, Denmark) and antibodies against histamine (1:400; Merck Millipore, Billerica, MA, US), mast cell tryptase (1:100; AbD Serotec, Oxford, UK), and Ki-67 (clone MIB-1; 1:100; Dako, Glostrup, Denmark) were selected for an immunohistochemical study. Heat-induced antigen retrieval was performed with citrate buffer for histamine, mast cell tryptase, and MIB-1 antibodies or with ethylenediaminetetraacetate (pH=8) for the CD117 antibody.

DNA was isolated from a formalin-fixed, paraffin-embedded skin sample. Polymerase chain reaction (PCR) amplification and a sequence analysis of c-*kit* was performed for the entire coding regions of *c-kit* exons 8, 11, 17, and 18 and for some of the region (1445–1537) of exon 9. As a sequence analysis of c-*kit* from canine CM has never been reported, we used the findings from canine mast cell tumor

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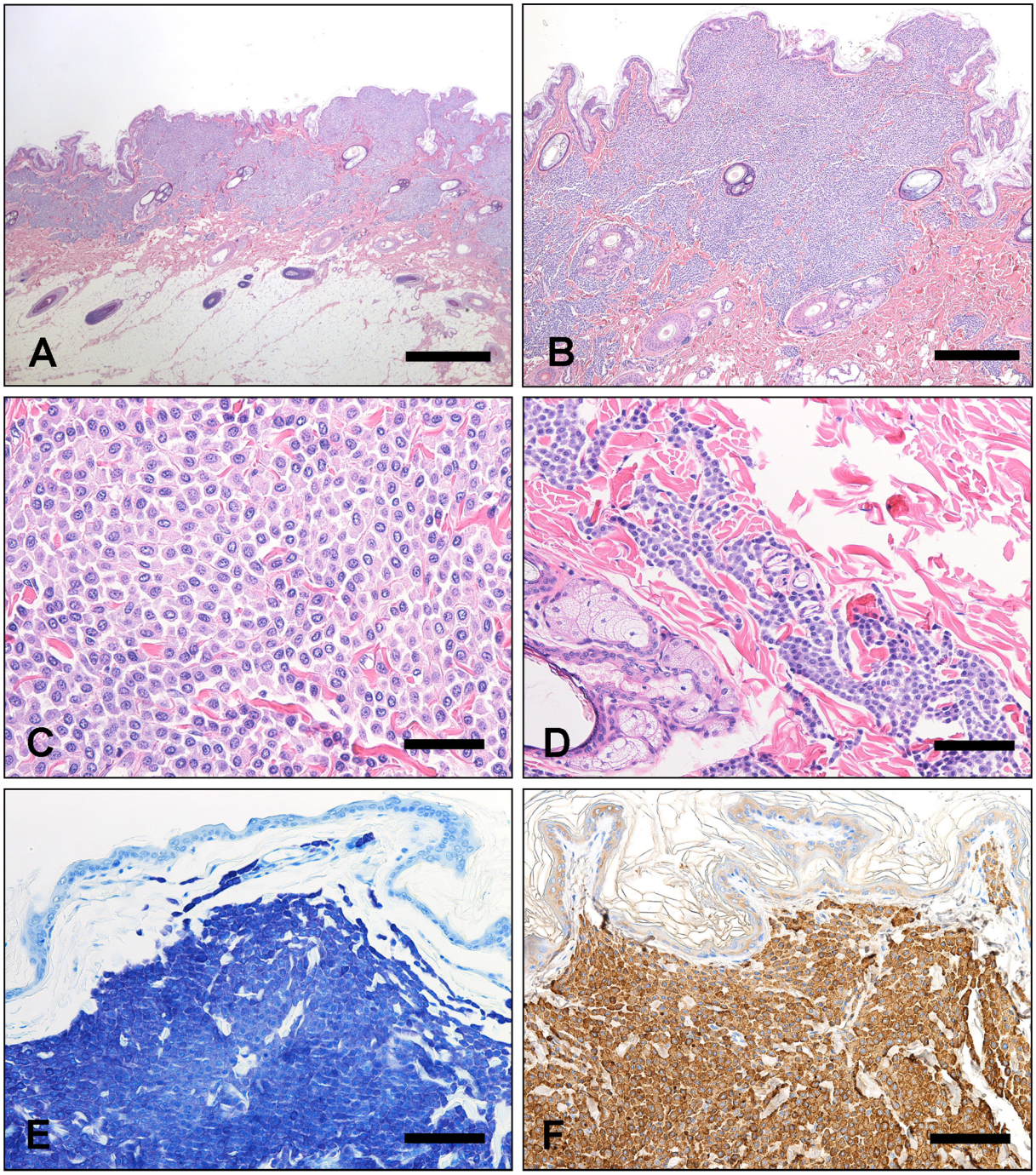

Fig. 1. Histological appearance of cutaneous mastocytosis. Hematoxylin and eosin staining (A–D). Toluidine blue staining (E). Immunostaining of mast cell tryptase (F). Diffuse extensive cellular infiltration of well-differentiated mast cells from the superficial to deep dermis in most parts of the skin around the fourth and fifth mammary papilla without nodule formation (A–B). Tumor cells were fairly monomorphic, well-differentiated mast cells with round nuclei of small distinct nucleoli and moderate to abundant, slightly eosinophilic and granular cytoplasm (C). A perivascular arrangement of mast cells was noted at the margin of the lesions, and these neoplastic cells were slightly small, like normal mast cells (D). Mast cells that infiltrated the dermis had cytoplasmic granules with metachromatic staining (E) and were immunopositive for mast cell tryptase (F). Bars: 1 mm (A), 500 µm (B), 100 µm (D–F), 50 µm (C).

and human CM as a reference for selection of the analysis region for *c-kit*.

Microscopically, diffuse extensive cellular infiltration was observed from the superficial (nearly the epidermal-dermal junction) to deep dermis in most parts of the skin around the fourth and fifth mammary papilla without nodule formation (Fig. 1A, 1B). Tumor cells were fairly monomorphic, well-differentiated mast cells with round nuclei of

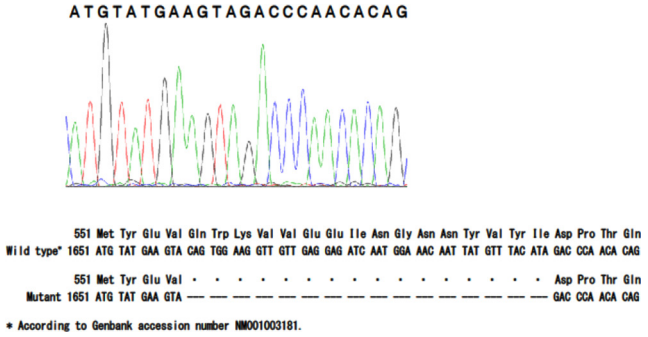

Fig. 2. Sequence of the canine c-kit gene in exon 11. The deletion of Gln_{555} to Ile_{570} (c.1663_1710 del) in this case is denoted by a dotted dashed line.

small distinct nucleoli and moderate to abundant, slightly eosinophilic and granular cytoplasm (Fig. 1C). A perivascular arrangement of mast cells was noted at the margin of the lesions, and these neoplastic cells were slightly small, like normal mast cells (Fig. 1D). Mitotic figures were rare, and the number of MIB-1-positive mast cells observed was smaller than that of cutaneous basal cells. Infiltration of eosinophils and collagen degeneration was not observed in the dermis. Furthermore, infiltration of mast cells was not present in any organs or tissues including bone marrow and peripheral blood, except for the affected skin. Toluidine blue staining revealed the typical metachromatic characteristics of cytoplasmic granules (Fig. 1E). Infiltrated mast cells were positive for CD117, histamine, and mast cell tryptase (Fig. 1F).

A sequence analysis of c-*kit* revealed the deletion of Gln_{555} to Ile_{570} within the protein sequence of the juxtamembrane domain in exon 11 (c.1663_1710del) (Fig. 2). No mutation was found in exon 8, 9, 17, or 18 in this case.

In children during infancy or early childhood, the skin lesions of UP, the most common form of CM, exhibit typical clinical findings including lesions that urticate when stroked (Darier's sign) and also show intraepidermal accumulation of melanin pigment, which is histopathological proof of abnormal mast cell infiltration of the dermis[3]. Canine CM was previously reported to be similar to UP in children[1, 2]. Dogs present with partially erythematous small papules to plaques on the head, neck, trunk, perineum, and legs[4]. Affected dogs are mostly under 1 year of age[1, 2, 4]. Histopathologically, the lesions are characterized by moderate to severe perivascular to diffuse infiltration of well-differentiated mast cells in the dermis[1, 2, 4]. Similar to UP in children, early onset CM in dogs may regress spontaneously; however, progression from CM to systemic mastocytosis has also been reported[1, 2, 4].

The diagnosis of CM in this case was based on the following characteristics: lack of typical clinical findings, absence of cutaneous nodules, the afflicted dog's young age, the apparent restriction of mast cell infiltration to the skin, diffuse extensive aggregation of well-differentiated mast cells from the superficial (nearly the epidermal-dermal junction) to deep dermis, slightly small mast cells infiltrated into the marginal zone of the lesions (perivascular arrangement), and no infiltration of eosinophils or collagen degeneration. We considered this clinical information and the histopathological features to be consistent with previously reported case of human and canine CM rather than mast cell tumors; however, it was difficult to differentiate its features from those of early stage of mast cell tumors because the lesions lacked the typical clinical findings of CM.

KIT is a type III receptor tyrosine kinase encoded by the c-*kit* gene, which includes an extracellular domain (encoded by exons 1–9), transmembrane domain (exon 10), and intracellular domain (exons 11–21)[5, 6]. The intracellular domain is further divided into a negative regulatory juxtamembrane domain (exons 11 and 12) and cytoplasmic tyrosine kinase domain that is spilt by an insert into ATP-binding (exon 13) and phosphotransferase lobes (exon 17)[6]. Mutations in the kinase domain or neighboring juxtamembrane domain have been shown to cause constitutive activation of c-*kit* in the absence of ligand binding, and they may result in the development and/or proliferation of certain canine mast cell tumors[5, 7–9]. For example, the most common somatic mutation in human (especially adult) CM, Asp816Val, is located in the kinase domain of KIT in exon 17 and results in augmented mast cell proliferation[3]. Previous studies on canine mast cell tumors identified mutations in exons 8, 9, 11, and 17 of KIT[6]. Most alterations within exon 11 were internal tandem duplications (ITDs) located in the distal part between residues 571 and 590, while other types, such as point mutations, deletions, and insertions in the proximal part between residues 553–562, have also been reported[5, 6, 8]. In the present study, deletion of Gln_{555} to Ile_{570} was observed in the proximal part of exon 11, and this may have resulted in augmented mast cell proliferation. Imatinib, a small molecule tyrosine kinase inhibitor, has been reported to be effective for treatment of canine mast cell tumors with *c-kit* mutations in exon 11; therefore, our case report indicates that imatinib is possibly effective for canine CM (in small animal practice)[5, 10].

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