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Expression of Stem Cell Factor in Feline Mast Cell Tumour

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Summary

Stem cell factor (SCF) is a ligand of the molecule Kit, which is expressed in mast cells and is important for mast cell proliferation, migration and survival. Mast cell tumours (MCTs) are associated with mutations of *c-kit*, a proto-oncogene encoding the Kit protein. In this study, we examined SCF expression in 23 samples of feline MCTs. SCF expression was detected in 10 cutaneous MCTs and a case of splenic mastocytosis. In the cutaneous MCTs, SCF-positive tumour cells were located at the margins. Kit was expressed in eight of the 10 cutaneous cases of SCF-expressing MCTs. In these cases, Kit-positive cells were located near to SCF-positive cells, and SCF/Kit double-positive tumour cells were found. Ki67-positive tumour cells were not found near to SCF-positive cells. These results suggest that SCF autocrine/paracrine mechanisms are involved in the expansion of cutaneous MCTs, but not in tumour proliferation.

Keywords: cat; kit; mast cell tumour; stem cell factor

Stem cell factor (SCF) is a ligand of Kit, a tyrosine kinase receptor that is expressed in various cells including mast cells (Lennartsson and Rönnstrand, 2012). SCF is important for mast cell proliferation, development, migration and survival (Okayama and Kawakami, 2006). Kit protein is encoded by the *c-kit* proto-oncogene. Although the detailed mechanisms are not fully understood, *c-kit* mutations result in the auto-activation of Kit protein without interaction with ligands (Lennartsson and Rönnstrand, 2012).

Mutations in *c-kit* are involved in the tumorigenesis of mast cell tumours (MCTs) (Lennartsson and Rönnstrand, 2012); however, such mutations are not always associated with human and animal MCTs (Lim *et al.*, 2009; Takeuchi *et al.*, 2013). A canine mast cell line (HRMC) is known to express wild type Kit protein (Ohmori *et al.*, 2008). The mechanisms of tumorigenesis in MCTs without *c-kit* mutations remain largely unknown. Recently, Amagai *et al.* (2013) suggested that autocrine/paracrine actions of SCF may be such a novel tumorigenesis mechanism in MCTs. In that study SCF was shown to be highly expressed in the HRMC cell line and it was suggested that SCF expressions contributed to the phosphorylation of Kit, resulting in proliferation of the HRMC cells. Additionally, SCF expression has been detected in clinical cases of canine MCT and been associated with tumour proliferation (Amagai *et al.*, 2014).

In cats, MCTs are common and occur as cutaneous MCTs or as visceral mastocytosis (Ginn *et al.*, 2007; Henry and Herrera, 2013). Reports of *c-kit* mutations in feline MCTs are limited, but feline MCTs without *c-kit* mutations are recognized (Dank *et al.*, 2002;

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Isotani *et al.*, 2009). Sabattini *et al.* (2017) showed no correlation between *c-kit* mutations and tumour differentiation, mitotic activity or survival in feline MCTs. As for canine MCTs, some feline MCTs may have tumorigenesis unrelated to *c-kit* mutations.

The aim of this study was to examine SCF expression in 23 formalin-fixed, paraffin wax-embedded samples of feline MCTs. The cases were assembled from the archives of the Veterinary Pathology Laboratory of Yamaguchi University (2012–2015) and Tokyo University (2014–2015). The cases comprised of 20 cutaneous MCTs and three examples of visceral mastocytosis (two involving the spleen and one involving a lymph node). The affected cats ranged in age from 1 year and 10 months to 17 years (mean 8 years and 5 months). Sections stained with haematoxylin and eosin (HE) were reviewed and in each case there was good tissue preservation with no evidence of autolysis. To confirm tissue preservation, immunohistochemistry (IHC) with anti-vimentin antibody (Dako, Kyoto, Japan) was performed and the tumour cells were clearly immunoreactive.

Sections (4 μm) were prepared for IHC and double immunofluorescence using primary antibodies specific for SCF (rabbit polyclonal antibody; Abcam Japan, Tokyo, Japan), Kit (Dako) and Ki67 (Dako). For IHC, the sections were subjected to boiling in sodium citrate buffer (pH 6.0) for 20 min in a pressure cooker. After blocking endogenous peroxidase activity, sections were preincubated with 10% normal goat serum and then primary antibodies were overlaid overnight in a refrigerator. After incubating with goat anti-mouse or anti-rabbit immunoglobulins conjugated to peroxidase-labelled dextran polymer (Envision + HRP TM, Dako), labelling was 'visualized' using 3, 3′ diaminobenzidine in a DAB substrate kit (Roche, Mannheim, Germany). Finally, sections were counterstained with haematoxylin. For double immunofluorescence, Alexa Fluor 488-conjugated goat anti-rabbit IgG antibody (dilution 1 in 500; Life Technologies, Carlsbad, California, USA), Alexa Fluor 555-conjugated donkey anti-mouse IgG antibody (dilution 1 in 500; Life Technologies) and Alexa Fluor 555-conjugated goat anti-rat IgG antibody (dilution 1 in 500; Life Technologies) were used as secondary reagents. Staining with 4′, 6-diamidine-2′-phenylindole dihydrochloride (DAPI) was used to visualize the cell nuclei. Negative controls were prepared by replacing primary antibodies by phosphate buffered saline.

SCF expression was detected in 10 cutaneous MCTs and one case of splenic mastocytosis. Consistent with a previous report of canine MCTs (Amagai *et al.*, 2014), SCF-positive tumour cells were mainly distributed in the marginal region of the cutaneous MCTs (Figs. 1 and 2). Tumour cells that infiltrated into surrounding tissue also often expressed SCF. Kit expression was found in 11 cutaneous MCTs including eight of the 10 cases that expressed SCF. Kit labelling was either membranous or cytoplasmic (Table 1). In the eight cutaneous MCTs expressing both SCF and Kit, Kit-positive cells were located near to SCF-positive cells and SCF/Kit double-positive cells were identified by double immunofluorescence (Fig. 3, Supplementary Fig. 1). However, SCF expression was not clearly related to proliferative activity as mitotic figures and Ki67 labelling were not present in the SCF-positive cells or tumour cells located near to the SCF-positive cells.

These results suggest that SCF expression may be involved in the tumorigenesis of some feline cutaneous MCTs. However, unlike canine MCTs, SCF expression does not appear to be directly related to tumour proliferation in feline cutaneous MCTs.

Migration is one of the effects of SCF on mast cells. SCF is known to be a major chemotactic factor for mast cells and their progenitor cells (Nilsson et al., 1994; Lennartsson and Rönnstrand, 2012). Through the Kit receptor, SCF provides the critical signals for homing and recruitment of mast cells to various tissues (Meininger *et al.*, 1992). In the present study, cells in the marginal region of cutaneous MCTs had prominent SCF expression. According to the distribution of SCF-expressing cells, tumour cells may have migrated from the central to the marginal region, thus contributing to tumour expansion.

SCF is also known to be important for mast cell survival (Okayama and Kawakami, 2006) and SCF

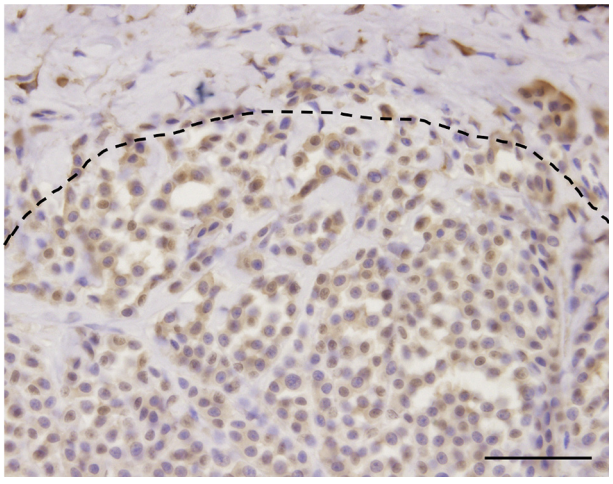

Fig. 1. Representative image of tumour cells in the marginal region of a cutaneous MCT showing cytoplasmic expression of SCF. The dotted line indicates the border between tumour tissue (below) and surrounding stroma (above). IHC. Bar, 50 μm.