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# Use of Kit Internal Tandem Duplications to Establish Mast Cell Tumor Clonality in 2 Dogs

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Mast cell tumor (MCT) is one of the most common tumors of dogs. Some affected dogs develop multiple cutaneous tumors in various locations over months to years. In these cases, it is not clear whether the tumors have arisen de novo, or if each tumor represents a recurrence of the previously excised original tumor (ie, distant metastasis). We used the presence of an internal tandem duplication (ITD) in c-*kit* to demonstrate that in 2 dogs with recurrent cutaneous MCT that had developed over 1–2 years, each recurrent MCT tumor possessed an identical ITD when compared to the original MCT, indicating that the multiple tumors were clonal in origin. This study demonstrates that similar to the situation in humans, specific somatic mutations identified in oncogenes found in canine neoplasms can be used to provide evidence of tumor clonality.

**Key words:** Canine; c-*kit* proto-oncogene; Mastocytoma; Neoplasm.

Mast cell tumor (MCT) is one of the most common tumors of dogs, occurring primarily in the skin.[1] The course of disease can range from benign behavior to an aggressive process with metastasis to lymph nodes, liver, spleen, and bone marrow.[1] Although the biologic behavior of MCT in dogs correlates most consistently with histologic grade, other factors such as tumor location, growth rate, clinical stage, concurrent treatment, and proliferative activity also have been associated with prognosis (reviewed by London and Seguin[1]).

Another well-studied potential prognostic factor for canine MCT is the presence of molecular alterations in the c-*kit* proto-oncogene. The protein product of c-*kit*, the receptor tyrosine kinase Kit, is expressed on mast cells and is critical for mast cell differentiation, proliferation, survival, and activation.[2,3] Activating mutations in c-*kit* have been described in both human and canine cancers.[4–6] Mutations in exon 11 (encoding the negative regulatory juxtamembrane domain) consisting of internal tandem duplications (ITDs) have been identified in 30–50% of grade II and grade III canine MCTs.[7,8] These mutations render Kit active in the absence of ligand binding, leading to constitutive or amplified Kit signaling, which ultimately results in aberrant proliferation and survival of cells expressing this mutation.[5,6,9] It previously has been shown that mutations in c-*kit* correlate with higher-grade (ie, malignant) tumors and occur in those tumors more likely to recur after surgery and eventually metastasize.[7,8]

One interesting aspect of MCT in the dog is the propensity of some animals to develop multiple cutaneous tumors in various locations over months to years. In these cases, it is not clear whether each tumor has arisen de novo, or if each tumor represents a local or distant recurrence of the previously excised original tumor (ie, the tumors are of clonal origin). In human oncology, molecular markers often are used to determine tumor clonality, and in veterinary oncology, genomic rearrangement of antigen receptor genes is used to evaluate dogs and cats for clonal lymphoproliferative disorders.[10] The purpose of this study was to determine if the presence of a Kit ITD in a canine MCT could be used as a molecular marker to determine the molecular origin of multiple tumors.

Fresh MCT biopsies were obtained from the 2 dogs in this study at the time of presentation to the Veterinary Medical Teaching Hospital (VMTH) at the University of California, Davis (UC Davis). For analysis of historical MCT samples from the 2 dogs for evidence of c-*kit* mutation, formalin-fixed paraffin-embedded tumors were obtained from the diagnostic pathology services to which they were originally submitted (Idexx[a] and Antech[b]).

Sections of each formalin-fixed paraffin-embedded tumor sample were cut at a thickness of 25 μm and placed in 1.5-mL microcentrifuge tubes. Paraffin was removed by the use of xylene treatment and ethanol washes and DNA was prepared as previously described.[8] For fresh tumor specimens, no xylene and ethanol treatments were used; the samples were placed in 500 μL of proteinase K buffer, and DNA was prepared as previously described.[9] For the C2 and BR canine mast cell lines (used as polymerase chain reaction [PCR] controls), $5 \times 10^6$ cells were collected from each, and genomic DNA was extracted by using proteinase K digestion and isopropanol precipitation.[8]

To screen for Kit ITDs in exons 11 and 12, the following primer pair was used: primer PE1 was based in the 5′ end of exon 11 (5′-CCC ATG TAT GAA GTA CAG TGG AAG-3′), and PE2 was based in the 5′ end of intron 11 (5′-GTT CCC TAA AGT CAT TGT TAC ACG-3′). The PCR assay was performed for 40 cycles consisting of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute. Resultant PCR products were analyzed by use of electrophoresis on a 4% agarose gel. PCR products were gel purified by using the Promega PCR Wizard Clean-Up Kit[c] and sequenced using both PE1 and PE2 primers at the core sequencing facility at UC Davis to determine the sequence of ITDs. Sequence alignment and comparison was performed by using the DNASIS sequence analysis program.[d]

Dog 1 was a 14-year-old, spayed female, yellow Labrador Retriever first presented to the VMTH at UC Davis in February 2002 for evaluation of a 2-cm dermal mass on the right side of the face just cranial to the right pinna. In January 2000, a cutaneous mass had been identified on the

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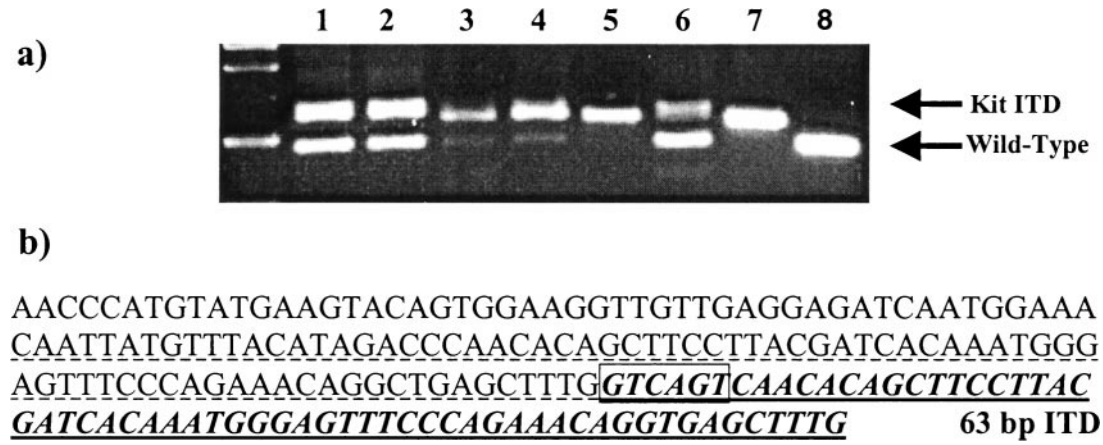


**Fig 1.** **(a)** Polymerase chain reaction (PCR) results from mast cell tumor (MCT) samples from dog 1. Lanes 1 and 2 represent PCR products derived from the original MCT (1/00), lanes 3–5 represent PCR products derived from the 2nd MCT (11/01), and lane 6 represents PCR products derived from the 3rd MCT (2/02). Lane 7 is the positive control for an internal tandem duplication (ITD), and lane 8 is the positive control for wild-type c-*kit*. **(b)** Shown is the nucleotide sequence for the 63-base-pair ITD in this MCT. The dotted underline represents the sequence that is duplicated, and the bold italic represents the actual ITD. The 6 nucleotides in the box represent those from intron 11 that were incorporated into the ITD.

dorsal midline at the level of the scapulae. This mass had been surgically excised and was diagnosed as a grade II MCT with clean margins. No further therapy had been given at this time. In November 2001, another grade II MCT was removed, this time from the caudal dorsum. Margins were found to be clean and no further therapy was given. Fine-needle aspiration cytology of the new mass on the right side of the face identified it as another MCT. A punch biopsy of this mass was obtained for c-*kit* mutation analysis, and the original MCT biopsy specimens from 2000 and 2001 were obtained for c-*kit* mutation analysis. Genomic DNA was prepared from all 3 tumor specimens and PCR was performed to determine if an ITD was present in the juxtamembrane domain of Kit. As shown in Figure 1a, all of the tumor specimens possessed evidence of an ITD, demonstrated by the presence of a larger PCR product in addition to the wild-type product. To determine if each presumed ITD was identical in nature, the larger PCR products generated from each tumor specimen were gel purified and directly sequenced. Identical Kit ITDs were found in each tumor specimen, consisting of an in-frame duplication 63 base pairs in length (Fig 1b). Within this ITD were 6 base pairs duplicated from the 5′ end of intron 11 of c-*kit*. Therefore, given that each tumor possessed an identical mutation in c-*kit*, all of the tumors that had developed in this dog over the 2-year time period were derived from a single malignant mast cell.

Patient 2 was a 4-year-old, intact male, black Labrador Retriever first presented to the VMTH at UC Davis in March 2002 for evaluation of several recurrent cutaneous MCTs. In October 2001, a grade III MCT was removed from the right pinna; no further therapy was administered at this time. In February 2002, 2 new cutaneous masses were found and removed. Histopathologic evaluation dem-

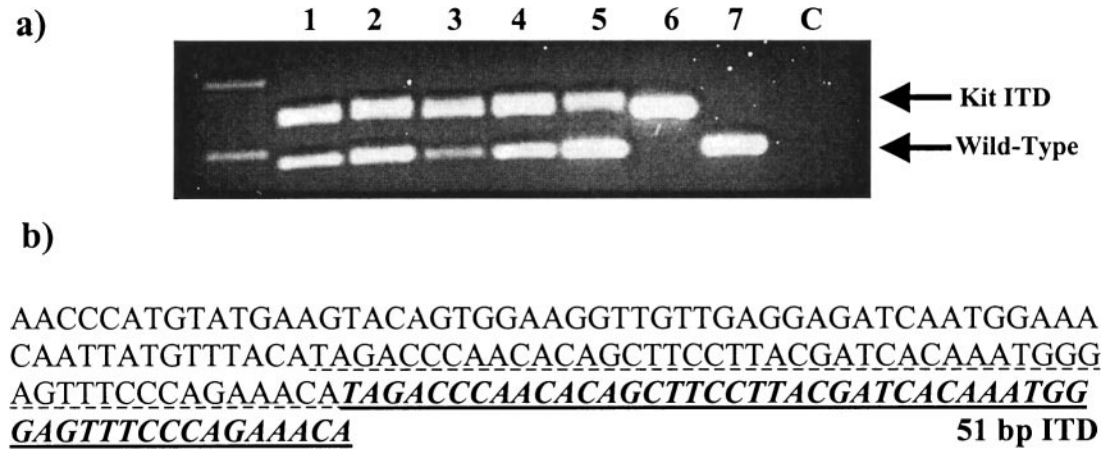


**Fig 2.** **(a)** Polymerase chain reaction (PCR) results from mast cell tumor (MCT) samples from dog 2. Lanes 1 and 2 represent PCR products derived from the original MCT (10/01), lane 3 represents PCR products derived from the 2nd MCT (2/02), and lanes 4 and 5 represent PCR products derived from the 3rd MCT (3/02). Lane 6 is the positive control for an internal tandem duplication (ITD), and lane 7 is the positive control for wild-type c-*kit*. C = control, no DNA added to the PCR. **(b)** Shown is the nucleotide sequence for the 51-base-pair ITD in this MCT. The dotted underline represents the sequence that is duplicated, and the bold italic represents the actual ITD.

onstrated completely excised grade III MCTs. The patient was begun on a chemotherapy regimen consisting of prednisone and vinblastine (given every 2 weeks), but within 1 month after surgery had developed several new cutaneous lesions. On presentation to the VMTH, masses involving the right neck, dorsal neck, and left hind paw (over digit 2) were identified. Fine-needle aspiration cytology of all masses revealed them to be poorly differentiated MCTs. A punch biopsy of the MCT over the digit was obtained for c-*kit* mutation analysis, and the original MCT biopsy specimens from 2001 and 2002 were obtained for c-*kit* mutation analysis. As demonstrated in Figure 2a, all of the tumor specimens possessed evidence of an ITD in c-*kit*. The larger PCR products generated from each tumor specimen were purified and directly sequenced. Identical Kit ITDs were found in each tumor specimen, consisting of an in-frame duplication 51 base pairs in length (Fig 2b). Therefore, given that each tumor possessed an identical mutation in c-*kit*, all of the tumors that had developed in this dog were derived from a single malignant mast cell.

The results of this study demonstrate that, similar to the case in humans, the presence of a unique somatic mutation in an oncogene or tumor suppressor gene can be used to determine the clonal origin of multiple tumors. In this instance, the multiple MCTs that arose over time in the 2 dogs evaluated were derived from a single cell, each with a distinct Kit ITD. The nucleotide sequence of each ITD was unique but was conserved within each patient and could be used to provide direct evidence that the MCTs represented metastatic disease rather than de novo tumors. Furthermore, this study suggests that malignant mast cells apparently can remain quiescent for long periods of time before appearing at distant cutaneous sites. Factors that play a role in the both the dormancy of metastatic mast cells as well as their sudden recurrent growth currently are unknown. The findings in these 2 dogs do not rule out the possibility that some dogs with multiple MCTs may develop independent tumors. However, the results of this brief report justify additional investigations in dogs that develop multiple MCTs over time. The ability to distinguish true recurrent MCTs from those that are considered new primary disease may be important because identifying recurrence or metastasis would provide further impetus to pursue additional treatments, such as chemotherapy.

## Footnotes

[a] Idexx, West Sacramento, CA
[b] Antech, Irvine, CA
[c] Promega PCR Wizard Clean-Up Kit, Promega, Madison, WI
[d] DNASIS sequence analysis program, Alameda, CA

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