REVISITING C-KIT MUTATIONS IN CANINE MAST CELL TUMORS

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Background

Mast cell tumors (MCTs) are the most frequent skin tumors in dogs, with an incidence of 16-21% of all tumors. Mutations in the proto-oncogene *c-Kit*, which encodes for the transmembrane stem cell factor receptor on the mast cells surface, induce constitutive receptor activation.

Up to 50% of MCTs in dogs exhibit internal tandem duplications (ITDs) either in exon 8 or 11 promoting cell growth and survival.

Objectives

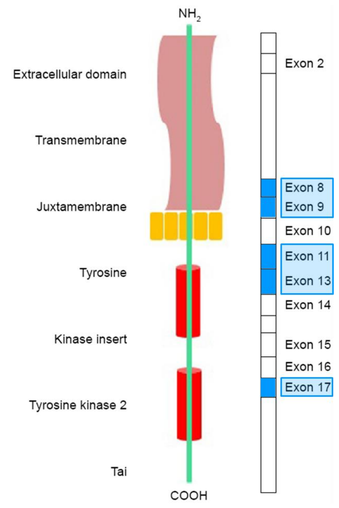
- Establishment of a time- and cost-efficient work routine to assess *c-Kit* mutations.
- Setting an indication for the treatment with tyrosine kinase inhibitors, based on the *c-Kit* mutation status of the canine MCT patients.

Methods

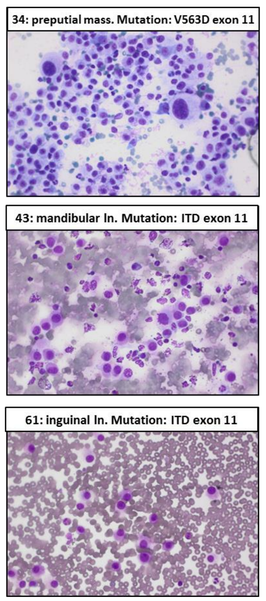
In 43 MCT dogs, the *c-Kit* exons 8, 9, 11, 13 and 17 were investigated. Total genomic DNA was isolated from remnant diagnostic material, PCR amplified and the obtained sequences were compared to healthy and malignant reference material.

Results

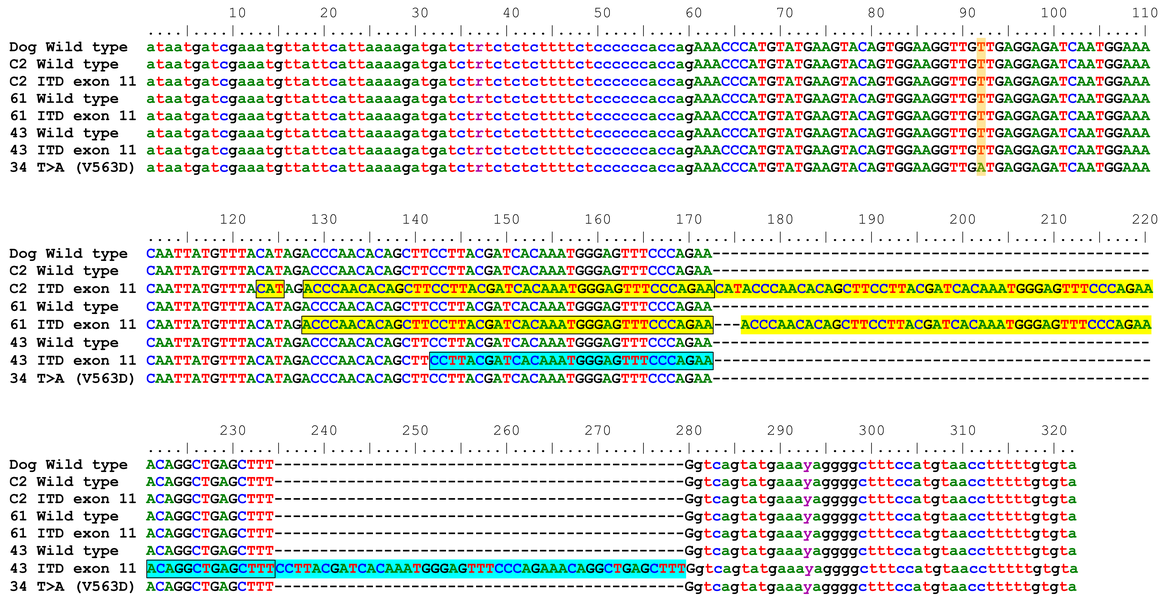
- In total, two ITDs (case 43 and 61) and one non-conservative missense mutation (case 34) were detected in exon 11.
- Case 34 showed the amino acid exchange V563D (T>A[1688]), which is a driving mutation in human gastrointestinal stromal tumors and could therefore also be an activating mutation in canine mast cell tumors.
- All three patients were classified as high-grade (Kiupel)/ grade III (Patnaik) tumor stage.
- For exon 8, neither the Q430R mutation nor ITDs, insertions and deletions were detected.
- The absence of point mutations S479I and N508I in exon 9 as well as of point mutation c.2443 G>C in exon 17 of the canine *c-Kit* gene were confirmed for all analyzed patients.
- Finally, for exon 13, no informative mutations were found among the studied cohort.



cDNA and protein structure of the tyrosine-protein kinase Kit (CD117), encoded by the proto-oncogene *c-Kit*. Mutations in exons 8, 9, 11, 13 and 17 are supposed to be associated with the development of cutaneous canine mast cell tumours (1).



Cytological analysis: Selected FNAB slides of cases 34, 43 and 61 (Diff Quick® 40x).



c-KIT sequencing results

C-Kit exon 11 multiple sequence alignment comparing mutated patient samples with appropriate reference sequences. Lower case letters indicate sequences of intron 10 and 11, respectively. Excluding potential ITDs, the obtained PCR products had a length of 277 base pairs (including primers). PCR was performed by using the forward primer 5'-CAT TTG TTC TCT ACC CTA AGT GCT-3' (Gregory-Bryson et al. 2010 BMC Cancer 15:559) and the reverse primer 5'-GTT CCC TAA AGT CAT TGT TAC ACG-3' (Jones et al. 2004 JVDI 1695-100).

V563D: Amino acid at position 563 in the canine *c-Kit* reference sequence (Accession number XP_005628025). Non-polar, aliphatic Valine is changed to the polar, acidic Aspartate. The detected ITDs are highlighted in yellow and blue, respectively.

C2 = canine mastocytoma tumor cell line (Lazarus et al. 1986 Am. J. Physiol. 251:C935-C944); 61, 43, 34 = canine mast cell tumor samples; ITD = Internal tandem duplication; SNP = Single nucleotide polymorphism; A = Adenine; C = Cytosine; G = Guanine; T = Thymine; SNP1: R = Purine (A or G); SNP2: Y = Pyrimidine (C or T); V = Valine; D = Aspartate.

Summary

(1) For *c-Kit* mutation analysis, a time- and cost-efficient work routine was established.

(2) ITDs in exon 11 were identified in 2 out of 43 cutaneous canine MCT samples.

(3) In exon 11, one patient showed the amino acid exchange V563D being described as an activating mutation in human gastrointestinal stromal tumors.

(4) The low incidence of exon 11 mutations (7%) and the overall absence of additional aberrations suggest that the *c-Kit* mutation status alone is not sufficient to make treatment decisions.

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