Author's response to reviews

Title: Evaluation of the Kinase Domain of c-KIT in Canine Cutaneous Mast Cell Tumors

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Author's response to reviews: see over
Dear BioMed Central Editorial Team,

We would like to resubmit our revised manuscript entitled “Evaluation of the Kinase Domain of c-KIT in Canine Cutaneous Mast Cell Tumors”, MS: 4551309108897823, by Webster et al. We would like to thank our reviewers for their insightful criticism of our manuscript, and have attempted to address both reviewers’ comments to the best of our ability. With regards to the comments made by reviewer 1, there were no compulsory major revisions, and we have attempted addressed all of the minor essential revisions that the reviewer has pointed out in the manuscript, as described below.

1. Further clarification regarding the phospho-transferase region of the kinase domain has been added to the abstract.
   a. “In order to characterize the prevalence of mutations in the phospho-transferase portion of c-KIT’s kinase domain in canine MCTs…”
   b. “In conclusion, mutations in the phospho-transferase portion of c-KIT’s kinase domain…”

2. The sample size from paraffin blocks is based on cubic measurements, and we have so noted this in the text.

3. The “in” that was part of the “for in” typo on line 143, page 7 has been deleted to correct the typographical error.
   a. “…products were pooled in groups of 7-10 for DNA sequencing…”

4. 4,000rpm has been converted to 1306 x g in line 163 of page 8.

5. Figure 4 has been revised showing both the residue numbers and the exon boundaries as suggested.

Although there are limitations when using a manual pool and sequencing method, this technique has been successfully used in our laboratory to identify single nucleotide polymorphisms and insertion/deletions for several years and we are confident in the reliability of this technique in revealing underlying variation. Recently other researchers in our laboratory used manual pool and sequencing to identify 79 SNPs, 9 indels, and 7 simple tandem repeats, further demonstrating the utility of this technique (Housley et al. (2004) Genomics 84: 248-264). In our current manuscript, we site the original reference for the technique (Brouillette and Venta Ref 45).

In response to the comments made by reviewer 2, we have previously screened all 33 of the mast cell tumors that were evaluated for mutations in exons 16-20 in this study, for mutations in the juxtamembrane domain of c-KIT. No mutations were found in any of the cases included in this study, and a statement regarding this has been added to the first paragraph of the results (page 8, lines 174-177). In regards to exons 8 and 9, we are interested in the possibility that these exons may contain activating mutations that might be important for the progression of canine mast cell tumors. At this time we have not looked at these exons, but we are planning on analyzing these in the future. We have added a statement in the discussion regarding the possibility of additional mutations in these exons, and the necessity of screening these exons in canine mast cell tumors (page 11, lines 238-242).

Again, we would like to thank both reviewers for their thoughtful comments as these comments greatly improve our manuscript. We sincerely hope our revisions sufficiently address the reviewers concerns. We would be happy to address any further questions or concerns.

Thank you,
Joshua Webster