Reviewer's report

Title: Gene Expression Profiling Identifies Inflammation and Angiogenesis as Distinguishing Features of Canine Hemangiosarcoma

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Reviewer: Bernard Mari

Reviewer's report:

General comments.
In this study, the authors used a dog cohort of hemangiosarcoma to investigate the molecular traits involved in this pathology. They first analyzed potential mutations of VHL and Ras and then performed genome-wide gene expression profiling of hemangiosarcoma versus i) non malignant endothelial cells and ii) bone marrow-derived tumors (lymphoma, leukemia, osteosarcoma). They found a specific signature including genes involved in angiogenesis, inflammation and invasion that discriminates hemangiosarcoma samples from all other malignant and non-malignant tested samples.

This manuscript contains some novel and interesting information in the field of hemangiosarcoma and provides genome-wide microarrays information in an animal specie with a high occurrence of this cancer that could help for a better understanding of the human disease. Nevertheless, there remains that most of the data related to pathways analysis were quite expected and some concerns detailed below.

Major compulsory revision :

1) It is mentioned in the Abstract that protein expression of VHL and Ras was examined using immunoblotting. I could not find data related to VHL/Ras western blots in the manuscript. Since the authors have derived culture from these cells, it would have been particularly interesting to also investigate the level of HIF-1 alpha and HIF-2 alpha subunits and of some well-known hypoxic markers as well as some Ras downstream effectors such as ERK or AKT to address the activation of these two pathways in the different cell lines.

2) Along the same lines, since the authors have an important collection of fixed and frozen tissues, the validation of some of the 58 genes from the microarray signature in vivo and at the protein level would have improved the manuscript.

3) It seems that some samples described in this study have already been submitted to the GEO data base along with the manuscript by Tamburini et al. in PloS One, 2009 e5549 with another accession number. We suggest to group all these samples in a SuperSerie including subseries corresponding to the different clinical subtypes to clarify these accessions for subsequent global analysis.

Minor essential revisions.
1) There is clearly a problem in the legend of Fig. 1A concerning the colors used in the graph: green should correspond to repressed genes and red to increased gene expression in order to fit with data shown in Table 1. Moreover, the scale indicates a range from -0.5 to 0.5 while the ratio mentioned in Table 1 appeared (hopefully) more relevant.

2) Results section: I would suggest to split the paragraph “Gene expression analysis segregates canine hemangiosarcoma cells from ....” into two distinct paragraphs, one focusing on the comparison with endothelial cells of splenic hematoma, the second one describing the comparison with other tumor samples.

Discretionary Revisions

Fig.3 is not very informative. In particular, Fig.3B should be improved at both the graph and legend level, in particular by adding the main pathways involved.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.