Author's response to reviews

Title: Enrichment of the Embryonic Stem Cell Reprogramming Factors Oct4, Nanog, Myc, and Sox2 in benign and malignant vascular tumors.

Authors:

Clarissa N Amaya (clarissa.amaya@ttuhsc.edu)
Brad A Bryan (brad.bryan@ttuhsc.edu)

Version: 5
Date: 13 August 2015

Author's response to reviews:

Thank you for the opportunity to resubmit this manuscript. We have completely addressed all reviewer comments. Please see itemized list of revisions below:

1) To address Reviewer #1’s concern about a lack of clinical characteristics of the examined population, we added a new table (now Table 1) that gives what we know about the clinical and demographic information associated with the 81 tumors tested in this study. We were not provided data on the clinical outcome of the patients in this study, thus we have no way of including this data in the table.

2) Reviewer #2 expressed concerns because “the percentage of stained cells for most protein markers was high…..and in the case of Myc the results are quite discrepant considering the HPA reports.” I respectfully request the reviewer to please revisit the published HPA reports and reconsider their concerns. The HPA reports that Myc is expressed in 78% of all cancers and the majority of the cancer staining was rated at medium to high levels. Similarly, the HPA reports Sox2 in 88% of all cancers, Klf4 in 28% of all cancers, Nanog in 7% of all cancers, and Oct4 in 88% of all cancers. These numbers are not discrepant with our reported findings, and if anything verify that these markers are detectable across a large number of more common tumor tissues. To clarify this concern for future readers, we have added the following sentence at the end of the Results section “The expression of Oct4, Nanog, Myc, Sox2, and Klf4 in the current study correlated well with data reported in the HPA which reveals expression of Oct4 in 88% of cancers, Sox2 in 88% of cancers, Myc in 78% of cancers, Klf4 in 28% of cancers, and Nanog in 7% of cancers.” In addition we have modified what was previously Figure 1, and now show it as a Table (Table 2).

3) Reviewer #2 asked us to clarify how the specificity of the antibodies was tested. We clarified this better in the Materials and Methods section showing that our original control staining was on tissues known to express the antigens, either stained with the antibody or in the absence of the antibody to show that the detection system did not cause non-specific staining. In addition, we added another negative control where antigen detection was performed on adipocyte tissue (shown to not express these proteins based on the HPA). We removed the positive and negative controls from each of the Figures and made a separate...
Supplemental Figure 1 that is referenced in the Materials and Methods section.

4) As requested by Reviewer #2, we changed “malignant cancers” to “malignant tumors.”

5) Reviewer #2 expresses concerns that despite a wealth of publications showing that cancer stem cells constitute a very small proportion of the total tumor cells, we show very high expression of these markers in our samples and asks us to further clarify this conundrum. This is an excellent point and we hope to make a more conservative statement in this manuscript. In this study, we do not provide evidence that these cells are “stem cells” or “cancer stem cells.” To make it very clear that we are not saying that there is a large stem cell population in these tumors, we have added the following sentences to the end of the discussion: “Our data additionally demonstrates that Oct4, Nanog, Sox2, Klf4, and Myc are widely expressed at high levels across a wide variety of sarcomas and benign vascular tumors at elevated levels. While the data reported in this study in no way indicate that the cells expressing these markers are cancer stem cells (which generally make up single digit or less percentages of the total cancer cell population in a tumor), the statistically significant increases in Oct4, Nanog, Sox2, and Myc expression in benign and malignant tumors relative to normal tissues provides correlative support that overexpression of these proteins could contribute to their overall tumorigenic properties. If future studies prove this statement true, therapeutic targeting of tumor cell populations that express embryonic stem cell reprogramming factors may disrupt tumor cell clonality, long term growth, and drug resistance.”

6) As requested by Reviewer #2, we reworked our statistics by applying the Mann-Whitney rank sum test as indicated in the Materials and Methods section. We removed what were previously histograms in Figure 1 and presented this data as Box and Whisker plots in our new Figure 6.