Author's response to reviews

Title: A combination of p300 and Braf expression in the diagnosis and prognosis of melanoma

Authors:

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Author's response to reviews:

Reviewer: VERA SADDI

Reviewer's report:

1. The question posed by the authors is clear and well defined, consisting in the analysis of the possible association between p300 and Braf expression in melanoma patients with an objective of exploring a possible opportunity to combine histone acetylation and Braf inhibition.

Author Response: We thank the reviewer for the positive comments.

2. Most of the methods were described in previous articles published by the authors and only the references are cited in the paper. Some important details are missing, including: the procedure for antigen retrieval; antibodies used in immunohistochemistry assays (clones, dilution, species, fabricators, etc); Fluorochrome and chromogenic labels.

Author Response: We now provide the details of the immunohistochemistry in the materials and methods section. Please refer to page 7, lines 17 to 23 and page 8, lines 1 to 6 for the details.

3. The authors used a classification and regression tree (CRT) to analysis the patient expression data in order to differentiate nevi and melanoma; however, this was not mentioned in the statistical analysis. We suggest a better description of this method.

Author Response: We now provide the description of CRT analysis in the materials and methods section. Please refer to page 8 lines 14 to 21 for the details.

4. The data are well described, and the results are presented in a very logic way. The results are very relevant, and a combination of Braf and p300 analysis by immunohistochemistry will certainly be very helpful for the clinical pathologist in order to distinguish melanoma from nevi.
Author Response: We thank the reviewer for the positive comments

5. The discussion and conclusions are well balanced; however, the basic relationship between MAPK pathway, Braf and p300 could be further discussed. Could the authors hypothesize this relationship? How does the cross-talk between Braf and p300 happen? How could this relationship be experimentaly tested?

Author Response: We have now provided a detailed hypothesis in our introduction and described the previous reports showing the relationship between MAPK pathway and p300. Please refer to page 4, lines 12 to 19 for the related description.

6. Investigations on Braf mutational status are crucial to elucidate the relationship between Braf and p300, and this is probably the main limitation of the study. But, we agree that this subject can be investigated in future studies.

Author Response: As we pointed in the discussion, Braf mutational status was a limitation of our study. We thank the reviewer for agreeing that this subject can be investigated in future studies.

7. Previous studies developed by the authors and by others are cited in the paper and some of them are crucial for the conclusions presented by the authors. The title and the abstract definitely describe what was found by the authors. The article is very well writing and easily comprehensible.

Author Response: We thank the reviewer for the positive comments.

Reviewer: Kenneth Tsai

Reviewer's report:

This report details findings on BRAF and p300 expression in melanoma. They find that BRAF expression is correlated with cytoplasmic p300 expression and inversely correlated with nuclear p300 expression. Nuclear p300 expression is independently correlated with patient survival. The combination of BRAF and p300 expression appears to be able to predict survival as well.

While the data appear compelling there are some major issues with the paper:

1. If nuclear p300 expression is independently predictive of survival, this should be shown with K_M curves (not only split into BRAF hi / BRAF lo, etc..) - show a KM curve that has just high and low p300 nuclear expression and a difference.

Author Response: We now provide additional figure showing the KM curve for high and low p300 nuclear expression. Please refer to Figure 4 to find the details.

2. The decision trees figures have no legends and are not well described. What do all the numbers mean?
Author Response: We now provide figure legends to decision trees. Please refer to page 19, legends of Figure 2 and 3 to find the respective description.

3. Distinguishing nevi from melanoma is rarely difficult on routine histology. It is unclear why using p300 / BRAF expression to distinguish them is clinically important. If applied to ambiguous lesions this would be very valuable.

Author Response: We agree with the reviewer that routine histology is fairly successful in distinguishing nevi from melanoma. We have now rephrased our discussion to explain that p300/BRAF expression could provide a clarification when the tissue sections show overlapping morphologic and histologic features. Please refer to page 12, lines 16-20 and page 13, lines 1 to 4 for the rephrased discussion.

4. Distinguishing primary from metastatic melanoma is valuable particularly if the data are all from the samples of the primary tumors - is this the case or are the metastatic samples from the metastatic lesions?

Author Response: We would like to point out that the metastatic samples are from the metastatic lesions. We pooled the data from primary and metastatic tumors and tested the efficacy of p300 and BRAF in classifying the data into primary and metastatic melanoma.

5. The functional consequences of these expression patterns of BRAF and p300 are overinterpreted throughout the paper. These are only correlations of expression - they have no actual functional information associated with them as determined by the authors - thus using this data to justify combination therapy and statements like "Cytoplasmic p300.. did not oppose the effects of BRAF" are overinterpretations of their data and must be avoided.

Author Response: We have toned down our conclusions and interpretation throughout the manuscript and pointed to the limitations of the study. Please refer to pages 10 and 11, and page 13 lines 13 to 20 for the rephrased description.