Author's response to reviews

Title: Candidate genes influencing liver metastasis of human uveal melanoma

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Author's response to reviews: see over
Dear Jesus Ervin Cenzon
Academic Editor
BMC Medical Genetics

Thank you for reviewing our manuscript entitled “Candidate genes influencing liver metastasis of human uveal melanoma” for publication in BMC Medical Genetics. We appreciate your comments. Having read you and the reviewer’s comments we are happy to respond as follows:

For Editor:
1. The title should be more specific. Note that the plural of "metastasis" is "metastases" and it should be changed in many places, including tables.

We have changed the manuscript title to “Expression analysis of genes and pathways associated with liver metastases of the uveal melanoma” as suggested and corrected the "metastasis" to "metastases".

2. The paper should compare the findings with the results of original papers which published the microarray sets used.

We have compared the differently expressed gene list with original papers that published the microarray sets and found that PTP4A3/PRL3 still has significant difference while SDCBP dropped out with too little fold change. In our opinion, this is caused by the enlargement of sample number which may impact larger in the previously smaller sample group for GSE27831 has only 29 samples. We have added this part in discussion detail in page 7.

3. Limitations of the study should be clearly discussed.
The largest limitation of the study in our opinion is the lack of experiment validation
of the selected genes and pathways, and the way of regulation is based the currently
known database. We will focus on the experiment validation and function
investigation of these genes in the following study. And we have put this information
in the discussion part. Thanks a lot.

4. Tables 2 and 3 - the title should be changed to " top upregulated (or down
reagulated) genes in ... where compare to where?” the title and the legend should
provide information on the type of analysis and samples compared, such as metastatic
(n=) vs. non-metastatic tumors (n=). Mean intensities should be provided with 2 digits,
not 7.

We have rebuilt Table 2 and 3, and changed title as “Most Obviously Dysregulated
Genes Sorted by P Value in Liver Metastatic Uveal Melanoma Compare to
Non-metastatic Tumors” and ” Most Obviously Dysregulated Genes with Fold
Change Absolute Value > 3 in Liver Metastatic Uveal Melanoma Compare to
Non-metastatic Tumors”, respectively. And the legend of each table has also been
changed as “Table 2. Most obviously dysregulated genes sorted by p value in liver
metastatic uveal melanoma (n=45) vs. non-metastatic tumors (n=46)” and “Most
obviously dysregulated genes with fold change absolute value > 3 in liver metastatic
uveal melanoma (n=45) vs. non-metastatic tumors (n=46)”, respectively. The mean
intensities were also changed on request detail in page 14 and 15.

5. Figure 3 in the current form is unreadable and not informative. I am not sure if Fig.
2 provides any information either.

We have changed the Figure 3 into Table 4 and 5 for more readable and informative.
Figure 2 had been changed to figure 1. From the figure we can clearly find these
differential probes could clearly separate the two groups from the whole samples and
have good consistency in the group. We have added this to manuscript detail in page
5.
6. Fig. 3, 4 - please use meaningful title, not some abbreviations.

We have changed the figure 3 into table 4 and 5 and renamed as “Different Gene Significant Upregulated GO” and “Different Gene Significant Downregulated GO”, respectively. We changed the figure 4 to figure 3 and renamed as “different gene significant pathway”.

For Reviewer #1:

1. The detail methods in this paper are too simple, which will result the false positive candidate genes. For example, in differential gene expression analysis, the authors used simple t-test between two groups. There are many methods shown some advantages than simple t-test, such as limma, RVM and SAM (PMID: 20838429).

For the sample size in our manuscript is 45 vs 46, we though it is not a small sample. T-test is one of the most widely accepted methods in discriminating two sets of data. We carefully studied the article provide by the reviewer and found when evaluating large samples t-test has very good performance in false-positive rate, power and in practice detail in table 3 of the article. For above reason, we choose t-test as the method for differential gene expression analysis.

2. Authors only highlighted top down/up-regulated gene between two groups, and most of the genes with fold change <3 (sorted with P-value). Missing the genes with large fold change and very significant genes (P-value <1e-3) would decrease the interesting of this paper.

We have reconstructed the table 2 and 3 for highlighting the top dysregulated genes
with the largest p value and fold change detail in page 5, 14 and 15. Thanks for the comments to let us further investigate the candidate gene list.

3. The paper didn’t compare the differentially expressed gene list in here with original data published paper.

We have compared the differently expressed gene list with original papers that published the microarray sets and found that PTP4A3/PRL3 still has significant difference while SDCBP dropped out with too little fold change. In our opinion, this is caused by the enlargement of sample number which may impact larger in the previously smaller sample group for GSE27831 has only 29 samples. We have added this part in discussion detail in page 7.

4. Also, this paper is not well organized in discussion with too many background information. The authors should focus on the their findings from result and describe the more association between these candidate genes and metastasis occurrence in uveal melanoma.

Thanks to the reviewer’s comments, we tried our best to reorganize the discussion part as the reviewer’s comments detail in page 7 to 9. For uveal melanoma is a rare disease and still lack of investigation, most of the candidate genes have no information about their roles in uveal melanoma. For most of the genes have been found play roles in cancer cell migration and liver metastases, we tried to put our attention in this aspect.

5. In abstract, 1138 genes or probes should be checked and corrected.

We have changed the genes to probes as comments detail in page 7.

6. Figure 1 should be in supplement since the outlier did not go though the next analysis.
We put the figure 1 to the supplement place as supplement figure 1 detail in page 4.

7. In the methods section, the normalization method for microarray data should be mentioned.

MAS5 was used to normalize the original microarray data and we have put this in page 4.

8. The paper used P-value < 0.05 as significant criteria in significant Differential Gene Analysis, Pathway analysis, but P-value <0.01 as the criteria in GO analysis. Then, in the result 4, the author change to O-value <=0.05 in Significant pathway analysis. So Author need to use the same significant criteria.

We reformed the GO analysis with P-value < 0.05 as the criteria and put in table 4 and 5 detail in page 16 to 19 to use the same significant criteria.

9. Table 1 summary should be revised according the real published data. Description or marks need make under the table If referring other data.

We got the sample information from the published articles and GSM information in the GEO database and put these in the legend of table 1 detail in page 13.

10. In methods section, delete “All the patients have informed written consent forms” . The method for Signal-Net analysis is redundant. Considering revised or deletion second part of this method. And the last paragraph should be revised.

We delete “All the patients have informed written consent forms” and the second paragraph in Signal-Net analysis methods section as comments.
11. In Figure 2, what’s the heatmap legend key means? standard deviation? It better to understand the gene expression pattern if added two groups with a colored horizontal bar between heatmap and cluster.

The legend key means a range of signal values after normalization. The more red means the larger of the signal values. We tried to add the color horizontal bar for the figure.

12. In Table 2 and Table 3, the titles need to clarify up/down-regulated gene in which group (non- metastatic and liver metastatic patients).

We have rebuilt Table 2 and 3, and changed title as “Most Obviously Dysregulated Genes Sorted by P Value in Liver Metastatic Uveal Melanoma Compare to Non-metastatic Tumors” and “Most Obviously Dysregulated Genes with Fold Change Absolute Value > 3 in Liver Metastatic Uveal Melanoma Compare to Non-metastatic Tumors”, respectively.

13. In Figure 3, P-value should be added to shown the significant GO terms.

We added the p-value of the significant GO items in the table 4 and 5 detail in page 16 to 19 as comments.

14. In Sigal-network analysis (Figure 5), the interesting nodes (YWHAZ, ATM, etc.) need to add fold change and P-value.

Only 2 of 5 concerned genes were in the signal-network and we had added the fold change and P-value for the interesting nodes.

15. The first and second paragraphs in discussion should be integrated into background.
We integrated the first and second paragraphs in discussion and added into the background detail in page 3 as comments.

16. “mir-15b and miR-16” change to “miR-15b and miR-16”.

We corrected the mir-15b to miR-15b detail in page 7 as comments.

For Reviewer #2:

1. In the abstract, in the methods sections, the selected cases is not well described, because the samples seem to originate from liver metastasis. Correct it.

We corrected the methods section in abstract as “Expression profiling of ocular tumor tissues from 46 liver metastatic uveal melanoma samples and 45 non-metastatic uveal melanoma samples were got from GEO database” detail in page 2 as comments.

2. According the role of CXCR4 I suggest other two papers:

We have added these to references to the manuscript detail in page 3 as comments.

We hope that forgoing addresses the reviewers’ comments to your satisfaction but we would be happy to respond to any further queries that may arise.

Your sincerely,

Yong Yang