Peer Review File

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Review comments

Comment1: You mention some deregulated miRNAs in retinoblastoma disease in the Introduction, can you also mention some genes and transcription factors?
Reply1: We made some changes in the text.
Changes in the text: See R60-62

Comment2: Can you clarify the aim of the study?
Reply2: We hope to provide peer researchers with potential biomarkers and research ideas for retinoblastoma by collating and analyzing bioinformatics data.

Comment3: Please describe the different datasets in short. Also you could compare your results to others who have used the same dataset, for instance;
Reply3: First of all, as mentioned in the text, we integrated different sequencing data to find the different expressed genes (DEGS), which I think is more scientific than that in a single database. Secondly, the paper you illustrated did not predict the microRNA regulatory network like our paper, which was only limited to non-coding RNAs or mRNAs.

Comment4:GSE111168 and GSE125903 are sequencing data, so the title should be changed and the title 'microarray data' in materials and methods section, because to me it seems you have not used multiple microarray datasets.
Reply4: This was an oversight in our writing, which we acknowledge and have changed "microarray data" to "bioinformatics data".
Changes in the text: the title 'bioinformatics data' in materials and methods section, R72.

Comment5: In the materials & methods section, please indicate version and the correct citation for the following tools; Cytoscape, clueGO, R, STRING, MCODE, miRtarbase, transmiR.
Reply5: we have modified our text as advised
Changes in the text: we have modified our text as advised (see materials and methods section, R92 107 114 118 120).

Comment6: Please indicate manufacturer and preferably catalogue number for the following; RPMI-1640, DMEM/F12, FBS, penicillin, streptomycin.
Reply6: we have modified our text as advised
Changes in the text: see materials and methods section, cell culture (See R139-144).

Comment7 and 8: Can you specify which cDNA synthesis kit have you used from Takara? Include the program for cDNA synthesis and PCR (temperature and time).
Reply7 and 8: We have modified our text as advised
Changes in the text: see materials and methods section, Total RNA extraction and qRT-PCR validation (R150-155).

Comment9: Change ‘qRT-PCR’ to ‘RT-qPCR’ according to MIQE guidelines (PMID: 19246619)
Reply9: We have modified our text as advised.

Comment10: Include more information on the nucleic acid extraction, how many cells were extracted? How many replicates were analyzed? How many replicates were analyzed in RT-qPCR?
Reply10: We extracted RNA using 6-well plates with $1*10^7$ cells in each well, and each set of experiments was repeated at least three times.

Comment11: On r162, is there a ‘respectively’ missing, did you normalize miRNA to U6 and mRNA to GAPDH?
Reply11: We normalize miRNA to U6 and mRNA to GAPDH, and we added respectively in proper position.
Changes in the text: R156

Comment 12: In figure 2 legend, specify that only aberrant genes are included.
Reply12: We have modified as suggested.
Changes in the text: See figure legend R13.

Comment 13: Is the color legend a bit too short in figure 3? I do not understand the colors in the figure.
Reply13: We have changed a new version of figure3 and the color means p-value.

Comment14: R232, change comma to semicolon: “in five pathways;”.
Reply14: We have modified our text as advised.
Changes in the text: See R224

Comment15: Maybe it would be interesting to look at up- or downregulated genes separately in a GO and KEGG enrichment analysis?
Reply15: We also thought this way before, but since we used RRA method, the final number of genes was small, and we could not obtain very effective information after separate analysis, so we chose to integrate DEG for analysis.

Comment16: The phrase ‘screen out’ seem to indicate to remove something, and you have used this phrase when you meant that you kept those. This phrase is used on rows 139,144,185,259,271,345. Maybe ‘identify’ could be used?
Reply16: We have modified our text as advised, changing “screen out ” to “identify”.
Changes in the text: See R129,133,178,251,263

Comment17: Should the y label be ‘miRNA’ instead of ‘mRNA’ in figure 6.
Reply17: We have modified our figure as advised.
Changes in the text: See Figure 6.

Comment18: Specify cancer type on r307.
Reply18: We have modified our figure as advised and added a specfic cancer type.
Changes in the text: R299

Comment19: Can you mention some limitations to your paper in the Discussion section?
Reply19:
Changes in the text: See main text R356

Comment20: Can you include a clear conclusion?
Reply20: We have modified the conclusion in the main text.
Changes in the text: R34-39.

Comment21: To me the aim and conclusion and the different parts of the paper are not clear. The title indicates that the main essence about the paper is to understand microRNA-target gene-TF regulation in retinoblastoma. And you have built this network, but I do not see the connection between this and the RT-qPCR verification. Instead you chose the top 5 hub genes and verified those. Would it be of interest to verify some of the targets in the network in figure 5?
Reply21: We choose some genes from figure5 to verify their expression as you advertised.
Changs in the text: See figure6.

Comment 22: If there are more retinoblastoma cell lines available, it would be valuable to look at expression in more than one cell line. Maybe there is some publicly available microRNA and or gene expression data in retinoblastoma cell lines that you could look at?
Reply22: We tried to find some bioinformatics data for other retinoblastoma cell lines in the public database, and the results obtained by the query were all treated cell lines, which did not meet the requirements. As for your suggestion, we really want to do it, but unfortunately, there is no appropriate data for us to conduct further analysis.

Comment 23: Please refer to additional figure 1 in the main text.
Reply23: We have already modified.
Changes in the text: R229.