Author's response to reviews

Title: Comprehensive analyses of imprinted differentially methylated regions reveal epigenetic and genetic characteristics in hepatoblastoma.

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Author's response to reviews: see over
Dear Prof. Solera,

Thank you very much for your consideration of our manuscript entitled, “Comprehensive analyses of imprinted differentially methylated regions reveal epigenetic and genetic characteristics in hepatoblastoma” by Rumbajan et al. (MS: 2038377211937522). We appreciate the helpful comments of the reviewers. We revised our manuscript according to the comments. Our responses are presented on the following pages, and we believe that we have addressed all the reviewers’ comments.

The points changed from the previous version are outlined as follows and indicated by red text in the manuscript.

Thank you for your consideration of our manuscript. We really look forward to hearing from you as soon as possible.

Sincerely Yours,

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Reviewer #1
The authors addressed all the concerns except the Major Comment #1. Considering the issue of multiple testing, the authors are requested to describe P values after the correction of multiple testing using an appropriate method, such as Bonferroni correction.

We have to apologize for not addressing the Major Comment #1. We consulted Dr. Keitaro Tanaka, a professor of Department of Social Medicine and an authority of statistical analysis in our university, and performed statistical analyses of our data again. When we applied Bonferroni correction to some comparisons of aberrantly methylated DMRs and to the comparison of the methylation levels of CpG sites of LINE-1 described on pages 20-22, some of significant differences were suspended. We corrected the texts on pages 20 and 22, and also Figure 3 and Figure 4 along with their legends accordingly.

Reviewer #2
Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct) Abstract: In the following: “Aberrant methylation at imprinted differentially methylated regions (DMRs) in humans 11p15.5” change to human, not humans.

We changed “in humans” to “in human” according to the comment.

Methods/MALDI-OF MS: correct spelling of “rangde”.

Thank you very much for pointing out the miss-spelling. We corrected it.

Results: p17. Please provide a reference for hot-stop PCR. “Hot-stop” is not a widely used expression (Uejima H, Lee MP, Cui H and Feinberg AP. (2000). Nat. Genet., 25, 375–376.)

We added the reference as reference 17 on page 17.
P17/18: Change “We found seven genetic alterations in four tumors resulting in the aberrant methylations:” to “We found seven genetic alterations in four tumors resulting in aberrant methylation:”

We made the suggested correction on page 18.

P20: Change “In the TxC comparison, both hypermethylation and hypomethylation were observed in tumors in the frequencies of no significant difference.” To “In the TxC comparison, both hypermethylation and hypomethylation were observed in tumors (no significant difference).”

We changed the sentence on page 20 according to the comment.

P22: change “However, other CpG sites did not show hypomethylation although bare hypermethylations (less than 2.5%) were found…” to “However, other CpG sites did not show hypomethylation although bare hypermethylation (less than 2.5%) was found…”

We corrected the sentence according to the comment.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

P22. I recommend deletion of the last sentence of the results section – it is not needed and it suggests more certainty that it should. Line-1 methylation has been used as a surrogate for genome-wide methylation but it is not the same as quantifying total methylation.

We keep the last sentence and replaced “overall methylation level” with “genome-wide methylation level” in the sentence according to the comment.

Fig 1 Legend: It is useful to be reminded of “Aberrant methylation of a DMR was defined as when more than 60% of total CpG units or CpG sites were aberrantly methylated. Aberrant methylation of a CpG unit or CpG site was defined as
occurring when the difference of its methylation indexes in two samples exceeded 0.15.” However, it would be adequate to state “Aberrant methylation is defined in Methods”.

In the first manuscript we did not describe the definition of aberrant methylation in the legend, however, we added the description in the revised manuscript because reviewer #4 requested to describe the definition in the legend.

Fig 4 legend: The last sentence of the legend (starting CpG#1) is not needed. The figure is clear.

We deleted the last sentence.

**Reviewer #3**

Abstract Background:

“Aberrant methylation at imprinted DMRs in humans 11p15.5 has been reported in many tumours including hepatoblastoma” – does not make sense, removal of the s from humans would make sense, alternatively removal of “in humans” would be more concise and just as clear.

We changed “in humans” to “in human” according to the comment.

**Figure 4:**

The error bars seem to have been slightly shifted so that they are not coincident with the dots to which they refer

We apologize for the problem of Figure 4. We aligned the error bars and the dots in the figure.

**Reviewer #4**

The small sample number is still a problem and most of the issues that the other reviewers had (such as expressing data in %) stem from the small sample number.
Only one data expressed in % is “18 (55%) of 33 DMRs” in line 1 on page 19. We agree with the comment and deleted “(55%)”.