nature portfolio

Peer Review File

Harnessing DNA replication stress to target RBM10 deficiency in lung adenocarcinoma

Editorial Note: This manuscript has been previously reviewed at another journal. This document only contains reviewer comments and rebuttal letters for versions considered at Nature Communications.

REVIEWERS' COMMENTS

Reviewer #1 (Remarks to the Author):

The authors have satisfactorily addressed most of my concerns with additional experimentation. However having said that, I still feel that the part Aurora kinase is too preliminary and off track from the main focus of of the manuscript.

Reviewer #2 (Remarks to the Author):

The authors have now convincingly addressed all my concerns and/or disagreed whenever appropriate (point#4).

Reviewer #4 (Remarks to the Author):

Machour et al., explore the use of replication stress to target RBM10 deficient lung tumours. I have several specific comments on this paper.

1. The paper uses cell line models, patient material and also in vivo system and is thus comprehensive in its analysis of the link between RBM10 and WEE1 in SL. It would be useful for the reader if more of the blue dots in Figure 1c were labelled. i.e. what is the blue dot that is beyond WEE1 in significance? I am mindful not to introduce yet more questions into the review process at this stage but I wonder if the authors could elaborate in the text why WAPAL and SF3B2 which are common essential don't reduce the fitness of WT HCC827 cells? This could be due to the timing of the assay etc. etc. It would be useful if the authors released the raw count files in their supplementary data – this is the easiest way for others to pick up and replicate their analysis.
2. It is pleasing to see that work was done in multiple cell line models; HCC827 and NCI-H1299. I presume (although I can't see) that the lethality phenotype (i.e. the SL interaction) was also validated in this second model? I can't see it in the text but where would a reader see the genetic lesions introduced into each of the engineered cell lines?
3. The data in Figure 2g is very nice and clear. The obvious experiment here (and I am very mindful that I am joining this reviewer at a later stage) is that a rescue experiment would really nail the association seen here. It is of course up to the editor to decide if this is in scope at this stage or not. This has been beautifully done for the MK1775 work in figure 4. As above the majority of the work in these figures has been done in just 2 cell lines.
4. A generally note on the figures – Figure 2C for example – I can't see the scale bar. In several of the figures – for example figure 5 there are grey boxes around figure panels that should be removed.

Focusing specifically on comments made by the reviewers and the authors responses.

1. I like the Aurora A section. I think it further speaks to the role of replication stress in the RBM10-associated phenotype. I agree with the authors that it could stay.
2. It is good that the authors have improved the depth of sequencing of the CRISPR screen but I do feel that Reviewer 3 is being a bit harsh in saying that the WEE1/RBM10 is not obvious in the primary data. I also disagree with them and think that RNAi is a good say to validate the CRISPR interactions identified from the screen. It is an orthogonal technology. The rescue experiments that reviewer 3 is asking for are validate (as above) but these experiments are performed in figure 4 (not in exactly the same context but they do convince me of the phenotype.
3. The authors are also correct about the toxicity associated with WEE1/AURKA inhibition and on this point I think Reviewer 3 is mistaken.

Minor
1. The authors really should know to italicise gene names.
2. In places the "tense" used is incorrect and I would suggest the authors look at this carefully. Similarly, I would suggest they look carefully at the grammar of the article as it is occasionally wrong.

Point-by-point response to Reviewers Comments

Reviewer's comments are in **Black**, our responses are in **Blue**.

Reviewer #1 (Remarks to the Author):

The authors have satisfactorily addressed most of my concerns with additional experimentation. However having said that, I still feel that the part Aurora kinase is too preliminary and off track from the main focus of of the manuscript.

We thank the reviewer for his time and effort in reviewing our manuscript. Indeed his constructive comments dramatically improved the findings of our manuscript. Regarding the part with Aurora A inhibition, and following the editor and Reviewer #4 comments, we decided to keep this part and expanded the discussion to reflect the limited mechanistic understanding of WEE1-Aurora A synergy in RBM10-deficient cells.

Reviewer #2 (Remarks to the Author):

The authors have now convincingly addressed all my concerns and/or disagreed whenever appropriate (point#4).

We would like to express our gratitude for Reviewer #2 whose insightful comments also greatly improved our manuscript.

Reviewer #4 (Remarks to the Author):

Machour et al., explore the use of replication stress to target RBM10 deficient lung tumours. I have several specific comments on this paper.

1. The paper uses cell line models, patient material and also in vivo system and is thus comprehensive in its analysis of the link between RBM10 and WEE1 in SL. It would be useful for the reader if more of the blue dots in Figure 1c were labelled. i.e. what is the blue dot that is beyond WEE1 in significance? I am mindful not to introduce yet more questions into the review process at this stage but I wonder if the authors could elaborate in the text why WAPAL and SF3B2 which are common essential don't reduce the fitness of WT HCC827 cells? This could be due to the timing of the assay etc. etc. It would be useful if the authors released the raw count files in their supplementary data – this is the easiest way for others to pick up and replicate their analysis.

We thank the reviewer for his careful examination of our manuscript. We would like to draw the reviewer's attention that in Figure 1e we did observe a mild decrease in fitness of RBM10-proficient cells following the depletion of WAPAL and SF3B2, consistent with their "essentiality" in high-throughput screens. However, RBM10-deficeint cells show a severe reduction in cell

fitness under the same experimental conditions. Moreover, the raw read counts of the screen are included in Supplementary Table 1.

2. It is pleasing to see that work was done in multiple cell line models; HCC827 and NCI-H1299. I presume (although I can't see) that the lethality phenotype (i.e. the SL interaction) was also validated in this second model? I can't see it in the text but where would a reader see the genetic lesions introduced into each of the engineered cell lines?

We would like to draw the reviewer's attention that we have tested and presented the sensitivity of both HCC827 and NCI-H1299 (along with other cell lines) to WEE1 inhibition as shown in Figure 4.

3. The data in Figure 2g is very nice and clear. The obvious experiment here (and I am very mindful that I am joining this reviewer at a later stage) is that a rescue experiment would really nail the association seen here. It is of course up to the editor to decide if this is in scope at this stage or not. This has been beautifully done for the MK1775 work in figure 4. As above the majority of the work in these figures has been done in just 2 cell lines.

We thank the reviewer for suggesting a rescue experiment for hydroxyurea treatment. However, we believe that the rescue in replication stress phenotype and sensitivity to WEE1 inhibition described in Figures 3 and 4 confirm the association between RBM10-deficiency and replication stress. Therefore we believe that there is not sufficient added value to this experiment at this stage.

4. A generally note on the figures – Figure 2C for example – I can't see the scale bar. In several of the figures – for example figure 5 there are grey boxes around figure panels that should be removed.

These issues are resolved using the original High-resolution Figures.

Focusing specifically on comments made by the reviewers and the authors responses.

1. I like the Aurora A section. I think it further speaks to the role of replication stress in the RBM10-associated phenotype. I agree with the authors that it could stay.

We thank the reviewer for his opinion and support.

2. It is good that the authors have improved the depth of sequencing of the CRISPR screen but I do feel that Reviewer 3 is being a bit harsh in saying that the WEE1/RBM10 is not obvious in the primary data. I also disagree with them and think that RNAi is a good say to validate the CRISPR interactions identified from the screen. It is an orthogonal technology. The rescue experiments that reviewer 3 is asking for are validate (as above) but these experiments are performed in figure 4 (not in exactly the same context but they do convince me of the phenotype.
3. The authors are also correct about the toxicity associated with WEE1/AURKA inhibition and on this point I think Reviewer 3 is mistaken.

We sincerely thank the reviewer for his careful assessment of our manuscript and agreeing with us on these points.

Minor
1. The authors really should know to italicise gene names.
2. In places the “tense” used is incorrect and I would suggest the authors look at this carefully. Similarly, I would suggest they look carefully at the grammar of the article as it is occasionally wrong.

We acknolwedge these points and have addressed them where appropriate.