Author's response to reviews

Title: Both Talin-1 and Talin-2 correlate with invasion and migration in human hepatocellular carcinoma

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Version: 6 Date: 29 December 2015

Author's response to reviews: see over
Dear Editor,

Many thanks to you and reviewers for the feedback regarding our manuscript entitled "Both Talin-1 and Talin-2 correlate with invasion and migration in human hepatocellular carcinoma" submitted for publication in your journal.

The reviewers provided sound and constructive comments, which have helped us improve our manuscript substantially.

A point by point response to all reviewer comments can be found below. Meanwhile, changes have been made in the revised version accordingly, we hope to your entire satisfaction.

Looking forward to hearing from you, we thank you in advance for the opportunity to contribute to your prestigious journal. Please contact us with any other questions regarding this manuscript.

Yours sincerely,

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Reviewer's report
Title: Both Talin-1 and Talin-2 correlate with invasion and migration in human hepatocellular carcinoma
Version: 2 Date: 29 May 2015
Reviewer: Ka Wai K Au-Yeung
Reviewer's report:
Comments to the Author
This manuscript is not well prepared because of poor English writing. The article may be accepted after revision.

Major compulsory revisions

1. Grammatical mistake were found in the text.

Response: Thanks for this remark. The whole manuscript has been reviewed and corrected by an experienced native English speaking scientist. The resulting text is substantially improved.


Response: Thanks for this remark. The whole manuscript has been polished as indicated above.

3. The differential expression of TLN-1 and TLN-2 in different cell lines should be provided (at least as supplementary data).

Response: Thanks for this comment. The data have been published in Ref#8 “Kun-Peng Fang, Jian-Lin Zhang, Yan-Hong Ren, Ye-Ben Qian. Talin-1 Correlates with Reduced Invasion and Migration in Human Hepatocellular Carcinoma Cells. Asian Pac J Cancer Prev. 2014; 15(6): 2655-2661”

4. Figure 2C, the mRNA expression of TLN-1 and TLN-2 were not in line with the histogram provided. No significant difference can be seen from the bands Provided.

Response: Thank for the remark. We apologize for the error. Indeed, mRNA expression was determined by real-time RT-PCR, and calculated using the $2^{-\Delta\Delta CT}$ method. The figures are now corrected.

5. Figure 2F and G, the y-axis stated with gene expression are not appropriate. Are they protein level?
Response: The figures have been revised.

6. Please quantify the results in Figure 3 in histograms and the quality of scratch assay are poor
Response: The figures have been revised.

Reviewer's report
Title: Both Talin-1 and Talin-2 correlate with invasion and migration in human hepatocellular carcinoma
Version: 2
Date: 6 July 2015
Reviewer: Sulagna Dr. Banerjee

Reviewer's report:
In this manuscript the authors Fang et al elucidate the role of Talin1/2 in hepatocellular carcinoma. The authors show that this protein may be involved in invasion, migration and tumorigenesis in HCC cells.

Major comments:

1. An article by the same group shows that Talin1 correlated with reduced invasion in HCC. How does that study fit in with the current study?

Response: Thanks for the question. Our previous investigation assessed the levels of TLN-1 and 2 in the five HCC cell lines and the human normal liver LO2 cell line, as well as invasion and migration. We found that high Talin-1 expression is correlated with a reduced invasion and migration ability, which hinted that TLN-1 might be related with invasion and migration of HCC. However, the current report demonstrated that Talin-1/2 expression knockdown using RNA interference reduces the malignancy of MHCC-97L cells in vitro and in vivo experiments (mouse xenografts). The observed changes of cell cycle distribution, anoikis, migration and invasion, and tumor formation inhibition in vivo were likely associated with the down-regulation of TLN-1 and TLN-2, not just TLN-1. In our future studies, we will be careful to investigate the underlying mechanism.

2. How does Talin ½ regulate invasion and migration in HCC. Is it possible to do a mechanistic analysis?

Response: Yes, we believe this is the case. The underlying mechanisms were beyond the scope of this work, and will be explored in future experiments. This can be found in the revised Discussion: “...These corroborate previous reports that TLN-1 is involved in integrin activation[7–8], which leads to the regulation of adhesion, invasion, proliferation, anoikis, survival, tumor progression and metastasis [7–8, 9–11, 33]. Interestingly, our in vivo mouse xenograft model confirmed the in vitro findings as smaller tumors were obtained in animals inoculated with the TLN-1/2 knockdown MHCC-97L cells in comparison with those of the control (blank and negative) groups. Interestingly, KIF14 and TLN-1 inhibition was found to sensitize triple-negative breast cancer (TNBC) cells to therapeutic intervention [20]. However, Talin 1 was recently proposed
to be a novel player in the anti-metastatic signaling network of miR-12434. Taken together, these findings suggest that Talin1/2 might regulate HCC invasion and migration through complex mechanisms that are not completely understood.”

3. The authors have very compelling animal data showing that Talin plays a significant role in tumorigenesis. An accompanying histology in the main figure panel would add to the message.

Response: This is an interesting point. However, we did not perform histology in this study. Since tumor samples were no more available, we could not provide those data this time. In our future studies, we will be careful to investigate that as well.