Author's response to reviews

Title: miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells

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

   Thanks for you and the reviewers for their helpful comments and suggestions. We have edited our manuscript according to the reviewers’ comments point by point, spelling and grammatical errors have been corrected, and some important experiments data have been also provided. All changes were highlighted with red color. The following are our responses to each of their comments.

   We believe that we adequately address all of the reviewers’ comments and it is ready for publication in *BMC Cancer*. We would like to take this opportunity to thank you and reviewers again.

Sincerely,

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Reviewer 1:
Although several important issues still need to be clarified, for example, the roles of miR-183 and its target gene PDCD4 in HCC development, the authors describe an interesting story between miR-183 and HCC apoptosis. Therefore, it could be accepted for publication by BMC Cancer. 
Although these issues can not be clarified now, we will continue to focus on these problems.

Reviewer 2:
1. The authors examined the PDCD4 mRNA expression in the tumor and corresponding peritumoral tissue according to the several reviewers? request and concluded miR-183 and PDCD4 mRNA expression showed ?significant? negative correlation. However, there is no parameter to show the ?significance? of negative correlation. At least, correlation coefficient and P value is necessary to show the ?significance?. Furthermore, although the authors add the Table 2 showing the quantitative mRNA data of miR-183 and PDCD4, same data of miR-183 are also listed in Table 1. It seems to be redundant and these data should be summarized in one table.
As suggested, the expression data of miR-183 and PDCD4 were summarized in Table 1. The correlation between expression levels of miR-183 and PDCD4 was analyzed and shown in Fig. 1C. U indicates up-regulation of miR-183 in HCC tissues, and NU indicates down-regulation and no significant change of miR-183 in HCC tissues. The mean value is shown as a horizontal line. Statistical analysis was performed with Student’s t-test. For the number of samples was limited, HCC specimens (miR-183 upregulated or no significant change) were combined into one group.

2. In the reply to the reviewer?s question about miR-21, the authors concluded that miR-21, a known regulator of PDCD4 mRNA, was not involved in the TGF-β1-induced PDCD4 regulation in Huh7 cells because there was no change in miR-21 level after TGF-β1 treatment. However, the no change of miR-21 levels does not directly mean that miR-21 are not involved in PDCD4 mRNA regulation. If the authors insist so, it is required to show that miR-21 does not affect the PDCD4 mRNA level after TGF-β1 treatment in Figure 3. Otherwise changes of miR-183 levels in Huh7 after TGF-β1 treatment should be demonstrated.
As you mentioned, PDCD4 is the target gene of miR-21, and miR-21 can be induced by TGF-β1 in smooth muscle cells (Davis MN et al, Nature 2008; 454: 56-61). Then, we detected the expression level of miR-21 in Huh7 cells treated with TGF-β1. Although miR-21 can not be induced by TGF-β1 in Huh7 cells, PDCD4 should be regulated by endogenous miR-21 as reported before. Not only that, many other factors could affect the expression of PDCD4. Here, we focus on the regulation of PDCD4 by miR-183, though it could be regulated by miR-21 simultaneously. In addition, we detected the expression of miR-183 in Huh7 cells treated with TGF-β1, the results were shown in Fig. 3B.

Reviewer 3:
1. Author should analyse the data of table 2 by statistic method, not raw data. The PDCD4 mRNA levels in hepatoma specimens seem be not significant negative relationship with miR-183 level.

The correlation between expression levels of miR-183 and PDCD4 was analyzed and shown in Fig. 1C. U indicates up-regulation of miR-183 in HCC tissues, and NU indicates down-regulation and no significant change of miR-183 in HCC tissues. The mean value is shown as a horizontal line. Statistical analysis was performed with Student’s t-test. For the number of samples was limited, HCC specimens (miR-183 upregulated or no significant change) were combined into one group.

2. The response to question 4 ?If the transfection of synthetic miR-183 regulates apoptosis by targeting PDCD4, it may need to over-expression of PDCD4 without UTR to rescue this phenomena.? The answer: Changed as suggested. But I can?t find the data in the figure. It should use PDCD4 expression constructor to rescue the apoptotic effect of miR-185, not report assay

We are sorry for our negligence. A rescue experiment was performed in the revised manuscript (Fig. 4C).