Dear Editors,

Thank you for reviewing our manuscript and for the reviewers' comments concerning our manuscript entitled "MiR-1181 inhibits invasion and proliferation via STAT3 in pancreatic cancer" (ESPS Manuscript NO: 28441). All the comments are valuable and very helpful for revising and improving our paper. We have revised the manuscript according to the comments and suggestions. Please see the point by point responses to the comments as listed below.

Replies to Reviewer #:

Reviewer # (Reviewer Comments to the Author):

1. The authors should clearly state the differences between the present study and their previous work "MiR-1181 inhibits stem cell-like phenotypes and suppresses SOX2 and STAT3 in human pancreatic cancer" published in Cancer Letters 2015.

Response: Thanks for the suggestion. We have further stated the differences between the present study and our previous work in the introduction section as below:

We previously found that miR-1181 could suppress the sphere formation rate, side population, and expression of the CSCs marker CD133. Furthermore, miR-1181 suppressed the tumorigenicity of pancreatic cancer cells in human derived pancreatic xenografts. Moreover, we demonstrated that miR1181 inhibited CSC phenotypes by directly suppressing SOX2 and STAT3 in pancreatic cancer cells. But we have not elucidated miR-1181's role on proliferation and invasion of pancreatic cancer cells, which was important malignant biological behavior of pancreatic cancer. And CSCs regulator always play an important role in the process of growth and invasion. As a result, We hypothesized that miR-1181 may suppress the invasion and proliferation of pancreatic cancer and explored it.

2. Could the authors provide evidence for the specificity and efficacy of the used miR-1181 adenoviruses? Any references that could be cited? Same for si-STAT3.

Response: Thank you for recognizing this question. We have validated the specificity and efficacy of miR-1181 adenoviruses and si-STAT3 before carrying out subsequent experiments. But the results of the verification experiment were not included in the manuscript. As a result, we have added the relevant content in Figure 1 and Figure 4 in modified manuscript.

3 To use HPDE as a 'normal' control for pancreatic cancer cells is questionable, since pancreatic cancers most likely develop from acinar cells, through ADM and PanIN. This should at least be discussed. Are there any data available regarding miR-1181 expression in acinar cells?

Response: We agree with this comment. It was more appropriate to take acinar cells as a normal control. But it is diffcult to isolate and culture pancreas acinar cells. And we have not detected miR-1181 expression in pancreas acinar cells. As a result, we took HPDE cells as ‘normal’ control and many published studies took HPDE cells as ‘normal’ control. Here are some of the publications:

1. Li M, Zhang Y, Liu Z, Bharadwaj U, Wang H, Wang X, Zhang S, Liuzzi JP, Chang SM, Cousins RJ, Fisher WE, Brunicardi FC, Logsdon CD, Chen C, Yao Q. Aberrant expression of zinc transporter ZIP4 (SLC39A4) significantly contributes to human pancreatic cancer pathogenesis and progression. Proc Natl Acad Sci U S A. 2007 Nov 20;104(47):18636-41.

2 Wang H, Song X, Logsdon C, Zhou G, Evans DB, Abbruzzese JL, Hamilton SR, Tan TH, Wang H. Proteasome-mediated degradation and functions of hematopoietic progenitor kinase 1 in pancreatic cancer. Cancer Res. 2009 Feb 1;69(3):1063-70.

Li Y, VandenBoom TG, Wang Z, Kong D, Ali S, Philip PA, Sarkar FH. Up-regulation of miR-146a contributes to the inhibition of invasion of pancreatic cancer cells.

Cancer Res. 2010 Apr 15;70(8 Suppl):5703.

Chang Z, Li Z, Wang X, Kang Y, Yuan Y, Niu J, Wang H, Chatterjee D, Fleming JB, Li M, Abbruzzese JL, Chiao PJ. Deciphering the mechanisms of tumorigenesis in human pancreatic ductal epithelial cells. Clin Cancer Res. 2013 Feb 1;19(3):549-59.

4. Methods: “human primary pancreatic cancer cells or subcutaneous xenograft”. Where were these cells/xenografts utilized?

Response: Thanks for recognizing this issue. We utilized PANC-1 and MIA-PaCa-2 cells in western blot and corrected this error.

5 The authors demonstrate that miR-1181 is up-regulated around 2-fold using the adenoviral vector. That seems to be a rather weak effect. Could the authors comment on this?

Response: Thanks for recognizing this issue. We evaluated the relative expression of miR-1181 after transfecting the adenovirus for 72h, which is shown in Figure 1. We started the experiment after transfecting for 1 week, when achieving the best transfection effect. Actually, we also evaluated the relative expression of miR-1181 after transfecting for 1 week and presented the results in revised Figure 1. what’s more, we have re-transfected the adenovirus follow the previous procedure and re-verified miR-1181 relative expression after transfecting for 1 week. miR-1181 was up-regulated around 5-fold using the adenoviral vector.

6. Could the authors quantify figures 2 C and D, as well as 3C?

Response: Thank you for the suggestion. We have quantified Figure 2C, Figure 2D and Figure 3C.

7. Figure 4A: was there any difference in STAT3 phosphorylation between groups?

Response: We appreciate this suggestion and detected the expression of p-STAT3. We presented the new results in Figure 4A.

8. Could the authors state the exact negative controls used for figure 4C and D?

Response: Thank you for the suggestion. In Figure 4C and Figure 4D, the negative control cells were transfected with negative-control (NC) adenovirus.

Sincerely yours,

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