Point-to-point response

Dear editors and reviewers,

Enclosed in the revised manuscript entitled “KLF16 promotes pancreatic adenocarcinoma cell proliferation and migration by positively regulating SMAD6” by Zhi Zheng et al. We thank the editor and the reviewers very much for their comments and suggestions. We are also pleased to note the favorable comments of reviewers in their opening sentence. We have studied these comments and suggestions carefully and have made revision, which are marked red in the revised manuscript. Following are the changes we made according to your suggestions point by point. If you have any questions, do not hesitate to contact me.

Regards,

Jun Zhang, MD, PhD.
Chief Doctor, Professor, Surgeon, Department of General Surgery, Beijing Friendship Hospital, Capital Medical University
E-mail: zhangjun5986@ccmu.edu.cn

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Accept (General priority)
Specific Comments to Authors: The study adds new data regarding the genetics in pancreatic adenocarcinoma. It clearly and well written.

Response： Thanks for your kind suggestion.

Reviewer #2:
Scientific Quality: Grade C (Good)
Language Quality: Grade C (A great deal of language polishing)
Conclusion: Rejection
Specific Comments to Authors: In this work, the authors have investigated the expression of KLF16 as potential oncogene/biomarker of pancreatic adenocarcinoma (PAAD). In particular, the authors showed that KLF16 and SMAD6 overexpression promoted the malignant function of PAAD and the KLF16/SMAD6 axis might be explored as a therapeutic target for PAAD therapy. The work treats an interesting topic, however after a careful reading through the text, I found several issues that should be addressed. My suggestions are below described in a point-by-point list:

1. In the introduction, the authors should better describe the rationale that led them to study KLF16 as a potential biomarker for PAAD.

Response： Thanks for your kind suggestion. We have revised the description of the rationale that led them to study KLF16 as a potential biomarker for PAAD. The revised statement was "It has been reported that KLF16 promoted progression of breast cancer via activating MAGT1 (8). Ma XD et al found that KLF16 enhanced colorectal carcinoma progression by modulating nucleolar homeostasis and translational reprogramming (9). On the other hand, in some malignancies, KLF16 functions as an oncogenic suppressor. It has been reported that KLF16 suppressed human glioma cell proliferation and tumourigenesis by regulating TFAM (10). However, the role of KLF16 on PAAD is not well understood. The present study was intended to explore the role and possible mechanisms of KLF16 in PAAD."

2. In figure 2, the control band of figure 2H (western blot) is much lower than the control band in figure 2B. Please clarify.

Response： Thanks for your kind suggestion. We have re-performed the western blot in figure 2. A total of 30 ug proteins was loaded in each lane. Please find the revised results in Figure 2.

3. Also in figure 4, microscope images in the control are very different. Please clarify.

   Response： Thanks for your kind suggestion. We checked the raw data and find the number of seeded cells were different in knockdown and overexpression experiments, while we did not clarify it in the methods section. To address this issue, we re-performed the transwell in figure 4. A total of 5.0×10^4 cells were planted in each group. Please find the revised results in Figure 4.

4. In figure 5, the control band of overexpression experiments (western blot, figure 5D) is much lower than the control band of knockdown experiments. Please clarify.

   Response： Thanks for your kind suggestion. We have re-performed the western blot in figure 5. A total of 30 ug proteins was loaded in each lane. Please find the revised results in Figure 5.

5. To support the potential role of KLF16 as biomarker for PAAD, the authors analysed their expression in both normal and PAAD tissues in the TCGA dataset. However, if survival data can be acquired from the TCGA PAAD dataset, an association analysis between KLF16 expression and survival rate should be performed.

   Response： Thanks for your kind suggestion. We have analyzed the association between KLF16 expression and survival in TCGA database. Unexpected, there was no significate association between KLF16 expression and survival of PAAD. The result was showed as below.