Dear Editor:

Thank you very much for your letter and for the reviewers' comments concerning our manuscript entitled "Genome-wide CRISPR-Cas9 screening identifies HIF-1a-meditated CBX8 promotes pancreatic cancer progression via IRS1/AKT Axis." (manuscript NO.67060). We appreciate the time and effort that you and the reviewers have dedicated to providing your valuable feedback on my manuscript. Those comments are all valuable and very helpful for revising and improving our paper. We have modified the manuscript in accordance with the comments. In this cover letter, the reviewers' comments are laid out below in *italicized font* and our response is given in normal font. Point by point responses to the reviewers' comments are listed below this letter.

Reviewers 1's comments:

The topic of this manuscript falls withon the scope of World Journal of Gastroenterology. Chrombox 8 (CBX8) is upregulated in pancreatic tumor tissues and shown to drive pancreatic cancer cells proliferation. higher expression of CBX8 is correlated with worse outcomes of pancreatic cancer patients. CBX8 is a promising therapeutic target for pancreatic cancer patients. The Authors demonstrated that hypoxia-inducible factors (HIF-1a) induce CBX8 transcription by combining with the promoter of CBX8. CBX8 is a key gene which is regulated by HIF-1a, and could IRS1/AKT pathway. CBX8 maybe a promosing therapeutic strategy for pancreastic cancer. Introduction is good. Method and Materials are well explained. Discussion sound well. Complete the references. The manuscript is interersting for surgeons and oncologists.

Our reply:

Thank you very much for your constructive comments. We went over our manuscript carefully and corrected some clerical errors.

Science editor's comments:

1. *The "Author Contributions" section is missing. Please provide the author contributions;*

Our reply:

Thank you very much for your comments. The "Author Contributions" section had been put into our manuscript.

2. *The authors did not provide the approved grant application form(s). Please upload the approved grant application form(s) or funding agency copy of any approval document(s);*

Our reply:

Thank you very much for your comments. We have re-uploaded the approved grant application form.

3. *The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;*

Our reply:

Thank you very much for your suggestions. We have re-uploaded the PowerPoint which contains all our original figures.

4. *PMID and DOI numbers are missing in the reference list. Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout;*

Our reply:

Thank you for your suggestions. We have corrected all the right citation format in our manuscript.

5. *The "Article Highlights" section is missing. Please add the"Article Highlights" section at the end of the main text;*

Our reply:

Thank you for your suggestions. The "Article Highlights" section had been put into our manuscript as following.

ARTICLE HIGHLIGHTS

Research background

Pancreatic cancer is one of the most lethal cancer in the world. It has become the second most fatal cancer in the United States. It was also found that CBX8 promoted tumor growth and metastasis in other cancers. However, whether CBX8 is involved in the proliferation of PC cells remains unknown.

Research motivation

Many studies have shown that the prognosis of patients with pancreatic cancer remains poor after complete surgical resection. Therefore, it is of great significance to study the occurrence and development of pancreatic cancer itself and the corresponding targeted therapy. We hope to provide a novel therapeutic target for patients with pancreatic cancer.

Research objectives

The present study aimed to investigate the function of CBX8/IRS1/AKT axis in Pancreatic Cancer.

Research methods

A genome-wide clustered regularly interspaced short palindromic repeats-Cas9 screening was performed to select genes which could facilitate PC cells proliferation. A total of 244 candidate genes were identified as responsible for proliferation of PC cells using deep sgRNA sequencing. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expression of CBX8 in Pancreatic Cancer tissues and cells. The regulatory roles of CBX8 in cell proliferation, migration and invasion ability were verified by Cell Counting Kit-8 and transwell assays.

Research results

Chromobox 8 (CBX8) was upregulated in pancreatic tumor tissues and shown to drive PC cells proliferation. Higher expression of CBX8 was correlated with worse outcomes of PC patients from two independent cohorts containing a total of 116 cases. Besides, CBX8 was also proved to serve as a promising therapeutic target for PC xenografts model. Moreover, we demonstrated that HIF-1a could induce CBX8 transcription by combining with the promoter of CBX8. CBX8 efficiently activated the PI3K/AKT signaling by upregulating IRS1.

Research conclusions

CBX8 could promote Pancreatic Cancer cell progression by activating the IRS1/AKT pathway.

Research perspectives

CBX8 could promote Pancreatic Cancer progression, which might provide a potential treatment strategy for patients with Pancreatic Cancer.

Thank you and best regards.

Yours sincerely,

Zhengjun Qiu

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