Dear editor,

We would like to thank you very much for your valuable comments and good suggestions that greatly helped to improve our manuscript. Thank you very much for your time and efforts.

We have substantially revised our manuscript after reading the comments provided by the reviewer. In the following we are going to explain how your comments have been taken into account in the revision. The specific modification methods and explanations for the questions raised by the reviewer are introduced in detail below.

In light of the pandemic currently ongoing, we hope you are doing great and always maintain good hygiene to avoid corona virus. Lastly, our manuscript has been polished in the institution recommended by the magazine. Once the polishing is completed, we will submit our manuscript immediately.

Kind regards，
Yours sincerely

Corresponding author: Zhengjun Qiu, M.D E-mail: qiuzjdoctor@sina.com

Answers to the reviewer:

Reviewer #1:

[1] Pancreatic cancer (PC) includes several types of carcinoma. The author should use "pancreatic ductal adenocarcinoma (PDAC)" instead of "PC".

Answers: Thank you for pointing this out. According to the pathological molecular phenotype of tumors, pancreatic cancer includes several types. Among them, pancreatic ductal adenocarcinoma (PDAC) is the most common type, accounting for about 90% of pancreatic cancer. And what we study in this paper is PDAC, so we will use "pancreatic ductal adenocarcinoma (PDAC)" instead of "pancreatic cancer (PC)" in this paper.

[2] MicroRNA (miR)-299-3p appeared suddenly. The author should explain the role of the miR-299-3p.

Answers: An increasing body of evidence has shown that lncRNAs function as competing endogenous RNA to exert their biological functions. This study discussed the role of lncRNA NRAV, which acting as a sponge of miR-299-3p in PDAC. In the part of introduction, we introduced the role of miR-299-3p in tumors.

[3] The analysis of clinical survival of PDAC patients using TCGA data base was not described in the Methods.

Answers: This question is similar to the sixth one. We will answer it later.

[4] In introduction, the author should explain the role of the miR-299-3p.

Answers: Thank you for pointing out the deficiency. We added a new paragraph in the part of introduction to further introduce the role of miR-299-3p.

[5] Are the cell lines, PANC-1, AsPC-1, Mia Paca-2 and BxPC-3 all derived from pancreatic ductal adenocarcinoma?

Answers: Yes. According to the relevant research, these cells were derived from the pancreatic ductal epithelium of patients with pancreatic ductal adenocarcinoma.

[6] The analysis of clinical survival of PDAC patients using TCGA data base was not described in the Methods. How did the authors divide the low NRAV and high NRAV group?

Answers: The analysis of clinical survival of PDAC patients using TCGA data base was obtained from "GEPIA," which is an online bioinformatics tool for gene expression analysis according to The Cancer Genome Atlas (TCGA) database and Genotype-Tissue Expression (GTEx) database. To sum up, the samples were divided into high expression group and low expression group equally, according to the expression level of NRAV in each sample, and then the correlation between the expression level of NRAV and patient prognosis was analyzed by log-rank test or Mantel–Cox test.

[7] In addition, the fig.1C was hard to understand.

Answers: GEPIA plots gene expression by pathological stages based on the TCGA clinical annotation. By comparing the expression levels of NRAV in patients with different clinical stages,

the correlation between the expression of NRAV and tumor clinical stages was further analyzed.

Reviewer #3:

[1] In 2016, the US National Cancer Institute has declared to stop screening most drugs using the panel of human cancer cell lines grown in culture. So, they recommended to refocus its drug screening on patient-derived xenografts by implanting small chunks of human tumors in mice. One drawback of this manuscript is that the analysis was done mostly in vitro. In addition, there is no description how we use NRAV in our clinical setting since the present study was still preliminary project.

Answers: We agree with your comment. As you said, our research is still in the preliminary stage. In our study, only the subcutaneous tumor formation experiment of mice was done to detect the proliferation of tumor cells. With the deepening of follow-up experiments, we will consider transplanting human tumors into mice for further xenotransplantation experiments.

[2] How about the overall survival of mice in the experiment for Figure4? As the authors know, tumor size is not only crucial factor in pancreatic cancer. An environment of cancer influences the development of cancer and invasion.

Answers: In the stage of animal experiment, the overall survival rate of mice was 100% by the time of being sacrificed. Clinically, the tumor size is not the most critical factor affecting tumor progression, metastasis and prognosis. As reported in recent years, the tumor microenvironment plays a significant role in the progression of the cancers. Therefore, we will pay more attention to the influence of tumor microenvironment in future research and will further explore it in the follow-up work.

[3] The authors described the following “these results demonstrated that NRAV might act as an oncogene and could be used as a new biological marker and therapeutic target in PC”. How much the expression level of NRAV in human pancreatic cancer patient? Almost of the pancreatic cancer patient express NRAV? The expression of CA19-9 and Span-1 are around 70-80% in pancreatic cancer patient. How about NRAV? It is incomplete, but potentially important paper if the authors added some comments in discussion section.

Answers: Thank you for raising this question. Our research suggested that NRAV might play a role as an oncogene in the progression of pancreatic cancer. However, this study is still in the preliminary stage. More tumor samples from patients with pancreatic cancer need to be collected. We need to detect the expression of NRAV in tumor samples from patients with pancreatic cancer and normal pancreatic tissues, and further analyze the correlation between the expression of NRAV and clinicopathological parameters and prognosis.

In the part of discussion, we will also clarify the limitations of the current research. Lastly, we also hope that our research can provide some help for the diagnosis and treatment of pancreatic cancer.

Reviewer #4:

[1] Some references missing. For example “An extremely aggressive malignancy, pancreatic cancer (PC) has shown a rapidly increasing incidence rate in recent years worldwide”.

Answers: OK, we will add some references appropriately.

[2] I suggest including clear limitations of the study in the discussion. I suggest drawing a figure for explaining the data. It will help to understand the findings.

Answers: Thank you for your comment. This study is still in its preliminary stage, and there are indeed many limitations. In the discussion section, we will further clarify the limitations of this study. At present, one drawback of this manuscript is that the analysis was done mostly in vitro, and in vivo experiments are still lacking. Finally, we will draw a brief pattern diagram so that readers can better understand the findings.