Dear Editor,

Please find enclosed the edited manuscript (File name: 40811-Revised manuscript).

Name of Journal: World Journal of Gastroenterology

Manuscript NO: 40811

Manuscript Type: BASIC STUDY

Title: Typing of pancreatic cancer-associated fibroblasts identifies different subpopulations

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Thank you very much for the opportunity to submit a revised manuscript. We thank the reviewers for their constructive comments. The manuscript has been improved according to the suggestions of reviewers. All changes to the manuscript have been marked with the Track Changes function.

To reviewers:

Reviewer #1 (Code: 02951605):

- Abstract – I am surprised that you distinguished four types of stroma but you did not distinguish between the type of pancreatic tumors themselves. The tumors may originate from different cell types and those in different locations within the pancreas have also different properties and prognosis. Thus, it would be very helpful if you could reflect current classification of pancreatic tumors in your manuscript.

Thank you for this valuable comment, this is an important point. We have now included an additional table describing the patient and tumour characteristics (Table 1).

- Introduction – "CAFs have been suggested to undergo an "unholy alliance" with the PC cells, each mutually promoting each other's proliferation [10, 11]." – a recent review on the signaling from tumors to fibroblasts was published in Crit Rev Oncol Hematol. 2016 Jan;97:303-11. It should be cited here in addition to those older papers.

Thank you, we have now cited the suggested paper.

- For the signaling from fibroblasts to the tumors, there are dozens of reviews, some even with the focus on the pancreatic cancer itself, such as the one in Future Oncol. 2015 Sep;11(18):2603-10.

Thank you, we have now cited the suggested paper.

- Introduction – recent research on pancreatic cancer-associated fibroblasts should be reflected. This includes Cancer Lett. 2018 Sep 28;432:227-236, Cancer Sci. 2018 Jun 14. doi: 10.1111/cas.13694 or Cancer Res Treat. 2018 Apr 20. doi: 10.4143/crt.2018.031.

Thank you for highlighting these important papers. We have included the following paragraph in the introduction and cited the first two papers mentioned:

"Further, combined targeting of CAFs and cancer cells has shown promise as a therapeutic option in in vitro studies [22-23] "

Regarding the last suggested paper (Cancer Res Treat. 2018 Apr 20. doi: 10.4143/crt.2018.031): This is an interesting paper, but we would rather not cite it since it is performed on gastric cancer.

- Methods – CD117 is present also on stem/progenitor cells, it is not mast cell-specific.

- Methods – CD163 is specific also for monocytes, not just macrophages - Methods – check the specificity of all the markers that you have used.

Thank you, we have modified the method section according to your suggestions.

- Methods – RNA concentration cannot be "determined" using nanodrop. Just estimated…

Thank you, we modified the description according to your suggestion.

- Methods – specify, whether you conducted multiple independent qPCR experiments. If not, you need to perform them.

Thank you for this valuable comment. If we understand your comment correctly you point of the importance of performing the qPCR experiments independently on RNA isolated from each specimen as opposed to performing the analyses on pooled RNA from the different specimens. We did perform the qPCR independently on RNA from each patient. Independent qPCR experiments were performed in triplicates on RNA isolated from 9 PC specimens and 3 normal pancreatic specimens. We specified this in the method section.

- Methods – You indicate the use of nonparametic tests but some values clearly were not of parametric nature. Please comment on why the parametric values were not tested or why they were tested by the non-parametric test.

In response to this comment, we would like to state that we used the Shapiro-Wilk test to test for normality, and found the data to be of both parametric and non-parametric nature. The use of this test is now described in the Methods section. Using non-parametric tests is in general more conservative than using parametric tests, and relies on fewer assumptions about the shape of the parameters. Especially, parametric assumption of normality should be avoided when using small sample sizes (which is the case in this study). In order to perform the same statistical approach on all data without assuming normal distribution, we maintain the non-parametric approach. Additionally, we can point to the following papers where a similar statistical approach has been used: 1) PLoS One. 2017 Aug 24;12(8):e0182954, 2) Cancer Chemother Pharmacol. 2013 Feb;71(2):293-9, and 3) Acta Cir Bras. 2017 Sep;32(9):712-725.

- Results – "44 markers (32 CAF IHC markers," – avoid starting the sentence with a number.

Thank you, we have now modified the sentence.

- Results – the results need to be rewritten in a way that the text will be fluent and most of the data (numbers) will be moved to some tables or graphs.

Thank you. We have modified the results section and removed all numbers from the text since it can already be found in tables and graphs.

- Raw data are completely undisclosed. These are needed in order to convince the readers that the reported differences were real. Provide supplementary figures regarding series of all the markers.

The results of the analysis of the "insignificant" markers and their expression in juxtatumoral and peripheral stroma are now presented in Supplementary Figure 1.

- I miss there a thorough discussion and interpretation of the findings supported by the state-of-the-art knowledge. For example, when you look at nestin, the text does not comment on its prognostic value at all, it just mentions that it was somewhere and that is it. I do not consider such data interpretation as sufficient.

Thank you. A paragraph on nestin has now been included in the Discussion.

- The last para of the Results belongs rather to a Discussion.

Thank you for this valuable comment, we have deleted this paragraph

- The study suffers from a focus on details (individual markers) and is poorly set into the broader context. The reader does not get any takehome message, which could be easily derived from the text. The current text looks rather like some work-in-progress notes allowing to write a paper instead of a paper itself. In my view, there is some value hidden in this work, but to present it in a standard manner, I suggest to team-up with some experienced science writer, who would be able to identify key takehome messages and re-write the text in a fluent way attracting the reader and leaving out the details in the supplementary materials and/or figures and tables. Actually most of current tables could also be moved to the supplementary materials.

Thank you for your comment. We have modified the manuscript according to your comments, which includes:

- *Discussion: Unnecessary details regarding the markers have been removed.*
- *Materials and Methods: Table 1, 2, and 3 have been transferred to Supplementary Materials.*
- *Results: Table 4 has been transferred to Supplementary Materials.*

Reviewer #2 (Code: 00053888):

This is an excellent scientific paper that is well conducted and presented. The information is valuable and helps to explain some of the clinico-pathological features that we see in pancreatic adenocarcinoma. The authors should be congratulated. My only criticisms are the length of the discussion and there are too many figures and tables. Tables 1 & 2 could be provided to the reviewer but I am not certain that they add anything to the manuscript.

Thank you for your comments.

- *We have now reduced the length of the Discussion.*
- *Table 1, 2, 3, and 4 have been moved to Supplementary Materials.*

Reviewer #3 (Code: 00503834):

This paper identify different subpopulations of cancer-associated fibroblasts in pancreatic cancer. 2. It also firstly reported the cytoglobin expression in human pancreatic cancer. 3. Although it's effect on future was uncertain at present, the basic and pioneer study ought to be accepted.

Thank you for your comments.

Other changes:

- *The manuscript was edited for English language usage, grammar, spelling and punctuation by Nature Research Editing Service.*
- *The Institutional review board statement has been modified according to the latest changes.*
- *The picture in Fig 6H has been replaced with the correct illustration.*

On behalf of the authors,
Kind regards,
Yours sincerely,

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