

Peer Review File

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Reviewer A

The manuscript by Mu and co-authors identified the neuronal development genes are associated with changes in the tumor immune cell infiltration in pancreatic cancer. In this study, they focused on the 20-neuronal development gene panel and evaluated the genetic alterations based on Whole Exome Sequencing (WES) and Whole Genomic Sequencing (WGS) data. Overall, this study presents some interesting insights. There are several points that need to be addressed and improved:

Comment 1: It is not clear how the authors analyzed the percentage of immune cell infiltration.

Reply 1: Thanks for reminding us to provide the important information. The method to analyze the percentage of immune cell infiltration was described in the study by Li et al. We have added related content in the METHODS section.

Changes in the text: see Page 4, line 20

Comment 2: “Specimens from a total of 89 pancreatic cancer patients treated at the Johns Hopkins Hospital, comprised of 41 surgically resectable and 48 locally advanced pancreatic cancer (LAPC) patients” Could the authors please present the demographics of the human cohort?

Reply 2: We appreciate your constructive advice. The demographics of the human cohort were described in the study by Li et al. We have added the contents in the METHODS section.

Changes in the text: see Page 4, line 14-15

Comment 3: “Welch's t-test was performed to compare the two groups.” Could the authors please confirm if and how they assessed the assumption of normality for the application of parametric statistical methods?

Reply 3: Thank you for your important questions and advice! We have described it in the METHODS section.

Changes in the text: see Page 4, line 26-28

Comment 4: Could the authors please expand on the discussion of the main study limitations in the Discussion section?

Reply 4: Thank you for your valuable advice! We have described our study limitation in the DISCUSSION section.

Changes in the text: see Page 12, line 17-19

Comment 5: There were some inconsistencies and typo mistakes in the manuscript, careful proofreading is strongly recommended.

Reply 5: Thank you for your essential recommendation! We have made careful amendments accordingly.

Reviewer B

This study is based on the previous report (PMID: 35902743) and continues to explore the relationships between specific genetic alterations in neuronal development genes and the TME. Hwang et al. discovered 20 neurodevelopmental genes, which were associated with PNI and poor prognosis through single-nucleus and spatial transcriptome profiling. However, these genes and immune cell infiltration have not been reported, so this is an interesting topic. Before the article is accepted, there are still a small number of contents that need to be revised.

Comment 1: Check the manuscript carefully, there are some spelling mistakes. For example, in the introduction section, Huang et al. was misspelled, which should be Hwang.

Reply 1: Thanks for patiently reviewing our manuscript and pointing out our mistakes. We have corrected it accordingly.

Changes in the text: see Page 3, line 37

Comment 2: In the method part, IHC is used to explore the infiltration of tissue immune cells, such as CD8T cells, CD4T cells and NK cells. It is better to increase the marker that marks immune cells.

Reply 2: Thank you for your important advice! The immune cells markers were described in the study by Li et al. We have added related content in the METHODS section.

Changes in the text: see Page 4, row 20

Comment 3: In the discussion part, the author briefly describes the research progress of 20 neuronal development genes in various diseases. The purpose of this study is to explore the relationship between these genes and immune cell infiltration in pancreatic cancer. Please focus on describing the reasons why these genes affect immune cell infiltration in the discussion part.

Reply 3: Thank you so much for your comment and recommendation. We have made the contents of DISCUSSION more concise.

Comment 4: Please check the accuracy of Figure drawing. For example, 'loss' in

Figure 1A ZNF667 should be compared with 'unchanged', not with 'gain'.

Reply 4: We appreciate your reminder. We have confirmed the accuracy of every figure. For some neuronal development genes (ZNF667, CFTR, NRG1, KCNMA1, PKHD1, ADCY5, MAPK10, SCN9A, NRXN3, HIF1A, PRKCE), there are significant changes in some immune cells between gain and loss of the gene.