Peer Review File

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Reviewer A

Comment 1: Data presented in the methods section and Figure 1 strongly suggest that these requirements were not met in this analysis. Only one siRNA was used and there is no information about the “control” condition. It is possible that the phenotypes observed are due to off-target effects of siRNA or the procedure of transducing cells. Additional controls are required for the correct interpretation of this data.

Reply 1: According to the standardized procedure for siRNA experiments, we utilized siNC (Negative control) as the control group. Unfortunately, due to our oversight, we apologize for only using the term "control" condition in the Figure 1 instead of providing accurate information to the readers.

Changes in the text: we have modified our text (see Page 4, line 85-86) and Figure 1 as advised.

Comment 2: Technically, the single-cell RNA-seq analysis is performed to a sufficient standard, however, the study would benefit from the addition of a public repository for the code to better understand individual steps taken to process the data. Unfortunately, the interpretation of data in Figures 2-6 is difficult as the fonts used are too small to read in the current format.

Reply 2: Regarding the preprocessing of single-cell data, we have provided a detailed description in the Methods section of the article. As for the code used in the study, we have stated in the data sharing statement that it can be obtained by contacting the corresponding author's email within two years after the publication of the article, based on the readers' demand. Additionally, we have adjusted the font size of Figure 2-6 as suggested.

Changes in the text: we have modified our Figure 2-6 as advised.

Comment 3: Secondly, data supporting the role of FKBP10 in iCAFs originated from a single patient. It would be advantageous to include additional analysis. For example, Kumar et al recently published scRNA-seq from 31 gastric cancer patients (PMID: 34642171)

Reply 3: Due to limited computational resources, we only analyzed the expression of FKBP10 on iCAFs from one patient, which resulted in a lack of credibility in our results. Therefore, we utilized the GSE183904 dataset to include paired tumor-adjacent samples from nine patients for further validation.

Changes in the text: we have modified our text (see Page 5, line 123-125; Page 7, line 192-195) and Figure 4 (4D, 4E) as advised.

Comment 4: Thirdly, although module iCAFs-M16 was derived from the analysis of iCAFs, it is surprising to see its expression in other cells types (especially, endothelial cells). It would be helpful to demonstrate the expression of individual

genes in that module to ensure that it is not an artefact.
Reply 4: We also believe that validating the expression of FKBP10 on iCAFs through multi-color immunofluorescence technology will enhance the persuasiveness of our study. In 2019, we collected 40 paired gastric cancer tissues and adjacent non-cancerous tissues. Through immunohistochemistry, we first discovered high expression of FKBP10 in cancer tissues, which was associated with poor prognosis (Ref. 19, PMID: 31233188). However, due to the reorganization of the hospital, we lost the permission to use the paired paraffin blocks of the 40 previously collected gastric cancer patients, and we regret that we were unable to perform the multi-color immunofluorescence experiments. In the future, we hope to collect samples and clinical data from gastric cancer patients with the approval of our institutional ethics committee, to demonstrate the expression of FKBP10 on iCAFs using multi-color immunofluorescence technology. Additionally, we plan to explore the impact of FKBP10 on the tumor immune microenvironment located on iCAFs and its potential mechanisms through further experiments. Moreover, to strengthen the persuasiveness of our study, we obtained an additional single-cell sequencing dataset (GSE183904) containing data from 9 gastric cancer patients to validate the expression of FKBP10 on iCAFs.
Changes in the text: we have modified our text (see Page 5, line 123-125; Page 7, line 192-195) and Figure 4 (4D, 4E) as advised.

Reviewer B
Comment 1: Insufficiency in terms of storytelling leading to conclusions.
Reply 1: Our research aims to investigate the role of FKBP10 in the progression and metastatic potential of gastric cancers. By analyzing the migration and invasion capabilities of cell lines with FKBP10 knockdown, we discovered a strong correlation between high expression of FKBP10 in gastric cancer and tumor migration and invasion. With the rapid development of single-cell sequencing technology in recent years, we further explored publicly available single-cell data on gastric cancer. We found that FKBP10 is primarily expressed on iCAFs in the gastric cancer microenvironment. Additionally, through high-dimensional weighted gene co-expression analysis of iCAFs, we identified gene modules co-expressed with FKBP10 that are primarily involved in extracellular matrix remodeling, cell adhesion, and pathways in tumors. Subsequently, we used FKBP10 co-expressed genes derived from iCAFs to divide stomach adenocarcinoma samples from the TCGA database into two clusters (low and high FKBP10 co-expression gene module clusters). We observed poorer survival statistics and predicted lower immune therapy response in the two clusters of patients, along with higher expression of FKBP10. In conclusion, our study reveals the expression characteristics and potential functions of FKBP10 in the tumor immune microenvironment, enhances our understanding of FKBP10-dependent biological changes specific to human gastric cancer cells, and provides

clues for guiding ICB treatment decisions. In addition, to enhance the completeness of the story, based on the recommendation, we have included an additional single-cell dataset consisting of 9 patients to validate the expression of FKBP10 in iCAFs. Furthermore, we performed GO and KEGG enrichment analysis on differentially expressed genes in iCAFs from primary tumors and metastatic tumors, elucidating the functionality of iCAFs in tumor progression. Eventually, we analyzed the expression of FKBP10 in various subtypes of gastric cancer.
Changes in the text: we have modified our text (see Page 5, line 123-132; Page 7, line 192-195, line 199-204) Table S1, and Figure 4 (4D, 4E, 4I) as advised.

Comment 2: Can we discern the importance of icaf from the single-cell data?
Reply 2: Cancer-associated fibroblasts (CAFs) have been recognized as an integral component of the tumor microenvironment, playing multiple roles in promoting tumor progression. In this study, we identified two major subtypes of fibroblasts, mCAFs and iCAFs, in primary tumor samples and metastatic tumor samples based on the expression of specific cell markers. To elucidate the importance of iCAFs, we performed differential gene enrichment analysis of iCAFs in primary tumor patients and metastatic tumor patients. The enriched GO and KEGG terms of genes differentially expressed in the metastatic tumor group included extracellular matrix organization, focal adhesion, CXCR chemokine receptor binding, IL-17 signaling pathway, and TNF signaling pathway. This suggests that iCAFs are involved in remodeling the extracellular matrix, promoting cancer cell metastasis, and exacerbating the inflammatory response in tumor tissue.
Changes in the text: we have modified our text (see Page 5, line 126-132; Page 7, line 199-204) Table S1, and Figure 4I as advised.

Comment 3: Does the initial cell line represent icaf? I am curious about its expression levels compared to single-cell data.
Reply 3: The initial cell line we used was gastric cancer cells, with the main purpose of investigating the impact of FKBP10 on the biological behavior of gastric cancer cells. We were also curious about the expression level of FKBP10 in tumor-associated fibroblasts (CAFs). We made efforts to isolate and extract CAFs from mouse gastric cancer tissues, but due to the lack of an SPF-level animal facility in the current experimental platform, the isolated cells were contaminated and difficult to proceed with subsequent validation. We hope that in the future, with improved experimental conditions, we can determine the expression and biological effects of FKBP10 through the application of CAFs isolation and cultivation techniques. To enhance the persuasiveness, we incorporated the GSE183904 dataset as supplementary validation. In this dataset, we observed prominent upregulation of FKBP10 in iCAFs across samples from nine patients.
Changes in the text: we have modified our text (see Page 5, line 123-125; Page 7, line 192-195) and Figure 4 (4D, 4E) as advised.

Comment 4: It would have been helpful to demonstrate the actual expression of FKBP10 within tumors in protein or RNAseq format.
Reply 4: In 2019, a total of 40 pairs of gastric cancer and adjacent cancer tissues were collected. By utilizing immunohistochemical techniques, we made the pioneering discovery of high expression of the FKBP10 protein in tumor samples, which is closely associated with poor prognosis (Ref. 19, PMID: 31233188).
Changes in the text: we have modified our text (see Page 6, line157-158) as advised.

Comment 5: There are different types of gastric cancer; it would be interesting to know if their expression levels vary.
Reply 5: By analyzing different types of samples in the TCGA-STAD dataset, we discovered an upregulation of FKBP10 expression in tumor samples, including Adenocarcinoma (NOS), Adenocarcinoma (Diffuse), Adenocarcinoma (Signet Ring), Intestinal Adenocarcinoma (NOS), Intestinal Adenocarcinoma (Tubular), and Intestinal Adenocarcinoma (Mucinous). Additionally, we observed differential expression of FKBP10 across different stages and grades of tumors.
Changes in the text: we have modified our text (see Page 6, line148-150; Page 8, line 237-241) and Figure S1 as advised.

Comment 6: How is patient information related to each type of tumor?
Reply 6: We have organized the clinical information of the patients into a table, as recommended.
Changes in the text: we added some data as advised. (see Page 8, line 237-238, Table S5)

Comment 7: Cell labels are needed in Figure 2E.
Reply 7: we have modified Figure 2E as advised.
Changes in the text: we have modified Figure 2E as advised.

Comment 8: Can we visualize the expression of FKBP10 in icaf through actual fluorescent images?
Reply 8: We also agree that it is necessary to perform multi-color immunofluorescence to confirm the localization of FKBP10 on iCAFs. However, due to the transfer of administrative jurisdiction of the hospital branch, we have lost access to the paired paraffin blocks of the 40 gastric cancer patients previously collected. Therefore, we apologize for not being able to conduct the localization experiment. We hope that in the future, with the approval of our institutional ethics committee, we can collect samples and clinical information from gastric cancer patients to demonstrate the expression of FKBP10 on iCAFs using multi-color immunofluorescence techniques. Furthermore, we plan to explore the effects and potential mechanisms of FKBP10 on the tumor immune microenvironment located on iCAFs through further experiments. Additionally, to enhance the persuasiveness of our study, we have obtained an additional single-

cell sequencing dataset (GSE183904) containing data from 9 gastric cancer patients to validate the expression of FKBP10 on iCAFs.
Changes in the text: we have modified our text (see Page 5, line 123-125; Page 7, line 192-195) and Figure 4 (4D, 4E) as advised.

Comment 9: The visibility of the enrichment analysis in Figure 5 needs improvement. Increase font size and prioritize displaying actual p-values prominently.
Reply 9: As per the suggestion, we have conducted a reoptimization and adjustment of the font sizes within each image in Figure 1-6.
Changes in the text: we have modified Figure 1-6 as advised.