Peer Review File

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Reviewer A

Summary of the Study:

The study predominantly focuses on developing a prognostic model for pancreatic adenocarcinoma (PAAD) based on integrin subunit genes (ITGs), which is a significant contribution to understanding survival outcomes in this aggressive cancer. Leveraging RNA-sequencing data from the TCGA and GEO databases, the authors conducted a single-sample gene set enrichment analysis (ssGSEA) to classify PAAD samples based on ITG expression. A set of 22 key differentially expressed ITGs were identified and further analyzed in the context of KEGG pathways and immune-related markers. The authors constructed an eight-gene model based on integrin subunit genes (ITGs) — including the genes: EREG, FAM83A, DLGAP5, PADI1, LAMA3, MET, CP, and GALNT5 — that effectively predicted the survival and prognosis of pancreatic cancer patients.

Strengths of the Paper:

1. **Relevance and Novelty**:
   The study addresses an important gap by exploring ITGs as prognostic biomarkers in PAAD, a malignancy with notoriously poor outcomes. Given the lack of prior research in this specific area, the work provides a fresh perspective on potential prognostic models and treatment strategies.
2. **Data Utilization**:
   The use of comprehensive RNA-sequencing data from both TCGA and GEO databases strengthens the robustness of the results. The combination of datasets adds depth to the findings, enhancing their generalizability.
3. **Methodological Rigor**:
   The application of ssGSEA, differential expression analysis, and the identification of enriched pathways in the KEGG and GO databases demonstrates a well-rounded approach to understanding gene expression changes in PAAD. Furthermore, the statistical analyses appear thorough, which supports the reliability of the findings.
4. **Potential Clinical Impact**:
   The study proposes a novel prognostic model involving eight key ITGs, which could be clinically relevant, particularly in the realm of immunotherapy. The findings linking ITG expression to immune infiltration and the differential

expression of immune checkpoints (e.g., CD274 and LAG3) are promising for future therapeutic interventions.

Weaknesses:

1. **Mechanistic Insights**:
   The study predominantly focuses on gene expression data, but it lacks a deep mechanistic investigation into how ITGs influence tumor progression and immune infiltration. Further experimental work could be incorporated to better elucidate the pathways involved.
2. **Limited Discussion on Therapeutic Implications**:
   While the paper hints at potential immunotherapy applications, it would benefit from a more detailed discussion regarding how the identified ITGs could be leveraged for therapeutic purposes. Are there existing therapies targeting these pathways? What future strategies could be considered?

However, the way the authors refer to "PAAD subtypes" might create some confusion. Currently, the text seems to imply that the identified prognostic groups (high-risk and low-risk based on ITG expression) correspond directly to distinct biological subtypes of PAAD, when in fact the main focus of the study is on survival prediction, not on defining or characterizing molecular subtypes.
PAAD (or PDAC, pancreatic ductal adenocarcinoma) is known to have well-characterized molecular subtypes, such as the classical and basal-like subtypes, which have distinct biological features and clinical behaviors. These subtypes have been established in prior research, often in relation to specific gene expression profiles. In contrast, the authors of this study classify patients based on their ITG expression and prognostic risk, which is more of an ITG-specific survival probability analysis rather than a true subtype classification.

Suggestions:

1. **Rephrase PAAD subtypes for Clarity**:
   To avoid conflating prognostic risk groups with known molecular subtypes, it would be beneficial to rephrase the discussion. The distinction between grouping patients by prognostic risk and identifying specific biological subtypes of the disease should be made clearer. For instance, instead of using the term "PAAD subtypes," the authors could refer to "prognostic risk groups" or "survival-based classification" derived from ITG expression.

   Reply: Revised

Changes in the text: We changed "PAAD subtypes" to "survival-based subtypes" in the text.

2. **Correlate with Known PDAC Subtypes**:
   Since the study utilizes TCGA data, it would be a valuable addition to explore how the identified ITG-based prognostic model correlates with the well-established molecular subtypes of PDAC (e.g., classical and basal-like). Performing such a correlation analysis could provide further insights into whether certain subtypes of PDAC are more likely to have higher or lower ITG expression, and whether the prognostic risk groups are enriched in particular subtypes. This would enhance the biological relevance of the findings and could potentially open new avenues for targeted therapeutic strategies.
   For example, if a correlation is found between a high ITG expression and a particular PDAC subtype (e.g., basal-like), which is known to have worse outcomes, it would lend additional weight to the prognostic significance of ITGs. This type of correlation could also improve the clinical utility of the ITG model by providing subtype-specific prognostic information.

   Reply: We further analyzed the expression levels of the signature genes of PAAD subtypes (classical and basal-like) in the high- and low-risk groups, followed by a detailed discussion.
   Changes in the text: we have modified our text as advised (see Page 13, line 405-407; Page 16, line 538-546)

3. **More detailed discussion about potential therapeutic potential**

While the paper hints at potential immunotherapy applications, it would benefit from a **more detailed discussion regarding how the identified ITGs could be leveraged for therapeutic purposes**. **Are there existing therapies targeting these pathways? What future strategies could be considered?**

Reply: We further elaborated on various strategies for targeting integrins in cancer therapy to enhance the clinical relevance of the text.
Changes in the text: we have modified our text as advised (see Page 13, line 421-430)

Overall, rephrasing the term “PAAD subtypes” and correlating ITG expression with known PDAC subtypes would clarify the study’s focus and add an important layer of

depth to the analysis. A more detailed discussion of the therapeutic potential of the identified ITGs, including existing therapies targeting these pathways and future strategies, would strengthen the paper's clinical relevance.

Additional Comments:

Figure 6C: The size of the description in the graph appears to be too big – please adjust the size.

Reply: Revised
Changes in the text: we have modified our text as advised (see Figure 6C)

Conclusion:
This study provides significant insights into the role of integrin subunit genes in pancreatic adenocarcinoma prognosis. The identification of key ITGs and the development of a prognostic model are valuable contributions to the field, particularly in the context of immunotherapy. However, further validation and mechanistic studies are necessary to fully realize the clinical potential of these findings.

Reviewer B

I have read with great interest your manuscript about the application of integrin subunit genes in pancreatic cancer and the construction of a prognosis model.

In accordance with the previously published work of "Li A, Ye B, Lin F, Wang Y, Miao X, Jiang Y. A novel immunogenomic signature to predict prognosis and reveal immune infiltration characteristics in pancreatic ductal adenocarcinoma. Precis Clin Med. 2022 Apr 25;5(2):pbac010. doi: 10.1093/pcmedi/pbac010. PMID: 35694712; PMCID: PMC9172649.", you demonstrate a differential integrins (ITG) expression in pancreatic adenocarcinoma presenting a prognosis model as well.

Although your findings are based in external databases and downstream molecular mechanisms of these 8 ITGs were not further investigated, I suggest that your work should be accepted for publication in this journal.
Reply: Thank you very much. The downstream pathways of these 8 ITGs will be further studied in the future.