

Peer Review File

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**Reviewer A**

The manuscript fulfills the requirements and also the novelty, and I think it is good to be published.

**Reviewer B**

The paper titled “Liquid-liquid phase separation-related features of PYGB/ACTR3/CCNA2/ITGB1/ATP8A1/RAP1GAP2 predict the prognosis of pancreatic cancer” is interesting. Six genes were identified as having potential causal relationships with PC. These genes were integrated into a prognostic risk model, thereby serving as unique prognostic signatures. Our findings provide novel insights into predicting the prognosis of PC patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

**Comment 1:** Liquid-liquid phase separation (LLPS) is a novel principle for explaining the precise spatial and temporal regulation in living cells. It is recommended to summarize the recent major advances toward elucidating the multiple mechanisms involved in cancer cell pathology driven by aberrant LLPS.

**Response:** Dear reviewer, thank you for your valuable suggestions! The field of Liquid-Liquid Phase Separation (LLPS), which has emerged as a prominent area of research in recent years, plays an indispensable role in driving the progression of cancer. We have expounded on the role of LLPS in the process of cancer development in the introduction (lines 95-108) and further delved into current research on LLPS in PC in the discussion (lines 467-481). The crucial role of liquid-liquid phase separation (LLPS) in future research and treatment is discussed. We firmly believe that these modifications not only enhance the manuscript's quality but also elevate its scientific rigor and readability. Once again, we sincerely appreciate your recognition and support. Please don't hesitate to inform us promptly if there are any aspects that require further improvement or enhancement. We are fully committed to enhancing the manuscript based on your invaluable suggestions in order to elevate its overall quality.

**Comment 2:** What is the greatest advantage of the prognostic risk model in this study? What is the biggest problem we are facing? It is suggested to add relevant content to the discussion.

**Response:** Thank you for your interest and valuable comments on our research. The focal point of this article lies in the prognostic risk model, which warrants a more comprehensive introduction. In order to provide a clearer elucidation, I have appended pertinent content regarding the prognostic risk model in the discussion section of the manuscript (lines 643-661), which offers a comprehensive description. The present paper elucidates the benefits and challenges associated with prognostic risk models in our research, while also proposing future research directions. The expression of similar details in my future work will be given increased attention to prevent any potential misunderstandings. We appreciate your correction, as it greatly contributes to enhancing the quality of our research.

**Comment 3:** This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.

**Response:** Thank you for your interest and valuable comments on our research. We highly appreciate your suggestion to incorporate in vivo and in vitro research into this study, as it holds significant importance for us and will be given thorough consideration. Indeed, in vivo and in vitro studies can offer more intuitive and reliable evidence to further validate and enhance the robustness of our findings. However, given the current stage of research, we are temporarily unable to conduct extensive in vivo and in vitro experiments due to resource constraints such as time and funding. The field of bioinformatics offers a distinct advantage in elucidating disease mechanisms and identifying potential therapeutic targets. We will persistently employ bioinformatic methodologies to delve deeper into existing data, with the aim of unearthing more profound discoveries. Simultaneously, we are actively pursuing collaboration opportunities and financial support to conduct additional in vivo and in vitro experimental studies in the future, thereby further validating our bioinformatics analysis findings. We firmly believe that by integrating diverse research methodologies and techniques, we will be able to comprehensively elucidate the essence of diseases and their treatments. The expression of gratitude is extended once again for your invaluable comments and suggestions.

**Comment 4:** Please discuss the therapeutic perspectives of LLPS in cancer research and the most recently developed drug candidates targeting LLPS modulation that can be used to combat tumorigenesis.

**Response:** Dear Reviewers, we appreciate your interest in our research on the potential therapeutic applications of (LLPS) in cancer treatment and the latest drug

candidates developed to target LLPS regulation. We highly appreciate your valuable feedback and engaged in a comprehensive discussion regarding the potential of liquid-liquid phase separation (LLPS) as a promising direction for cancer treatment, drug candidates targeting LLPS, as well as future research directions and challenges. The main components of cisplatin, an RNA phase modulator, and a LLPS drug delivery system are introduced (lines 631-642). These modifications not only enhance the manuscript's quality but also improve its scientific rigor and readability. Thank you once again for your recognition and support.

**Comment 5:** What is the relationship of genes integrated into prognostic risk model and immune microenvironment in lung adenocarcinoma? It is recommended to add relevant content.

**Response:** Dear reviewer, I sincerely appreciate your valuable suggestions. Through further investigation, mounting evidence suggests that the incorporation of immune-related genes into prognostic risk models can enhance their predictive accuracy. The aforementioned genes may play a direct role in immune response regulation or exert influence on the immune microenvironment through interactions with other cellular components and molecular factors. The immune checkpoint analysis was complemented by incorporating immune infiltration and correlation analysis. The specific experimental methods for immune checkpoints are provided in the experimental methods section of the manuscript (lines 265-276) and described in detail within the results section (lines 421-425). The valuable comments you have provided are greatly appreciated, as they play a crucial role in enhancing the quality of our paper.

**Comment 6:** All supplement figures are not clear enough. It is recommended to provide clearer figures again.

**Response:** Dear reviewer, we greatly appreciate your valuable feedback. We acknowledge and take seriously your concerns regarding the lack of clarity in the supplemental charts. In order to ensure optimal presentation and facilitate comprehension and evaluation by readers, we will enhance the clarity and quality of all supplementary charts in our revised manuscript. We eagerly anticipate showcasing improved charts.