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Reviewer A

This study was basically a meta-analysis study using publicly available various data of gene expression. Original data of this study seemed to be just IHC of commercially available tissue microarrays, which could provide an opportunity of validation of the meta-analysis data. Therefore, the IHC data should be shown in more detail by providing more pictures for each score evaluated. Moreover, the authors found that CDK1, CDC20, CCNA2, and MAD2L1 were associated with PTTG1. They showed expression data of these molecules by citing Human Protein Atlas, which did not provide their actual associations with PTTG1. Hence, they should investigate actual associations of these molecules with PTTG1 using the tissue microarray samples used for IHC analysis of PTTG1.

Reply: Thank you for your insightful comments. We highly appreciate your recommendation to provide more detailed IHC data with additional pictures for each evaluated score. However, due to constraints in our current resources and the scope of this study, which primarily focuses on a meta-analysis approach with public data, it is challenging for us to extend our investigation to include a more detailed IHC analysis with tissue microarrays. Regarding the associations of CDK1, CDC20, CCNA2, and MAD2L1 with PTTG1, we acknowledge the limitation noted in citing the Human Protein Atlas data. However, our current study mainly focuses on a comprehensive meta-analysis to explore the broader gene expression landscape associated with PTTG1, rather than detailed molecular interactions in tissue samples. We believe our study still provides valuable insights within the scope of its methodology.

Reviewer B

The paper titled “Upregulated expression of PTTG1 is associated with progression of pancreatic cancer” is interesting. The upregulated expression of PTTG1 plays an essential role in PC’s progression as a biomarker. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the correlations between PTTG1 and pancreatic cancer staging, degree of differentiation, neural invasion and lymphatic metastasis? It is recommended to add relevant content.

Reply 1: The higher expression of PTTG1 is associated with the higher pancreatic cancer stages. Currently, no significant correlation is ensured between PTTG1 and degree of differentiation, neural invasion and lymphatic metastasis. We added relevant content in Introduction (Page 4-5, line 120-132)

Changes in the text: Add "For the present PTTG1 is ascertained to be highly correlated with various aspects of PC, including gender, clinical stage, and prognosis. Zhang et al. found that higher expression of PTTG1 was associated with higher clinical stages and worse prognosis of PC, the potential mechanism of which was the enhanced OAd5 transduction into PC cells by increasing CXADR expression on the cell surface. In a study of human PC tissues, the scholars discovered that PTTG1 was highly expressed in PC, and its expression was related to the gender of PC patients. A recent study showed that PTTG1 is highly expressed in PC, and the positive expression of PTTG1 is related to the gender of PC patients (21). However, no significant correlation was found between PTTG1 expression and perineural infiltration as well as age, tumor sizes, pathological styles and distant metastases in PC patients." in the Introduction.

2) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply 2: We rewrite the research background in the abstract.

Changes in the text: "Pancreatic cancer (PC) is an aggressive disease with a very poor prognosis. The insidious onset, rapid progression, and resistance to conventional therapies mark the imperious need for novel biomarkers and therapeutic targets. The pituitary tumor-transforming gene 1 (PTTG1), implicated in tumorigenesis and cellular transformation, has been studied in various cancers, however, its role and mechanisms in PC remain to be elucidated for better understanding the disease pathology and in enhancing patient management strategies."

3) Some fonts need to be enlarged, as shown in Figures 2,3,4,5.

Reply 3: Thanks for reminding us. The replaced figures now have larger fonts.

Changes in the text: Change Figures 2,3,4,5.

4) It is recommended to increase the functional study of PTTG1, which may be more meaningful.

Reply 4: With regard to your recommendation to increase the functional study of PTTG1, we fully acknowledge that this would indeed enhance the understanding of the role of PTTG1 in the relevant biological processes. However, we regret that due to current limitations in our experimental conditions and resource constraints, we are unable to conduct additional functional experiments. Nevertheless, we would like to emphasize that despite the lack of further functional analyses in our current work, the results still lay a solid foundation for future investigations into the function of PTTG1. Our study contributes to a better understanding of PTTG1's potential clinical significance in PC and relevant mechanisms, providing valuable preliminary data for future research endeavors. We commit to including more in-depth functional studies on PTTG1 in our subsequent research and will consider integrating your suggestion into our plans for future work.

5) Therapeutic advances in potential targets of the pancreatic cancer tumor microenvironment and the research progress of PTTG1 in tumors are suggested to be added to the discussion.

Reply 5: Thanks for your advice. We added related information in the Discussion part (Page 13, line 384-400)

Changes in the text: Add "The tumor microenvironment (TME) in PC is known to influence tumor progression and can be influenced by different tumor characteristics, leading to diverse mechanisms of immune evasion. Current therapeutic strategies targeting the TME of PC have been designed to modulate the cancer-associated fibroblast and immune compartments. These strategies include the use of CD40 agonistic monoclonal antibodies, chemotherapy, and immune checkpoint inhibitors." "Though the overexpression of PTTG1 is controversial in promoting or inhibiting cell proliferation in various kinds of tumors, PTTG1 is confirmed to be tumorigenic via cell transforming by the induction of chromosomal instability and aneuploidy. Moreover, overexpressed PTTG1 may act as a paracrine/autocrine activator, enhancing expression of growth factors that in turn further sustain tumor growth and contribute to the tumorigenic microenvironment."

6) Figures 8,9,11,12 are not clear enough. It is recommended to provide clearer figures again.

Reply 6: Thanks for reminding us. The replaced figures now are clearer.

Changes in the text: Change Figures 8,9,11,12.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "The expression and survival significance of sodium glucose transporters in pancreatic cancer, BMC Cancer, PMID: 35090421". It is recommended to quote this article.

Reply 7: Thanks for your precious advice. We now cite this wonderful paper in our Introduction, and we also add another citation.

Changes in the text: Add the citation "The expression and survival significance of sodium glucose transporters in pancreatic cancer, BMC Cancer, PMID: 35090421" and new content "For instance, Du et al utilized the overexpression of SGLT-2 to predict the prognosis of the patients with pancreatic ductal adenocarcinoma" in Introduction.

8) Could PTTG1 be an independent factor affecting the prognosis of pancreatic cancer? What research is needed for further proof?

Reply 8: In our research, the elevated levels of PTTG1 expression may correlate with poor prognosis and aggressive tumor behavior in pancreatic cancer. However, as you say, to substantiate PTTG1 as an independent prognostic factor, comprehensive research is needed. With multivariate analysis, we may conduct prospective trials or retrospective studies to accurately assess the prognostic value of PTTG1 independent of other confounding factors.

Mendelian randomization analysis can also be applied for proving the causal relationship between PTTG1 and poor prognosis of pancreatic cancer.

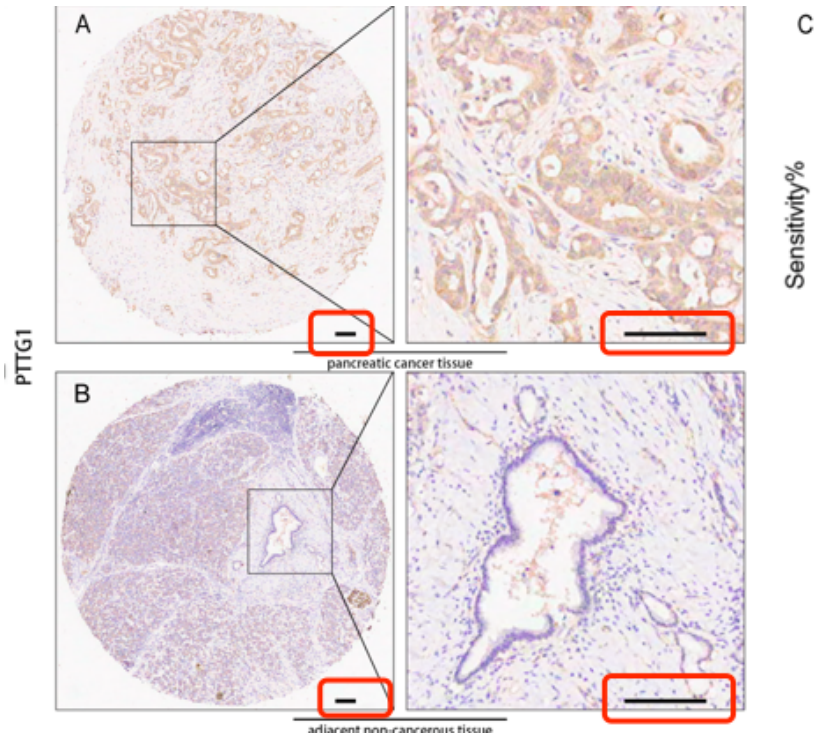
Changes in the text: Add "To substantiate PTTG1 as an independent prognostic factor, comprehensive research is needed. With multivariate analysis, we will conduct prospective trials or retrospective studies to accurately assess the prognostic value of PTTG1 independent of other confounding factors. Mendelian randomization analysis can also be applied for proving the causal relationship between PTTG1 and poor prognosis of pancreatic cancer." in the Discussion part.

Reviewer C

1. **Figure 6**

There are two different scale bars. Please also indicate the other one.

768 **Figure 6** Expression of *PTTG1* protein in PC and adjacent non-cancerous tissues by
769 IHC. (A) *PTTG1* protein expression in PC tissue. Scale bars, 100 μm. (B) *PTTG1*
770 protein expression in adjacent non-cancerous tissue. Scale bars, 100 μm. (C) ROC



Reply: Thanks for your warnings. We have indicates the other one.(Page 28 ,line 783-785)

2. **Figure 7**

Please add the description of the Y-axis.

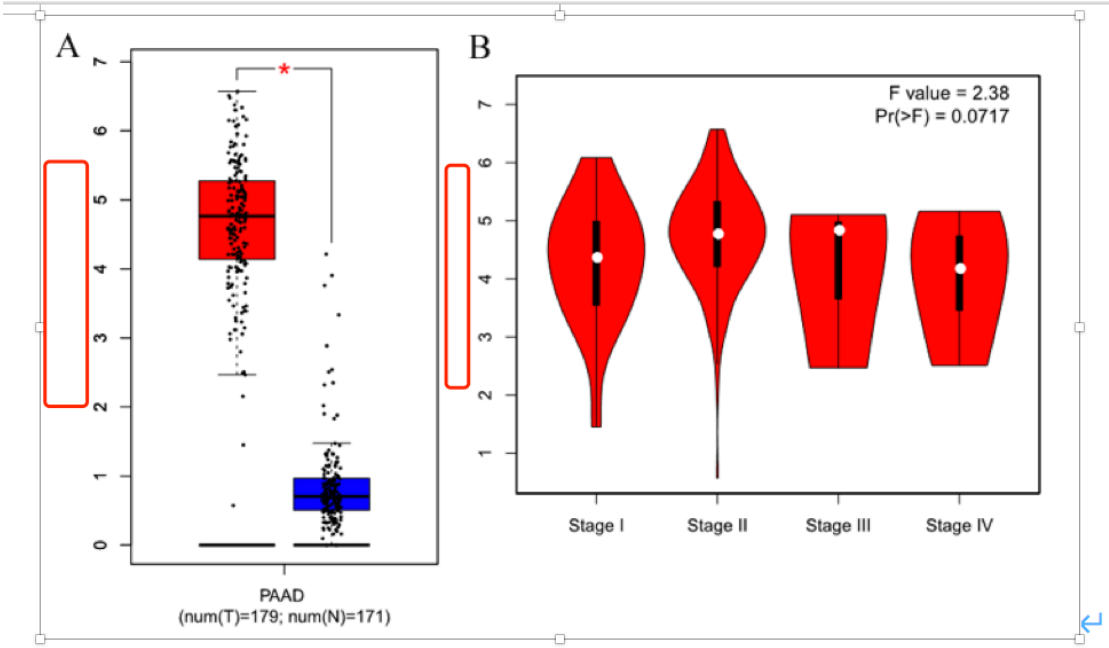


Figure 7 The expression level of *PTTG1* from GEPIA. (A) Cance

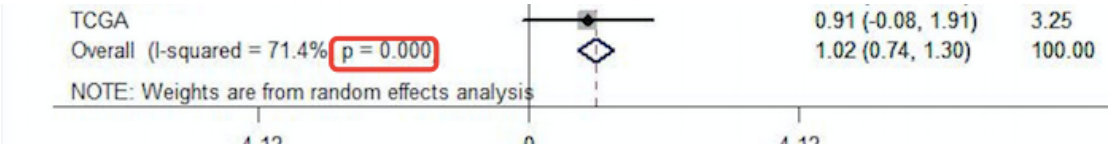
Reply: Thanks for your warnings. We have added the description of the Y-axis.

3. Figure 8

a. Please confirm which P value is correct.

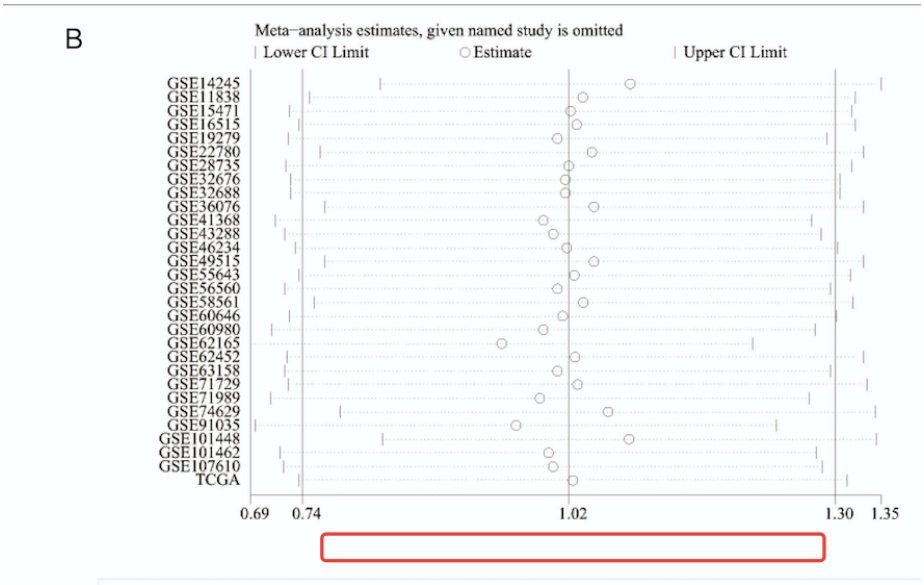
318 cancerous controls (SMD: 1.02, 95% CI: 0.74–1.30, *Figure 8A*). Due to the high
319 heterogeneity in this meta-analysis (I^2=71.4%, P<0.001), a random effects model was

787 **Figure 8** Meta-analysis of GEO microarrays and TCGA database. (A) Forest plot of
788 *PTTG1* expression data from GEO microarrays and TCGA database. The pooled SMD
789 of *PTTG1* was 1.02 (95% CI: 0.74–1.30) by the random effects model. The I^2 value was
790 71.4% (P<0.001). (B) Sensitivity analysis of GEO microarrays and TCGA database. (C)



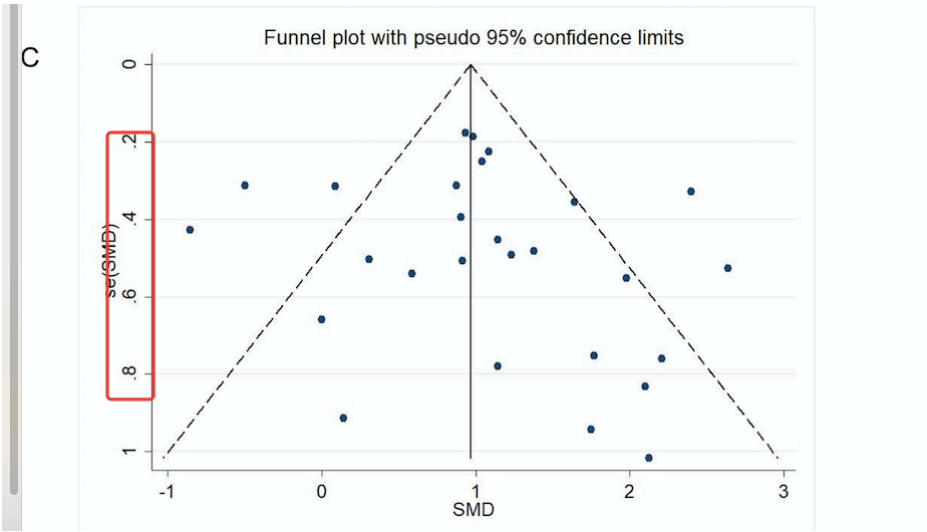
Reply: Thanks for your warnings. We have revised "p=0.000" of figure 8 as "P<0.001".

b. Please add the description of the X-axis.



Reply: Thanks for your warnings. We have added the description of the X-axis of figure 8B.

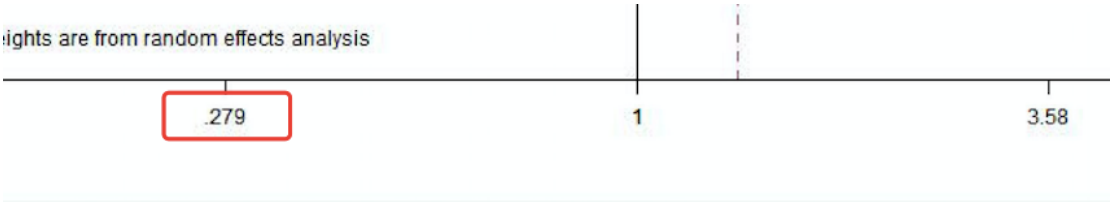
c. Some numbers are incomplete, please revise figure 8C.

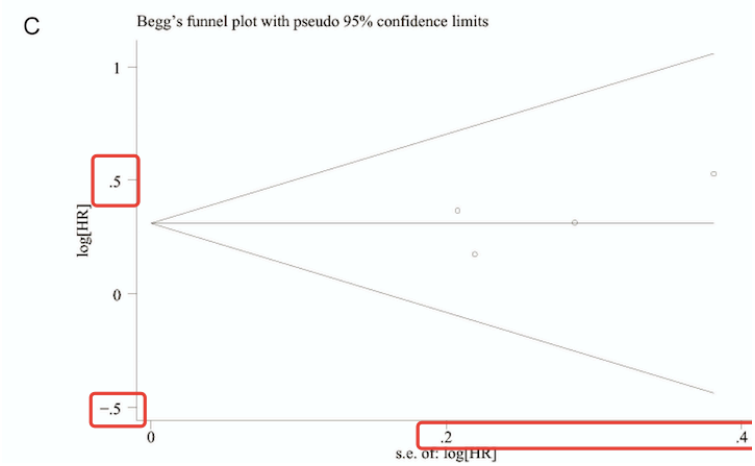


Reply: Thanks for your warnings. We have revised figure 8C.

4. Figure 9

Some numbers are incomplete, please revise.

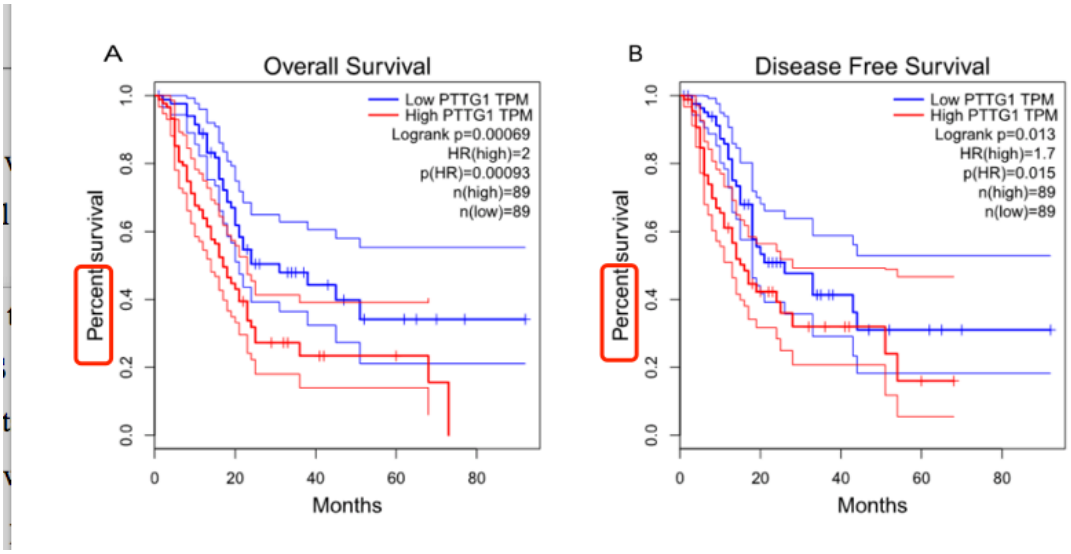




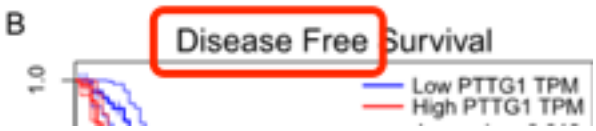
Reply: Thanks for your warnings. We have revised figure 9.

5. Figure 10

a. Since the numbers are range from 0-1, please delete 'percent'.



b. Please revise 'Disease Free' to 'Disease-Free'



Reply: Thanks for your warnings. We have revised Figure 10.

6. Figure 12

Some letters are overlapped. Please revise.

Reply: Thanks for your warnings. We have revised Figure 12.

7. Reference/citation

a. The author's name does not match the citation. Please revise.

Zhang et al. found that higher expression of PTTG1 was associated with higher clinical stages and worse prognosis of PC, the potential mechanism of which was the enhanced OAd5 transduction into PC cells by increasing CXADR expression on the cell surface (22).

22. Long L, Gao J, Zhang R. PTTG1 Enhances Oncolytic Adenovirus 5 Entry into Pancreatic Adenocarcinoma Cells by Increasing CXADR Expression. Viruses 2023;15.

Reply: Thanks for your warnings. We have revised it as "Long et al.".(Page 4, line 122)

b. The authors mentioned "studies...", while only one reference was cited. Please revise.

Most published studies have shown that PTTG1 in hepatocellular carcinoma may be an independent prognostic indicator and potential therapeutic target by upregulating c-myc (13).

Previous studies have shown that PTTG1 expression has an association with the expression of bFGF and VEGF, therefore contributing to cell proliferation and migration (14).

Specifically, previous studies have found that the accumulation of CCNA2 in the S phase is up-regulated in the S, G2, and early M phases (49).

Previous studies have shown that increased expression of CDC20 eliminated the cytotoxic functions induced by curcumin and enhanced cellular proliferation and invasion in PC cells (65).

Reply: Thanks for your warnings. We have changed "Studies" to "A study".(Page 4, line 108-110, Page 15, line 469-470, Page 16, line 490)