Peer Review File

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Responds to the reviewer's comments:

1. Thank you for you remind. We have changed our title into "Development of a basement membrane gene signature and identification of the potential candidate therapeutic targets for pancreatic cancer" (Page 1, line 2-3).

2. Thank you for your advice. We have cited the reference in the introduction section. And the prognostic value of biomarkers mentioned in the reference was also described in this section. (Page 3, line 83-85, line 93-95)

3. We have cited the typical references about the function of BMs in pancreatic cancer. And we described the representative mechanism in the introduction section. We hope this supplementary description can explain the function of BMs in PDAC. (Page 3-4, line 98-105)

4. I am sorry for the mistake in the subtitle. We have changed it into "RNA extraction". (Page 6, line 176)

5. We chose TINAG for the following validation because we found TINAG was the most upregulated gene among all the differently expressed BMGs which might indicate that TINAG play important role in cancer development. We also described the reason in the result section, and our analysis of the BMGs expression level in the TCGA database was attached as an appendix file in the manuscript. (Page 10, line 322-325).

6. Thank you for the recommendation that we look into TINAG's effect on the basement membrane. Your insight is very much appreciated. The new experiment would make the manuscript more integrated. However, because it is the Spring Festival in China and our university is still on winter break, we will be unable to complete the complementing experiments before the deadline. We also searched PubMed for all TINAG-related studies. We made an assumption based on our understanding, which will be validated in our future investigation. And the assumption is mentioned below.

   TINAG was defined as it was first identified as a nephritogenic antigen involved in anti-tubular basement membrane antibody-mediated interstitial nephritis in humans[1, 2]. It is reported that TINAG interacted with laminin and type IV collagen and promotes cell adhesion[3]. As is known this function might play important role in cancer cell invasion and migration. And one experimental study reported TINAG can promote proliferation, invasion and migration in hepatocellular carcinoma[4]. In their study, they found TINAG can be activated by

PI3K/AKT, but they didn't show the mechanism that how TINAG influenced the basement membrane in hepatocellular carcinoma. According to our knowledge, we found TINAG promote cell proliferation in PDAC, and as a basement membrane gene, it is interesting to investigate whether TINAG can activate the signal molecules in the cytoplasm and nucleus. IP- mass spectrum can be used to investigate the molecules interacted with TINAG and RNA-seq can be used to find the transcripts influenced by TINAG. And by combining IP/MS and RNA- seq, it is possible to find the mechanism that how TINAG promote cell proliferation in PDAC.

Meanwhile, according to the current studies, it is reasonable to suppose that TINAG might promote cell invasion and migration in PDAC. As EMT is very important for cell adhesion, so we guess whether TINAG could influence EMT process and then affects cell invasion and migration. Immunohistochemical and immunofluorescence will be used to detected the change of basement membrane during this procedure.

7. We re-summarized the role of 7 BMGs in PDAC in the discussion section (Page 11, line 347-354). And the individual role of each gene in PDAC from current studies were describe in the discussion section (Page 11-13, line 369-421).

8. According to your suggestion, we summarized the advantage of BMG signature and the innovation of our study which is shown in page 13, line 427-437.

References

[1] R.J. Butkowski, J.P. Langeveld, J. Wieslander, J.R. Brentjens, G.A. Andres, Characterization of a tubular basement membrane component reactive with autoantibodies associated with tubulointerstitial nephritis, J Biol Chem 265(34) (1990) 21091-8.
[2] K. Yoshioka, S. Hino, T. Takemura, H. Miyasato, E. Honda, S. Maki, Isolation and characterization of the tubular basement membrane antigen associated with human tubulo-interstitial nephritis, Clin Exp Immunol 90(2) (1992) 319-25.
[3] T.A. Kalfa, J.D. Thull, R.J. Butkowski, A.S. Charonis, Tubulointerstitial nephritis antigen interacts with laminin and type IV collagen and promotes cell adhesion, J Biol Chem 269(3) (1994) 1654-9.
[4] M.H. Zhang, H. Niu, Z. Li, R.T. Huo, J.M. Wang, J. Liu, Activation of PI3K/AKT is involved in TINAG-mediated promotion of proliferation, invasion and migration of hepatocellular carcinoma, Cancer Biomark 23(1) (2018) 33-43.