Peer Review File

<mark>Reviewer A</mark>

Major

1. For proposing the dominant effect of exosome, the add of exosome uptake inhibitor or endocytosis inhibitor was suggested for experiment design.

Reply 1：We are very grateful for the valuable suggestions. In this research, we used 0.22 μm filters and EXO Quick-TC™ exosome isolation reagent to isolate exosomes from culture medium. Before exosome treatment, MRC-5 cells were cultured with a medium containing 10% exosome-free FBS. Whether exosomes are added or not is the only variable in the experiment. The experiment protocol referred to the previous study in Molecular Cancer 2019 (PMID: 30866952). Besides, the main aim of this study is to investigate the function of fibroblasts active by exosomes of dormant cancer cells or normal cancer cells. So we had not set the exosome uptake inhibitor or endocytosis inhibitor group.

2. For pointing out significant of exosomal transfer ITGB6, please add the experimental group of co-culture A549 and fibroblasts and explain the possible differences.

Reply 2: We are very grateful for the valuable suggestions. We have performed the experimental group of co-culture A549 and fibroblasts but failed. After a short-term large dose of cisplatin, the dormant A549 cells were too weak to be reinoculated for the follow-up experiment. Therefore, we performed the experiment of exosome treatment directly.

3. Multi-omic method were employed in present study. The author initially evaluated exosome proteomics, then fibroblast RNA -sequencing, then connected the findings on fibroblast with exosomes proteomics. ECM remodeling, TGFβ then ITGB6 identification was sequentially described. What's the possible findings of conducting fibroblast proteomics and correlating with exosome proteomics?
Please also put the algorithm chart to show the thinking process.

Reply 3: Thanks for your attention. According to the results of our experiments, as for Figure 5D, exosomal proteins were detected in receptive cells after 6 hours of incubation with exosomes. Therefore, we speculate that a fair number of proteins in fibroblast proteomics would be consistent with exosome proteomics. Besides, the algorithm chart has been added in Figure 6.

4. For better describing CAF phenotype, what's the cellular effect of exosome- cultured

fibroblast on A549? Possible stimulation on A549 viability and migration? Please add associated experiments.

Reply 4: We are very grateful for the valuable suggestions. The study of crosstalk between CAFs and A549 is underway. We would tend to present this partial result in the next paper. Thanks for your kind support and understanding！

5. Exosomal transfer between cancer cells and tumor microenvironment had been published. Please add to the debate and the comparison should be described.

Reply 5：Revise. We have added this content in discussion part.

Change in the text: We have modified our text as advised (see Page 15-16, line 466-480).

Minor

1. What's the difference between dormant and common cisplatin-resisted lung cancer cell? Please add description.

Reply 1：We are very grateful for the valuable suggestions. In this research, we used a short-term single dose of cisplatin instead of long-term repeated cisplatin treatment. Long-term repeated chemotherapy can induce senescence, intended as a stable form of growth arrest. In this cellular model, we aim to study the molecular alterations during the process of dormancy induced by cisplatin. Short-term treatment can induce temporary growth arrest and reenter the cell cycle after withdrawal of chemotherapy. We optimized experimental parameters according to reference PLoS One.2014 May 20;9(5):e98021.

Change in the text: We have modified our text as advised (see Page 12, line 341-345)

2. Is there any statistical difference between exosomes obtained from A549 and dormant A549 cell? The size or exosome marker? The quantity of exosome secretion? Please add appropriate description.

Reply 2：As for figure1 F, NTA showed that both diameters of exosomes derived from A549 and dormant A549 ranged from 50 to 170 nm. In general, there are 2 traditional exosomal markers (Tetrapanins, heat shock proteins) and 11 conventional exosomal markers(HSPA8, Alix, HSP90b1, CD9, HSP90AA1, FLOT2, CD81, FLOT1, TSG101, HSPA4, CD63). According to the difference in size of EVPs, the source of EVPs, the expression of EVP markers can be different. There are 13 newly defined EVP markers including FN1, LGALS3BP, A2M, JCHAIN, HBB, GSN, ACTB, B2M, STOM, MSN, PRDX2, RAP1B and FLNA) (Cell. 2020 Aug 20;182(4):1044-1061). In this study, from the identified 1183 proteins in proteomics, we have identified EVP biomarkers including HSP90AA1, FN1, LGALS3BP, A2M, HBB, GSN, PRDX2 and RAP1B. HSP90AA1, FN1 and LGALS3BP are highly expressed in A549-secreted exosomes; A2M, HBB, GSN, PRDX2 and RAP1B are highly expressed in dormant A549-secreted exosomes. Although we did not detect CD63, HSP70, CD9 and TSG101 from the quantitative proteome analysis, we detected the marker's expression by western blot analysis.

We considered the discrepancy may be due to the sensitivity of method. We have added this information in result part.

Besides, in this study, we focused on the function of exosomes and did not detect differences in the quantity of exosome secretion.

Change in the text: We have modified our text as advised (see Page 12, line 354-356)

3. The description of LUAD specimen was ambiguous. What kind of tumor specimen was defined as LUAD? Why the patient had biopsies before chemotherapy and throughout progression? What's anatomical place of tumor? Please clarify.

Reply 3：Thanks for your attention. We add descriptions of tissue specimens. Two patients had histologically confirmed LUAD, which received neoadjuvant chemotherapy but progressed. The first biopsy was performed before therapy to identify the tumor tissue type. After tumor progression, a second biopsy was performed for tumor gene sequencing to guide the next step of treatment. We have added this information in method part.

Change in the text: We have modified our text as advised (see Page 5, line 138-142)

Reviewer B

The paper titled "Exosomal ITGB6 from dormant lung adenocarcinoma cells activates cancer-associated fibroblasts by KLF10 positive feedback loop and the TGF-β pathway" is interesting. The study demonstrated that CAFs were activated by exosomes from dormant lung cancer cells and reconstruct ECM. ITGB6 may be a critical molecule for activating the TGF-β pathway and remodeling ECM. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There have been many studies on lung cancer and CAF. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 1：Revise. We have added this content in introduction part.

Change in the text: We have modified our text as advised (see Page 5, line 106-118).

2) What is the role of the ECM in lung cancer dormancy and outgrowth? What role does Exosomal ITGB6 play in this process? It is recommended to add relevant content.

Reply 2：Revise. We have added this content in discussion part.

Change in the text: We have modified our text as advised (see Page 16-17, line 481-502).

3) What is the clinical relevance of CAFs? How to emphasis their value as prognosis factors and therapeutic targets? It is recommended to add relevant content.

Reply 3：Revise. We have added this content in discussion part.

Change in the text: We have modified our text as advised (see Page 18-19, line 553-580).

4) Why did the author choose ITGB6 for research? Please describe the reason.

Reply 4：Thanks for your attention. We add descriptions of choose ITGB6 for research.

Change in the text: We have modified our text as advised (see Page 14-15, line 430-437).

5) What are the direct and indirect crosstalk between CAFs and infiltrating immune cells? It is recommended to add relevant content.

Reply 5：Revise. We have added this content in discussion part.

Change in the text: We have modified our text as advised (see Page 17-18, line 524-534).

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Exosomal miR-125b-5p derived from cancer-associated fibroblasts promotes the growth, migration, and invasion of pancreatic cancer cells by decreasing adenomatous polyposis coli (APC) expression, J Gastrointest Oncol, PMID: 37201069". It is recommended to quote the article.

Reply 6：Revise. We have added the quote article in introduction part, located at citation No. 19.

7) What is the mechanism by which CAF regulate tumor drug resistance? It is recommended to add relevant content.

Reply 7：We are very grateful for the valuable suggestions. This manuscript focuses on the exosomes derived from cancer cells active the CAFs. The little section was involved with CAFs regulating tumor drug resistance. Further study is required to elucidate the mechanism of CAFs and drug resistance.

Reviewer C

1. Reference

The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

Previous studies show that the surviving cells reinitiated to proliferate for an additional 16 days(24).

Reply: Revise. Change "Studies" to "A study".

2. The figure 1E, figure 2B, figure 3D, figure 4 (A, B, C), figure 5 (C, D, E) in this file 'Western bot' is different from the one in the jpg format. Please confirm which version is correct.

Reply: The file shows the original picture of western blot, including the original exposure picture and white light picture. The WB pictures of the article are clipped from the original exposure picture. We add the relevant instructions to the file. Thanks for your attention.

3. **Figure 6** is a (A, B, C) combined picture, but the citation of figure 6C is missing in the main text. Please revise. Figures should be cited consecutively in the text and numbered in the order in which they are discussed. (example: Figure 1 contains 4 parts, such as Figure 1A, 1B, 1C, 1D, these parts should also be cited consecutively, unless Figure 1 is already cited before Figure 1A, 1B, 1C, 1D.)

Reply: Revise. We have modified our text as advised (see Page 15, line 458-459).

4. **Figure 1**

For cell map, please indicate the observation method in the figure 1A legend.

Reply: Revise. We have modified our text as advised (see Page 21, line 630).

5. **Figure 2**

a. For cell map, please indicate the magnification in the figure 2A legend.

b. No 'ns' in figure 2, please check and revise.

> 660 way ANOVA ns, no statistical significance; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****,

Reply: Revise. a, we have modified our text as advised (see Page 23, line 653); b, we have modified our text as advised (see Page 24, line 666)

6. **Figure 5**

Please indicate the meaning of (**) in the figure 5G legend.

Reply: Revise. we have modified our text as advised (see Page 23, line 653); b, we have modified our text as advised (see Page 28, line 738)

7. **When using abbreviations** in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. Please check and revise. Such as: α-SMA in figure 2; α-SMA, IL in figure 6.

Reply: Revise.

8. When using abbreviations, please mention the entire expression at its first occurrence in the paper.

> 289 China). The primary antibodies against α-SMA (14395-1-AP; Proteintech, Rosemont,

Reply: Revise.