Peer Review File

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Review A

The paper titled "Correlation of gene expression profiles to identify pancreatic cancer cell lines that best model primary human tumors" is interesting. The gene expression profiles of PAAD cell lines correlate weakly with those of primary pancreatic tumors. Through comparison of the genetic similarity between PAAD cell lines and human tumor tissue, we have provided a strategy for choosing the appropriate PAAD cell line. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the differences in mutation characteristics and drug sensitivity between PAAD cell lines and primary pancreatic tumors? It is suggested to add relevant contents.

Reply: In primary pancreatic tumors, mutations of 4 genes predominate:KRAS, TP53, SMAD4 and CDKN2A, each of which is mutated in >50% patients. However, mutations of SMAD4 and CDKN2A are <50% among these 33 PAAD cell lines (see Table 1). For example, if the role of KRAS mutation in pancreatic tumor is studied, then it is not reasonable to choose BxPC-3, KP-4 and Panc 10.05 cell lines.

Gemcitabine is the first line and gold standard drug for pancreatic cancer till now, but its efficiency is unsatisfactory. Most pancreatic cancer patients develop drug resistance. However, PAAD cell lines BxPC-3,CFPAC and SU86.86 are sensitive to Gemcitabine.

Changes in the text: In introduction part, paragraph 2, Line 12, "For example……are not suitable."

Table 1: Common 4 mutations in primary pancreatic tumor are showed in 33 PAAD cell lines.

Cell lines	KRAS	TP53	SMAD4	CDKN2A	Cell lines	KRAS	TP53	SMAD4	CDKN2A
AsPC-1	G12D	√	√	√	T3M-4	Q61H	√	×	×
BxPC-3	×	√	×	×	YAPC	G12V	√	√	×
PANC-1	G12D	√	×	×	HPAC	G12D	√	√	√
Capan-1	G12V	√	√	×	Panc 02.13	Q61R	√	×	×
Capan-2	G12V	√	×	√	Panc 03.27	G12V	×	×	×
CFPAC-1	G12V	√	×	×	Panc 04.03	G12D	√	×	√
DAN-G	G12V	√	√	×	Panc 05.04	G12D	×	×	×

Hs 766T	Q61H	×	×	√	Panc 08.13	G12D	×	√	×
HuP-T3	G12R	√	×	×	Panc 10.05	×	√	×	×
HuP-T4	G12V	√	×	×	PK-1	G12D	√	×	×
KP-2	G12R	√	√	×	SW1990	G12D	√	×	×
KP-3	G12V	√	√	×	SUIT-2	G12D	√	×	√
KP-4	G12D	×	×	×	SNU-410	G12D	√	×	×
MIA PaCa-2	G12C	√	×	×	SU.86.86	G12D	√	×	×
PK-45H	G12D	×	×	×	SNU-324	×	×	×	√
PK-59	G12D	×	×	×	SNU-213	G12V	√	√	√
PSN1	G12R	√	×	×					

2) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply: We provide clearer figures in attachment.

3) It is suggested to add the description of the whole genome characteristics of commonly used PAAD cell lines.

Reply: BxPC-3 and Panc-1 are commonly used PAAD cell lines, there are no fundamental differences lead to genome-wide transcriptional difference among them, but genes involved in EMT and carbohydrate metabolism are quite different. For example, 20 EMT-related alterations are more prevalent in mesenchymal-like cells (Panc-1) than in epithelial-like cells (BxPC-3); Mitochondrial respiration-impaired-BXPC-3 cell line is unable to sustain the metabolic adaptation required by glucose deprivation/substitution which lead to cell apoptosis while the mitochondrial respiration-competent-PANC-1 cell line is not show clear evidence of cell sufferance.

Changes in the text: In introduction part, paragraph 1, Line 10, “In two commonly ……are quite different. ”

4) What is the cellular composition of PAAD? What is the correlation and heterogeneity between primary pancreatic tumors and metastatic disease? It is suggested to add relevant contents.

Reply: Human PAAD tissues contained cell populations including epithelial tumor cells (ETCs), tumor cells with EMT characteristics (EMTs), cancer-associated fibroblasts (CAFs), dendritic cells (DCs), endothelial cells (Endos) , tumor-infiltrating lymphocytes (TILs) and tumor-associated macrophages (TAMs).

Heterogeneity between primary pancreatic tumors and metastatic disease:

Among them, primary tumors contained all 7 major cell populations while the metastatic lesions contained ETCs, TILs, and TAMs; Tumor cells from primary lesions showed

mesenchymal phenotype while the metastatic ones showed little mesenchymal characteristics; Macrophages in the primary tumors and in the metastasis were very different：TAMs from the primary tumors overexpressed genes related to extracellular matrix and late stages of the wound healing-related processes (HIF1A, RHOB, AXL, C3, SERPING1, LUM, COL1A1, and VEGFA), harboring M2-like characteristics; TAMs in the metastases expressed genes such as CD74, FCER1G, and MHC I/II-related genes that are related to the antigen-presenting function of macrophages.

Correlation between primary pancreatic tumors and metastatic disease:

The fraction of each cell type varied greatly from patient to patient , whether primary or metastatic ones ;Functional states and phenotypes of immune cells (TILs and TAMs) were similar in primary and metastatic tumors.

Changes in the text: In discussion part, paragraph 4, Line 1, "Recent study reported huge heterogeneity between primary pancreatic tumors and metastatic disease:……little mesenchymal characteristics. "

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Determining the effect of ellagic acid on the proliferation and migration of pancreatic cancer cell lines, PMID: 35116272". It is recommended to quote the article.

Reply: We add more reference into the introduction part including the recommended paper.

Changes in the text: In introduction part, paragraph 1, line 7, reference 6-8.

6) What is the difference between the immune microenvironment of PAAD cell lines and primary pancreatic tumors? It is suggested to add relevant contents.

Reply: In primary pancreatic tumors,there are not only tumor cells, but also stroma, fibroblast and various immune cells. PAAD cell lines do not mimic the real immune environment in primary pancreatic tumors. Study reports that CD105neg fibroblasts restrict tumor growth in a manner that is dependent on functional adaptive immunity; CD200 expression in the primary pancreatic cancer microenvironment limits responses to immunotherapy by promoting expansion and activity of myeloid-derived suppressor cells(MDSC).

Changes in the text: In discussion part, paragraph 3, line 8, "Fourthly, PAAD cell lines cannot mimic …… various immune cells are present in tumors".

Review B

1. Please check whether the full name of "PAAD" is correct.

(CCLE) project (15). However, pancreatic carcinoma (PAAD) was found to exhibit a weaker correlation between cell lines and primary tumors, with a correlation

Reply: Thank you for your advice, the full name of PAAD has been changed to "pancreatic adenocarcinoma".

Changes in the text: The abstract part, paragraph 2, line 5; In introduction part, paragraph 2, line 6.

2. Please check all abbreviations in the main text, such as "EMT" below. All abbreviated terms should be full when they first appear.

cell lines KURAMOCHI and OVSAHO (9). In two commonly used cell lines BXPC-3 and PANC-1, genes involved in EMT and carbohydrate metabolism are quite

Reply: Sorry for my carelessness, the full name of EMT has been added in the paper.

Changes in the text: In introduction part, paragraph 1, line 11.

3. Since patient tumor tissues were obtained from GEO, please add the statement "The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013)." **in both the "Methods" section of Main Text and the "Ethical Statement" section of Footnote.**

Reply: Thank you for your advice, the footnote has been added according to your request.

Changes in the text: At the bottom of page 5 and page 12.

4. Table S1:

It's suggested to revise "?" to "Unknown".

PK-45H	PK-45 H; PK45H	primary lesion	?	PubMed =11115575 (2001)

Reply: Thank you for your advice, "?" has been changed to "Unknown" in Table S1.

Changes in the text: Table S1.

5. Table S2:

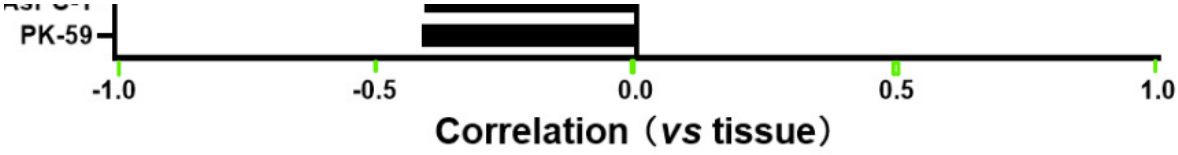
Please indicate the full name of "PAAD", "GEO" in Table S2 footnote.

Reply: Thank you for your advice, the footnote has been added in Table S2.

Changes in the text: At the bottom of page 22.

6. Figure 1:

Please add scale bars of each number in the x-axis as below.

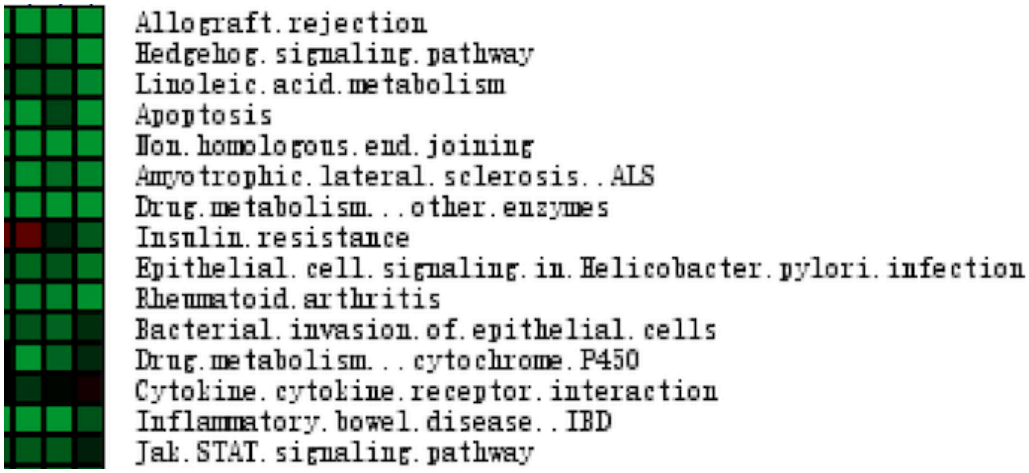


Reply: Thank you for your advice, the Figure 1 has been revised.

Changes in the text: Figure 1.

7. Figure 2-3:

The words in Figure 2-3 are not clear enough. Please resubmit Figure 2-3 in higher resolution.



Reply: We provide clearer figures in attachment.