

Thanks for peer review of our manuscript titled: Silencing profilin-1 inhibits the progress of gastric cancer via modulating integrin β 1/FAK pathway,ESPS Manuscript NO: 10919. We appreciate that you gave us a chance of revision to improve our manuscript to a level suitable for publication in your journal.

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated; however, Figure, made with photoshop, is saved in the state of merged layer, so it is unable to decompose. I hope that this can meet your requirements.

2 Revision has been made according to the suggestions of the reviewer and the responses to reviewers are attached below.

3 References and typesetting were corrected

We hope those responses could meet your approval.

We greatly appreciate both your help and that of the referees concerning improvement to this paper. We hope that the revised manuscript, being sent under separate cover, is a great improvement on the original, and is now suitable for publication.

Thank you for your patience and interest.

Sincerely yours,

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Review 1#:

1. Meaning of following sentences is not clear. "Therefore, silencing profilin-1 promotes inhibits the progress of gastric cancer via modulating integrin β 1/FAK pathway.", "At last, we speculate that silencing profilin-1 promotes inhibits the progress of gastric cancer via modulating integrin β 1/FAK pathway."

Answer:

Thanks your good advice. These mistakes have been corrected in my paper.

2. Graphical presentation is recommended for the suggested action mechanism of profilin-1 in the signaling pathway.

Answer:

Thanks your good advice. Graphical presentation has been shown in my paper.

Review 2#:

1. In the part of INTRODUCTION, the last paragraph is not suitable here, because it's a conclusion.

Answer:

Yes, this comment is very rational. The last paragraph about conclusion has been moved, the new paragraph about how to study has been presented.

2. In the part of RESULTS, there is not any description about FIGURE1C and FIGURE1D.

Answer:

I am sorry to this careless mistake. The description and legend about Figure 1c and Figure 1d has been shown in may paper.

3. In the part of RESULTS, the expression of PFN1 in the gastric cancer epithelial cell lines exhibited relatively higher levels of PFN1 expression than the human immortalized gastric mucosa epithelial cell line, why do

you select immortalized gastric mucosa epithelial cell line for comparison?

What is the P value?

Answer:

Thanks your good advice. The human immortalized gastric mucosa epithelial cell line (GES) was often as normal human gastric epithelial cell line to study or compare with the gastric cancer cell lines [1,2]. So, the GES was selected to study.

I am very sorry for this inconvenience to you. And P value has been shown in my paper.

[1]Du T, Qu Y, Li J, Li H, Su L, Zhou Q, Yan M, Li C, Zhu Z, Liu B. Maternalembryonic leucine zipper kinase enhances gastric cancer progression via the FAK/Paxillin pathway. Mol Cancer. 2014 May 4;13(1):100. doi: 10.1186/1476-4598-13-100.

[2] Sun M, Jin FY, Xia R, Kong R, Li JH, Xu TP, Liu YW, Zhang EB, Liu XH, De W. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. BMC Cancer. 2014 May 6;14(1):319. doi: 10.1186/1471-2407-14-319.

4. In the part of DISCUSSION, passage 8, “.....Our results in present study consistent with this, but different from others finds in other cancers such as breast [6], pancreas [7], liver [8]”. Is it a sentence? What’s the meaning?

Answer:

I admit that there are many mistakes in my paper. These mistakes have been corrected in my paper. Thanks your good advice.

5. Now that the present study demonstrate that PFN1 plays a critical role in cell proliferation and migration of gastric cancer cells by down-regulating integrin $\beta 1$ and inhibiting FAK signaling pathway, you’d better draw a diagram to clarify the relationship between PFN1 and FAK signaling pathway, and the tumorigenicity assays are necessary.

Answer:

Thanks your good advice. Graphical presentation has been shown in my paper.

Yes, this comment is very rational. Because only transient transfection with small interfering RNA was used to study and stably transfected cell lines were not obtained, it was difficult to carry out the tumorigenicity assays and the experiment in vivo would be carried out in next research project. Meanwhile, the tumorigenicity assays were not carried out by some authors in WJG[1]. Of course, if the tumorigenicity assays was put into effect, the conclusion will be more convincing.

Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basictranscription factor 3 is involved in gastric cancer development and progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

Review 3#:

1. I noticed that only SGC-7901 cell line was transfected with siRNA and the results were displayed in your article. If there are more cell lines involved, the conclusion will be more convincing .

Answer:

Thanks your good advice. Firstly, SGC-7901 is a representative gastric cancer cell line and often used to study compared with other cell lines [1,2]. So, the SGC-7901 cell line was selected to explore the function and role of PFN1 in gastric cancer. Next, when this project was designed, we referred this paper, only SGC-7901 cell line was used to study [3]. And the study with small interfering RNA was relatively not complicated for me, as a beginner. So, only one cell line was used to study. Meanwhile, other authors in WJG often elucidate the study only by one cell line [4,5]. Though there are only one cell line involved, clinical data may be good evidence to support our conclusion. In addition, a lot of experiments including Cell proliferation assay, Colony

formation assay, Cell cycle analysis, Cell apoptosis analysis, Transwell assay and Invasion assay have been used to explore the function of PFN1 in gastric cancer. Moreover, FN was used to verify the connection between alteration in integrin β 1/FAK pathway and changes in tumor cell aggressiveness by PFN1 silencing. Of course, if there are more cell lines involved, the conclusion will be more convincing.

[1] Du T, Qu Y, Li J, Li H, Su L, Zhou Q, Yan M, Li C, Zhu Z, Liu B. Maternalembryonic leucine zipper kinase enhances gastric cancer progression via the FAK/Paxillin pathway. Mol Cancer. 2014 May 4;13(1):100. doi: 10.1186/1476-4598-13-100.

[2] Sun M, Jin FY, Xia R, Kong R, Li JH, Xu TP, Liu YW, Zhang EB, Liu XH, De W. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. BMC Cancer. 2014 May 6;14(1):319. doi: 10.1186/1471-2407-14-319.

[3] Zhang Y, Zhang X, Wang X, Gan L, Yu G, Chen Y, Liu K, Li P, Pan J, Wang J, Qin S. Inhibition of LDH-A by lentivirus-mediated small interfering RNA suppresses intestinal-type gastric cancer tumorigenicity through the downregulation of Oct4. Cancer Lett. 2012 Aug 1;321(1):45-54. doi: 10.1016/j.canlet.2012.03.013.

[4] Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basic transcription factor 3 is involved in gastric cancer development and progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

[5] Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basic transcription factor 3 is involved in gastric cancer development and progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

2. In figure 2 A and B, the difference in Western blot and qRT-PCR of MKN28 cell line is obvious and should be explained.

Answer:

Thanks your good advice. PFN1 expression of MKN28 was highest in mRNA level, but its protein level is not highest. On the contrary, PFN1 expression of SGC-7901 was relatively higher compared to other lines both at the mRNA and protein level. In addition, SGC-7901, as a representative gastric cancer cell line, is often used to study compared with other cell lines [1,2]. So the SGC-7901 was selected to study, not MKN28.

[1] Du T, Qu Y, Li J, Li H, Su L, Zhou Q, Yan M, Li C, Zhu Z, Liu B. Maternalembryonic leucine zipper kinase enhances gastric cancer progression via the FAK/Paxillin pathway. Mol Cancer. 2014 May 4;13(1):100. doi: 10.1186/1476-4598-13-100.

[2] Sun M, Jin FY, Xia R, Kong R, Li JH, Xu TP, Liu YW, Zhang EB, Liu XH, De W. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. BMC Cancer. 2014 May 6;14(1):319. doi: 10.1186/1471-2407-14-319.

3. The effects of PFN1 knockdown on the proliferation of SGC-7901 cells were evaluated by CCK-8 kit within 3 days, and i suggest the authors to expand it to 5 days to show the complete effect.

Answer:

Thanks your good advice. Although The effects on the proliferation often evaluated by CCK-8 kit within 3 days[1,2], the experiment was to evaluate the effect of PFN1 knockdown on the proliferation within 5 days has been performed by us and the results has been shown in my paper.

[1] Wu N, Zhang W, Yang Y, Liang YL, Wang LY, Jin JW, Cai XM, Zha XL. Profilin 1 obtained by proteomic analysis in all-trans retinoic acid-treated hepatocarcinoma cell lines is involved in inhibition of cell proliferation and migration. Proteomics. 2006;6:6095-6106.

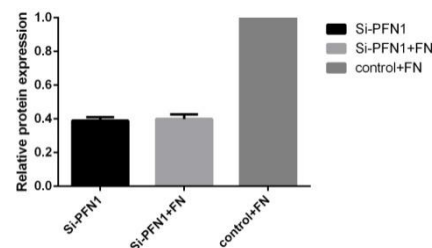
[2] Sun X, Qiu JJ, Zhu S, Cao B, Sun L, Li S, Li P, Zhang S, Dong S. Oncogenic features of PHF8 histone demethylase in esophageal squamous cell carcinoma. PLoS One. 2013;8(10):e77353.

4. In Figure 4A, The migration and invasion ability were assessed with

transwell assay and invasion assay, and in figure 6C, there is a lack of chart bar to count the cell numbers, as well as in figure 6A.

Answer:

Thanks your good advice. The chart bar of figure 4A, 6A and 6C has been drown and figure 4A and 6C have been shown in my paper, But it is no space for the chart bar of Figure 6A and differences in the Figure 6A is very significant. The chart bar of figure 6A has been shown on the following.



5. There are still some minor mistakes like, In figure 1A, there are 4 pictures with only two interpretation;

Answer:

I am sorry to this careless mistake. The description and legend about Figure 1c and Figure 1d has been shown in may paper.

6. The last sentence in your discussion part, "At last, we speculate that silencing profilin-1 promotes inhibits the progress of gastric cancer via modulating integrin β 1/FAK pathway" does not make sense. Please pay more attention to details.

Answer:

Thanks your good advice. This sentence has been deleted in my paper and some mistakes like this have been found and corrected in my paper.

Review 4#:

Major concerns:

1. The authors only used siRNA for transient knockdown of PFN1 expression in SGC7901 cell and function analysis, these results need confirm by

overexpressing PFN1 in gastric cancer cells with relative lower PFN1 expression throughout the manuscript.

Answer:

Thanks your good advice. Firstly, SGC-7901, as a representative gastric cancer cell line, is often used to study compared with other cell lines. Next, when this project was designed, we referred this paper, only small interfering RNA was used to study [1]. The study with small interfering RNA was relatively not complicated for me, as a beginner. So the study was put into effect by small interfering RNA. Meanwhile, other authors in WJG often elucidate the study only by small interfering RNA [2,3]. Though our results were not confirmed by overexpressing PFN1, clinical data might be good evidence to support our conclusion. In addition, a lot of experiments including Cell proliferation assay, Colony formation assay, Cell cycle analysis, Cell apoptosis analysis, Transwell assay and Invasion assay have been used to explore the function of PFN1 in gastric cancer. Moreover, FN was used to verify the connection between alteration in integrin β 1/FAK pathway and changes in tumor cell aggressiveness by PFN1 silencing. Of course, if our results were confirmed by overexpressing PFN1, the conclusion will be more convincing.

[1]Zhang Y, Zhang X, Wang X, Gan L, Yu G, Chen Y, Liu K, Li P, Pan J, Wang J, QinS. Inhibition of LDH-A by lentivirus-mediated small interfering RNA suppresses intestinal-type gastric cancer tumorigenicity through the downregulation of Oct4. Cancer Lett. 2012 Aug 1;321(1):45-54. doi: 10.1016/j.canlet.2012.03.013.

[2]Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basictranscription factor 3 is involved in gastric cancer development and progression.World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

[3]Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basic transcription factor 3 is involved in gastric cancer development and

progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

2. The authors found that silencing PFN1 expression could reduce the expression of integrin β 1 at the protein level with mRNA level unchanged. The underlying mechanism should be explored or at least discussed in the discussion section.

Answer:

Thanks your good advice. Previous study has found that PFN1 overexpression can increase the expression of integrin β 1 at the protein level with mRNA level unchanged. Next, a lot experiments has been carried out to explore this mechanism, and they has a conclusion that PFN1 contributes to the quantity of integrin β 1 linked to the cytoskeleton on the cell surface by promoting actin polymerization to increase the amount of F-actin[1]. So silencing PFN1 decreases the quantity of integrin β 1 linked to the cytoskeleton on the cell surface by inhibiting actin polymerization to decrease the amount of F-actin and it has been added into discussion.

[1] Yao W, Yu X, Fang Z, Yin P, Zhao C, Li N, Wang L, Li Z, Zha X. Profilin1 facilitates staurosporine-triggered apoptosis by stabilizing the integrin β 1-actin complex in breast cancer cells. J Cell Mol Med. 2012;16:824-835.

3. In the manuscript, the authors mentioned “Additionally, the colony formation assay also proved that the PFN1-transfected cells possessed significantly lower colony forming efficiency ($P < 0.05$) (Figure 3C).” Did the authors mean PFN1-siRNA transfected? Please make sure the consistency throughout the manuscript.

Answer:

Thanks your good advice. “PFN1-transfected”has been replaced by “PFN1-siRNA transfected”in my paper.

4. Figure 5C, the authors did not show what measurements have been used for SGC7901 cell before detecting the downstream molecular targets.

Answer:

I am sorry to this careless mistake. This mistake has been corrected in my paper.

5. Figure 6, these result lack control. The author should provide results of SGC7901 cell without treatment or treated with fibronectin to prove that silencing of PFN1 could reverse Fibronectin mediated FAK activation.

Answer:

Thanks your good advice. The experiment including Si-PFN1, Si-PFN1 treated with FN and SGC7901 treated with FN has been carried out again and the result has been shown in my paper.

6. The figure legend for figure 1 did not match the photographs shown. There are four subfigures in figure 1, however, only two were explained in the legend. Moreover, figure 1 was not correctly cited in the manuscript.

Answer:

I am sorry to this careless mistake. The description and legend about Figure 1c and Figure 1d has been shown in paper.

7. In Table 1, the total number of patients with high PFN1 expression according to tumor location was 54, which is inconsistent with total 53 patients with PFN1 high expression. Moreover, the p values for the correlation between PFN1 expression and nodal metastasis or TNM stage need recalculated.

Answer:

I am sorry to this careless mistake. The number of Antrum is 26, not 27, and it has been shown in my paper. All p value in Table 1 has been recalculated and verified again by a professional statistician. Thanks your good advice.

Minor concerns:

1. Abbreviations should be explained at their first occurrence in the text. For example, "PFN1".

Answer:

Thanks your good advice. All the abbreviation has been shown at their first occurrence in this paper

2. The written English is poor, and a lot grammar errors and misspellings should be corrected.

Answer:

I am very sorry for this inconvenience to you. The language has been edited by professional English language editing companies.

3. The primes used in the study for RT-PCRs should be shown.

Answer:

I am very sorry for this inconvenience to you. The primer sequences for PFN1 have been previously described in reference 16, not 8. This mistake has been corrected in my paper.

4. The definition for the cut off value of PFN1 high or low expression in the gastric cancer tissues was not shown throughout the manuscript.

Answer:

I am very sorry for this inconvenience to you. When composite score in cancer tissues greater than normal tissues was considered high expression, and composite score in cancer tissues less than or equal to the normal tissues was considered low expression. This has been shown in my paper.

5. SH-10-TC was used for detecting PFN expression, this should be mentioned in the result.

Answer:

I am sorry to this careless mistake. The cell line has been mentioned in my paper.

6. What is the meaning of the figure after table 1?

Answer:

I am sorry to this careless mistake. The figure has been deleted in my paper.

Review 5#:

1. The tissue microarray results may not be in accordance with the whole tissue caused by selection bias, so the tissue microarray results need to be validated by immunohistochemistry.

Answer:

I admit my mistake about selection bias, and the tissue microarray results has been choosed again for in accordance with the whole tissue. Thanks your good advice.

2. PFN1 mRNA expression needs to be validated in more cases.

Answer:

Thanks your good advice. The cases for qRT-PCR have been add up to 30 and the result has been shown in my paper.

3. The expression of Beta-actin in GES was relatively low, which made the result unbelievable.

Answer:

Thanks your good advice. The expression of GAPDH and PFN1 has been checked again and the results basically consistent with the previous.

4. The effects of silencing PFN1 on malignant behavior of gastric cell and downstream protein expression of the signaling pathway need to be validated in more than two cell lines.

Answer:

Thanks your good advice. Firstly, SGC-7901 is a representative gastric cancer cell line and often used to study compared with other cell lines [1,2]. So, the SGC-7901 cell line was selected to explore the function and role of PFN1 in gastric cancer. Next, when this project was designed, we referred this paper, only SGC-7901 cell line was used to study [3]. And the study with small interfering RNA was relatively not complicated for me, as a beginner. So, only one cell line was used to study. Meanwhile, other authors in WJG often elucidate the study only by one cell line [4,5]. Though there are only one cell line involved, clinical data may be good evidence to support our conclusion. In addition, a lot of experiments including Cell proliferation assay, Colony

formation assay, Cell cycle analysis, Cell apoptosis analysis, Transwell assay and Invasion assay have been used to explore the function of PFN1 in gastric cancer. Moreover, FN was used to verify the connection between alteration in integrin β 1/FAK pathway and changes in tumor cell aggressiveness by PFN1 silencing. Of course, if there are more cell lines involved, the conclusion will be more convincing.

[1] Du T, Qu Y, Li J, Li H, Su L, Zhou Q, Yan M, Li C, Zhu Z, Liu B. Maternalembryonic leucine zipper kinase enhances gastric cancer progression via the FAK/Paxillin pathway. Mol Cancer. 2014 May 4;13(1):100. doi: 10.1186/1476-4598-13-100.

[2] Sun M, Jin FY, Xia R, Kong R, Li JH, Xu TP, Liu YW, Zhang EB, Liu XH, De W. Decreased expression of long noncoding RNA GAS5 indicates a poor prognosis and promotes cell proliferation in gastric cancer. BMC Cancer. 2014 May 6;14(1):319. doi: 10.1186/1471-2407-14-319.

[3] Zhang Y, Zhang X, Wang X, Gan L, Yu G, Chen Y, Liu K, Li P, Pan J, Wang J, Qin S. Inhibition of LDH-A by lentivirus-mediated small interfering RNA suppresses intestinal-type gastric cancer tumorigenicity through the downregulation of Oct4. Cancer Lett. 2012 Aug 1;321(1):45-54. doi: 10.1016/j.canlet.2012.03.013.

[4] Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basic transcription factor 3 is involved in gastric cancer development and progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

[5] Liu Q, Zhou JP, Li B, Huang ZC, Dong HY, Li GY, Zhou K, Nie SL. Basic transcription factor 3 is involved in gastric cancer development and progression. World J Gastroenterol. 2013 Jul 28;19(28):4495-503. doi:10.3748/wjg.v19.i28.4495.

5. The result in Fig6A should show the results of the control group.

Answer:

Thanks your good advice. The experiment including Si-PFN1, Si-PFN1 treated with FN and SGC7901 treated with FN has been carried out again and the result has been shown in my paper.

6. There is no underlying mechanism explored.

Answer:

Thanks your good advice. Previous study has found that PFN1 overexpression can increase the expression of integrin β 1 at the protein level with mRNA level unchanged. Next, a lot experiments has been carried out to explore this mechanism, and they has a conclusion that PFN1 contributes to the quantity of integrin β 1 linked to the cytoskeleton on the cell surface by promoting actin polymerization to increase the amount of F-actin[1]. So silencing PFN1 decreases the quantity of integrin β 1 linked to the cytoskeleton on the cell surface by inhibiting actin polymerization to decrease the amount of F-actin and these has been added into discussion.

[1] Yao W, Yu X, Fang Z, Yin P, Zhao C, Li N, Wang L, Li Z, Zha X. Profilin1 facilitates staurosporine-triggered apoptosis by stabilizing the integrin β 1-actin complex in breast cancer cells. J Cell Mol Med. 2012;16:824-835.