Peer Review File

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Reviewer A

The manuscript has characterized genotypic and phenotypic differences between tumor and nearby stem cells. They describe several findings that could be interesting if presented in a more clear and rigorous way.

The main finding of the work "STOM slowed down the proliferation of MPCs, but promoted the proliferation of breast cancer cells" is unsubstantiated by the data. They reference Figure 4, but this data does not lead to such conclusion. Their claims need to be revised or experiments need to be added.

Reply 1: Thank you for your suggestion about "STOM slows down the proliferation of MPCs but promotes the proliferation of breast cancer cells", we have confirmed that MPCs transfected with STOM proliferate slowly by using multiple sets of tissue samples, therefore we believe that increasing STOM gene expression slows down the proliferation of MPCs, and we have modified the wording for this. Regarding the promotion of breast cancer cell proliferation by MPCs, we supplemented experiments with the MCF7 cell line and found that after co-culturing STOM-overexpressing MPCs with MCF7 breast cancers, MCF7 showed the same effect of enhanced proliferation. In this regard, we concluded that STOM high expression of MPC promotes the proliferation of breast cancer cell lines.

Changes in the text: page15 line12

The text needs to be extensively edited. There are many grammatical and contextual errors. For example, "Wang found that the stom gene...", which is too informal for a publication. Individuals should not be referred to and gene names should be capitalized.

Reply 2: Thank you for your suggestions regarding the text, we have checked the whole text in detail and corrected the language and wording problems.

Changes in the text: page 5 line 9

The figures need to be all in English. All figures need higher resolution or should be vectorized because they are currently too small to read. Figure 4 has overlapping text, and text of different sizes and font which looks unprofessional.

Reply 3: Thank you for your suggestions on the figures, we have revised and re-edited the figures to make the article look clearer and more professional.

Changes in the text: all figure

Reviewer B

A very interesting article.

1. Please conduct proofreading for grammar

Reply 1: Thanks for the suggestion, we've corrected the grammar error.

2. Standardise the usage of abbreviations (STOM vs stom).

Reply 2: Thanks for the suggestion. We normalize proper nouns such as gene names.

3. In the introduction, please combine line 4 (variety of cancers) with line 7 – 9 (the actual list of cancers). Do explain if these studies assessed overall STOM expression, which would include that in the TME or just tumour cell components for example. Comment on the expected variation/ difference between MSCs obtained from adipose in normal breast vs MPCs obtained from the adenocarcinoma itself. The focus on the later identified genes should be expanded.
Reply 3: Thanks for the suggestions on the introduction, we merged the tumors in line 4 and line 7 according to the suggestions, and also evaluated the STOM expression. We also add text about MSC and MPC expected difference evaluation. In addition to adding the target gene introduction and curtailing unnecessary content.
Changes in the text: page5 line9

4. The introduction needs to be rewritten to address the aim of the study.
Reply 4: Thanks for the suggestions on the introduction. We have corrected and rewritten the Introduction.

5. For Methods
a. 2.1. How long were samples stored in saline?
Reply 5.1: After removing the tissue specimen, we process the tissues as soon as possible. Tissue is stored in saline for no more than two hours or the cellular state will be affected.
Changes in the text: page6 line20

b. 2.2. Please give passage number of the MDA-MB-231 cell line used
Reply 5.2: The cell line is passed down from the laboratory for about twenty generations or less.
Changes in the text: page7 line3

c. 2.3 What is MSC basic medium and where did you purchase it from?
Give centrifugation in g not rpm. Ensure mathematical notation is correct (1X106 or 1E6).
Reply 5.3: MSC cultured in human MSC special medium, purchased from Beijing clin-Biotech Co. The centrifugation has been changed to g, while amending mathematical notation.
Changes in the text: page7 line14

d. 2.4. Give the pH of PBS; and catalogue numbers of antibodies used.
Reply 5.4: PBS with a pH of 7.4, and catalog of antibody numbers have been provided.
Changes in the text: page8 line3, table 3

e. 2.4. Correct that ‘cells were incubated with antibodies conjugated with fluorescein’ antibodies were conjugated to range of fluorochromes.
Reply 5.5: We've corrected the wording.
Changes in the text: page8 line4

f. 2.4. Explain the controls that were used for the flow cytometry. How many antibodies were incubated with the cells at a time given there overlap in fluorochromes? Was compensation etc. conducted? What software was used? No rationale is given for the choice of surface markers.

Reply 5.6: Flow cytometry control was cells that was not labeled for fluorescence, flow cytometry was performed with 0.5ul fluorescent antibody added to $1x10^6$ cells for incubation. Compensation adjustments had been made, and we utilized FlowJo for flow analysis. We selected common surface markers of stem cells for labeling, and excluded surface markers such as endothelial cells, immune cells etc.
Changes in the text: page8 line7

g. 2.4. On how many passages were the cells validated for surface markers?
Reply 5.7: We used third-generation cells for surface marker identification.
Changes in the text: page7 line22

h. 2.6. Please give company and catalogue number for Efluor670 solution. Please rewrite section for clarity. Why was a different flow cytometer used (out of interest).
Reply 5.8: We have added the company and catalog number of the Efluor670 solution and tried to describe it more clearly. We have two flow cytometers in our lab, one for multi-samples and the other for fewer samples.
Changes in the text: page9 line1

i. 2.7. Why were MSCs and MPCs digested? Rewrite paragraph in past tense
Reply 5.9: We have rewritten the article paragraph tense. Digestion of MSC and MPC cells because the CCK8 assay can only detect proliferation at different time intervals in 96-well plates.
Changes in the text: page9 line13

j. 2.8. How was RNA quality assessed? Give all kits, reagents etc. used.
Reply 5.10: We send our tissue samples to a technical company, which extracts and analyzes the quality of RNA.

k. 2.9. Rewrite paragraph appropriately. Give a few details on software used for primer design. Was the RNA quality and integrity checked? Give reference for the analytical method.
Reply 5.11: We have added primer design website. We generally assess primer quality and design primers using regular generalized methods.
Changes in the text: page10 line9

l. 2.10 and 2.11. What were the controls used? What is ‘walling’?
Reply 5.12: We simultaneously constructed a blank plasmid as a control, Walling means cell walling, we have changed the wording to make the article clearer.
Changes in the text: page11 line7

m. 2.12. What normality tests were used? What was the n for each experiment? Why choose a paired T-test when multiple variables were being assessed? How was the transcriptomic data assessed? All P<… should be small p
Reply 5.13: Each experiment n was greater than or equal to 3. We used t-test to detect two different groups of samples for comparison. For transcriptome data we screened for differential

genes with |log2FoldChange | > 1 and p < 0.05, and selected appropriate genes through literature reading. We have changed the writing style of p
Changes in the text: page12 line 1

6. Results:
a. Table 1 – what histological / molecular subtype did the patients present with?
Reply 6.1: We have added patient molecular subtypes.

b. 3.1. Figure 1A – can't see the morphology – use contrast. Figure 1B, please check Chinese characters. Images are blurry – check dpi.
Reply 6.2: We have modified the figure.

c. 3.2. Give references for phenotypic identification
Reply 6.3: We references *Transcriptome analysis of tumor-derived mesenchymal progenitor cells shows that CHST15 is a fibrosis regulator of retroperitoneal liposarcoma.*

d. 3.3. Is Dye670 the same as Efluor670? In Figure 2A, what does generation refer to? Proliferation index equation is not given in the methods.
Reply 6.4: Dye670 is the same as Efluor670, Dye670 can detect different cell generations, generation refers to different cell generations. We analyzed the Dye670 data with Modifit software, and the proliferation index was automatically calculated.

e. 3.4. Cell scratch assay not given in the methods. Please use greater dpi in the images.
Reply 6.5: Cell scratching experimental protocol has been added and we modified the images.
Changes in the text: page9 line21

f. 3.5. Refers to secreted factors – not gene expression. What did you do?
Reply 6.6: We used qPCR to detect the RNA of different secretory factors. We've changed the wording to make the article more accurate.
Changes in the text: page12 line16

g. 3.6.
Gene ontology shows enrichment in general pathways (cellular process etc.) and molecular function, the 'differences were mainly in the cell, organelle, and membrane' is very arbitrary (granted that is what the data says) but can you reword and be more succinct since KEGG is giving more direct information?
Reply 6.7: GO analysis and KEGG analysis are equally important methods of bioconfidence analysis, GO analysis will refer to differential expression sites, which it points out here as proteins mainly localized in the cytoplasm and cell membrane.

h. 3.7. Figure 3F shows MSC1/2 etc. -what does that mean? Matched MPC and MSC from patient 1? Then Patient 2? Please be clear. There is considerable variance. If the data was grouped and analysed as n=3 then would you find differences? Or is it passages and n=5 as in

the text? Very confusing.
Reply 6.8: Because of the heterogeneity across patients, in Figure 3F we paired the patient samples, e.g., MPC1 paired with MSC1 and MPC2 paired with MSC2. n=5 means that we experimented with 5 groups of specimens, out of which we took out the 3 groups with better results for the graphs, and we corrected the wording to make the article clearer.
Changes in the text: page14 line1

i. 3.8. Gene expression vs secreted protein – please be clear on what you looked for (text vs figures). Please ensure methods are comprehensive enough (including controls etc.). Figure 4- reorder.
Reply 6.9: We reordered Figure 4. And we used qPCR to detect RNA expression of different secreted factors and did not directly detect cytokine expression levels in the supernatant
Changes in the text: page15 line14

j. 3.9. Overexpression of STOM reduced cell proliferation
Reply 6.10: We've experimentally confirmed that Overexpression of STOM reduced MPC cell proliferation.
Changes in the text: page15 line12

k. 3.10. Why were MDA-MB-231s chosen?
Reply 6.11: The 231 cell line is one of the most common cell lines in human breast cancer, and we added another cell line, MCF7, to make our experimental conclusions more accurate.
Changes in the text: page15 line12

7. Discussion:
a. Are the terms ‘normal breast tissue’ and ‘paracancerous tissue’ interchangeable? Some may argue.
Reply 7.1: We replace the term 'paracancerous tissue' to ‘normal breast tissue’.

b. Please be cautious in referring to secreted factors when you assessed gene expression. This needs to be rewritten for accuracy.
Reply 7.2: We have rewritten a portion of the discussion with reference to secretion factors to more accurately assess gene expression.
Changes in the text: page16 line7

c. The discussion is light and needs to be thoroughly researched and expanded.
Reply 7.2: We expanded the discussion to make the article more profound.

Overall the work is of high quality but the manuscript needs thorough revision before I would recommend it for publication.

Reviewer C
Background

- TME spelled out twice
- MSCs not spelled out
- Clarify that data on how MPCs regulate tumor cells is preclinical
- Use STOM consistently (stom appears multiple times)
- I think the description of the genes at the end of the background section is a little confusing. It gives the impression that the authors selectively decided to investigate these genes. However, based on the abstract, it sounds like these genes were selected based on experiments indicating that they are overexpressed in MPCs. I would prefer that a more general paragraph about this is instead included in the background section and that these specific genes are discussed in the discussion instead.

Reply 1: We fix some detailing errors such as writing problems with TMEs MSCs(see Page 4, line 15), and STOMs, and we add a discussion of genes in Backgrounds(see Page 5, line 4),.Current studies have not been able to confirm that STOM is a preclinical causative agent of breast cancer or a pathological feature of the disease, but this does not influence the fact that STOM is a good predictive therapeutic target.

Methods
- Clarify if informed consent was obtained from patients
- Did the samples >5cm from the primary tumor constitute the stromal tissue samples? Or the "normal-tissue" samples? Please clarify. If the former, >5cm is quite far away. One can argue that it would be preferable to obtain samples of the stroma from the immediate peritumoral region instead.
- Samples were pathologically confirmed by a board- certified pathologist?
- The sections including the assay/experiment protocols are written in a different tense. Perhaps put the full protocols in the supplementary methods and summarize in the main methods section with a tense consistent with the remainder of the manuscript.
- Why did the authors limit themselves to 3 patients for the paired MSCs vs MPCs analysis when there was tissue from 10 patients? In addition, Fig 4 seems to include 5 samples which is confusing. Please clarify
- How did the authors select the differentially regulated genes to investigate? Did they choose the top differentially regulated genes? Or did they base it on previous literature etc.? Please also clarify this in the results section

Reply 2: We have obtained informed consent from the patients (see Page 6, line 15). We extracted stromal cells at the tumor tissue as the target cells, and cells larger than five in were paracancerous samples, which we considered to be normal tissue. The samples in this study were confirmed by a specialized pathologist (see Page 6, line 20). We have modified the tenses of the experimental methods section. Because of the heterogeneity across patients, in Figure 3F we paired the patient samples, e.g., MPC1 paired with MSC1 and MPC2 paired with MSC2. n=5 means that we experimented with 5 groups of specimens, out of which we took out the 3 groups with better results for the graphs, and we corrected the wording to make the article clearer. We selected meaningful target genes in differential genes through literature reading.
Changes in the text: Methods

Results

- It would be helpful to the reader to summarize briefly from how many patients the MSCs and MPCs were obtained and from what tissues (normal tissue (from where?) vs tumor tissue)
- Differences in differentiation abilities: would be preferable to include statistical measurements in results section
- It would be reasonable to have the same axes for the paired experiments (e.g. Fig 4I-K).

Reply 3: We wrote a short fact sheet about the patient's conditions(see Page 12, line 12),.We quantitatively differentiate the differentiation capacity of complementary cells （Figure 1B）.And we normalized the cells, with different kinds of cells having different growth multiplicities. So, the graphs have different vertical axes and the time points are on the same horizontal axis.

Changes in the text: Results

Discussion

- Comment on whether the effects of MPCs on cancer cells are clinically meaningful. Fig4K does not give the impression that there is a meaningful difference.
- Include a limitations section

Reply 4: We modify the content of the article to make the content clearer and increase the effectiveness of MPC evaluation. And we described the limitations of the article(see Page 18, line 11-17)

Changes in the text: Discussion

General comments

- There are several areas that need grammatical revision. In addition, there appears to be text from templates in different areas of the manuscript and the authors need to carefully read through the manuscript to remove these parts. For example, the following text is included at the end of the discussion: “Authors should discuss the results and how they can be interpreted from the perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible. Future research directions may also be highlighted”.

Reply 4: We modify the content of the article to make the content clearer and increase the effectiveness of MPC evaluation. And we described the limitations of the article.