Author's response to reviews

Title: Cell fusion between gastric epithelial cells and mesenchymal stem cells results in epithelial-to-mesenchymal transition and malignant transformation

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Author's response to reviews: see over
Dear Editor:

On behalf of my co-authors, I am submitting the revised manuscript *Cell fusion between gastric epithelial cells and MSCs results in EMT and malignant transformation* (MS: 3227807010995263) for publication in *BMC cancer*. We revised the manuscript in light of the reviewer’s comments and did our best to make substantial improvement in order to meet the merits for publication in *BMC cancer*.

The point-to-point responses to the concerns are listed below, and the revised manuscript conforms to the journal style of *BMC cancer*.

1. Response to reviewer Dr. Feng’s comments:

(1) We described the technique to identify the fusion cells in the revised manuscript. Fluorescence-activated cell sorting (FACS) to identify hybrids after cell fusion is a widely accepted technique, though the product may contain a few cells just binding without real fusion. To ensure the purity, the sorted cells were resorted for GSEPKH-26 cells as describe in material and methods.
(2) Figure 1 showed information collected by different techniques, immunofluorescence and H&E staining, which made it difficult to display the same region with overlaid image, so we prefer keep the original image.
(3) P value was provided for Figure 3E, F in the figure legend, and figure 3E was addressed in the results.
(4) Detailed notes for each panel was labeled in the corresponding figure legend.
(5) Because tumor mass were only observed in mice injected with hybrids, there were no mass observed in those injected with GES-1 or CM-MSCs, so we think that including volume curve for the tumor growth here is not necessary though we did had data about that.

For Minor points: (1) The concentration and company information were provide for CK-18 antibody and the corresponding second antibody in the method. (2) “BMDCs” in the discussion were changed to “BMDSCs”.

2. Responses to reviewer Dr. Lazebnik’s comments:

Reponses for primary concerns:

i) Although the GES-1 cells used in the study carried SV40 T antigen, we do not think CM-MSC transduced with SV40 is a critical control. The purpose of this study is to investigate the effect of cell fusion. Even the effects observed in hybrids was recapitulated by SV40, it was still the effect of cell fusion of which genetic information get transferred horizontally.
ii) Cell scratch test and transwell cell migration and invasion assay used in our study are widely accepted in vitro assays for studying cell invasion and metastasize. We do not agree with the reviewer’s concern.

iii) Cell fusion resulted in changes in gene expression in hybrids compared to parental epithelial cells. Genes involving in EMT expressed and the phenotype of hybrids showed characteristics of EMT, so we think the term EMT can be used here. However, in order to be less controversial, we change the title of the manuscript to “Cell fusion between gastric epithelial cells and MSCs results in EMT and malignant transformation”.

For other concerns: (Responses are underlined)

Page 2:

“The fusion hybrid showed increased proliferation, migration and invasion abilities compared with the control cells.” Referring to ‘parental cells’ would be more appropriate. Changed to “parental cells” as comments.

“Increased nuclear-cytoplasmic ratio with aneuploidy was observed in 84.1% of cells.” As stated, the sentence means that 84.1% of the cells had both increased nuclear-cytoplasmic ratio and were aneuploid. Changed to “Aneuploidy was observed in 84.1% of cells.”, which is more accurate.

“…cell fusion between gastric epithelial cells and mesenchymal stem cells may result in epithelial to mesenchymal transition” Because the term ‘epithelial to mesenchymal transition’ refers to epithelial cells that acquire properties of mesenchymal cells due to changes in gene expression.; Cell fusion resulted in expression of EMT related genes in hybrids compared to parental epithelial cells. The phenotype of hybrids showed characteristics of EMT, so we think the term EMT can be used here.

Page 3.

“Mutations are the principal pathway of malignant transformation.” Perhaps stating that “Mutations are thought to be …” would be more accurate. Changed as comments.

“Cell fusion has been proposed as one of the possible mechanisms of carcinogenesis.” This statement requires references. References are added.

“Cell fusion between healthy differentiated cells is usually cytostatic and fails to generate oncogenic cells.” This statement requires references. Reference is added.

“The potential pathological consequence of fusion between bone marrow-derived stem cells (BMDSCs) and epithelial cells remains to be known.” This statement should be expanded by reviewing previous studies that analyzed the hybrids between BMDSC and epithelial cells. This statement should be “The potential pathological consequence of fusion between bone marrow-derived stem cells (BMDSCs) and epithelial cells remains to be unknown.”
“We previously hypothesized that fusion between an “altered” pre-malignant cell and a bone marrow-derived stem cell results in malignant transformation of the hybrid progeny cells.” This statement requires a reference. Reference is moved here.

Page 9.

“H&E staining showed that the morphologies of GES-1 cells (Figure 1I) and fusion hybrids (Figure 1J) were oval, spindle-shaped and polygonal.” It would be helpful if the authors included the images of H&E and CK-18 stained CM-MSC. We did not have images of H&E and CK-18 stained for CM-MSC available.

“Both H&E and CK-18 IF results detected an increase in the nuclear/cytoplasm ratio in the fusion hybrid, which is a representative characteristic of tumor cells.” This statement should be supported by quantitative evidence. Data were added.

Page 10.

“These results indicate that the fusion hybrid acquired phenotypes from both parental cells, which can result in increased tumor-like characteristic in GES-1 cells.” This statement is unclear, as GES-1 cells are not tumorigenic. Changed to “These results indicate that the fusion hybrid acquired phenotypes from both parental cells. Compare to GES-1 cells, hybrids showed increased tumor-like characteristic.”

“MSCs can acquire the phenotype of GES-1 through cell fusion.” This statement is ambiguous, as the resulting hybrids are neither MSC or GES-1, and is redundant with the preceding sentence, which is more accurate. This sentence is deleted.

“Fusion cell ploidy disorder with increased metastatic and proliferation ability” This statement is unclear. Changed to “Fusion cells showed ploidy disorder and increased metastatic and proliferation ability”. This statement is much clear.

“DNA ploidy analysis was performed on the parental and progeny cells. GES-1 and CM-MSCs were diploid. The majority of fusion hybrids were aneuploid cells (84.10%). The remainder were diploid (12.09%) and polyploid (3.81%), a characteristic of tumor cells.” The original data should be provided as a figure along with the explanation of how the aneuploidy was defined. Supplementing the ploidy analysis with karyotyping would be highly desirable. The original data as figure is provided.

“In the cell scratch assay (Figure 2) the fusion hybrids had greater migration ability than GES-1… these results indicate that fusion of GES-1 with CM-MSCs not only increase the migration ability…” The results for CM-MSC should be provided to determine whether the increased migration is an emergent property of the hybrids or was inherited from one of the parents. Increased migration is not an emergent property of the hybrids, but was inherited from the CM-MSC. The result of CM-MSCs is provided.

“Immunocytochemistry was performed to evaluate the expressions of E-cadherin, N-cadherin and vimentin.” Please explain why these markers were chosen and why immunocytochemistry rather than more quantitative approaches, such as immunoblotting or flow cytometry, were used. Explanations are added.
“Increased proliferation and tumorigenicity in the fusion hybrid” The experiments presented in this section should be described in more detail. How many mice were used for each cell line? Was the size of the masses measured? What was the histopathology of the masses? Does it relate to any tumor type? What does the statement “…no tumor was found” (Fig. 5. Legend) refer to? Changes are made in the manuscript.

“Mutations are believed to be the principal mechanism of malignant transformation.” This statement requires references. Reference is added.

Page 13.

“In conclusion, our results show that fusion of gastric epithelial cells with mesenchymal stem cells induces EMT and malignant transformation.” Cell fusion does not induce EMT, it produces cells that retain epithelial and mesenchymal properties. Cell fusion produced cells that retain epithelial and mesenchymal properties and EMT related genes expressed in hybrids. To less controversial, we change the sentence “In conclusion, our results show that fusion of gastric epithelial cells with mesenchymal stem cells results in EMT and malignant transformation.”

How many times the experiments presented in Figures 2 - 4 were performed? What are the errors – standard deviation or the standard error of the mean – shown in figures 3 and 4 and mentioned throughout the text? The times of the experiments and the meaning of error bar are added in the figure legends.

Figure 3:

What are ‘Fusion(G)’ in 3F? “Graph (E) indicates the number of cells crossing the microporous membrane in the transwell.” Since both migration and invasion assays use the transwells, the authors should specify which assays was used in this case. Fusion (G) in Graph (E) indicates the number number of hybrids penetrated Matrigel. The figure legend were rewrited and specified it.

“…but only fusion cells (D) could penetrate through Matrigel and cross the microporous membrane in the transwell invasion assay.” This statement should be supported by quantitative evidence. The quantitative data was shown in Graph (E)

The hybrids of GES-1 and CM-MSC are referred throughout the text as “the fusion hybrid”. Since the authors used a pooled population of hybrids rather than a clone, it is more accurate to use the word ‘hybrids’ rather than ‘hybrid’. Since cell hybrids are a result of cell fusion, the word ‘fusion’ in ‘fusion hybrids’ is redundant. Changed as suggested.

Thank you in advance for your consideration.

Sincerely,

Xianghui He, MD, PhD