Reviewer's report

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

Version: 2
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Reviewer: Yuri Lazebnik

Reviewer's report:

This study tests the model that fusion of premalignant cells to normal stem cells can produce cancerous progeny. To this end, authors fused a cell line, GES-1, which was made by expressing the oncogene SV40 in human gastric epithelial cells, with normal mesenchymal stem cells, CM-MSC and compared the properties of the parental cells and the hybrids. The authors conclude that “Cell fusion ... induces epithelial-to-mesenchymal transition and malignant transformation”.

The hypothesis, the approach and the results are of interest, but the experimental design, the presentation of the results and the conclusions drawn require substantial improvement, which prevents this reviewer from recommending this manuscript for publication.

Primary concerns:

i) This study lacks of a critical control. Since GES-1 cells express SV40, while CM-MSC do not, it is possible that the effects observed in their hybrids, and the effects on tumorigenesis in particular, can be recapitulated by expressing SV40 in CM-MSC. To rule this out, the authors should compare the properties of the hybrids to that of CM-MSC transduced with SV40.

ii) Since malignant transformation implies the ability of the cells to invade tissues and/or to metastasize. Since the authors provide evidence for neither, the conclusion that cell fusion induced malignant transformation is unfounded.

iii) Epithelial to mesenchymal transition (EMT) is the conversion of an epithelial cell into a mesenchymal cell by changes in gene expression patterns. I am not sure that this term is applicable to the case that involves fusion between epithelial and mesenchymal cells. Perhaps stating that cell fusion produces a new cell type that shares some properties with both parents would be more accurate.

Other concerns:

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.

“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.

“…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,

Page 3.

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

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

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

“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.

“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.

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.

“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.

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.

“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.

“Fusion cell ploidy disorder with increased metastatic and proliferation ability” This statement is unclear.

“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.

“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.

“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.

Page 11.

“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?

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

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.

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?

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.

“…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 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.
Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests