Authors' Response to Peer Reviews

Authors' Response to Peer Reviews of "Establishment of a Novel Fetal Ovine Heart Cell Line by Spontaneous Cell Fusion: Experimental Study"

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This is the authors' response to peer-review reports for "Establishment of a Novel Fetal Ovine Heart Cell Line by Spontaneous Cell Fusion: Experimental Study."

Round 1 Review

Reviewer CU [1]

1. Enhancing the presentation for clarity and coherence in outlining the study's goals and results would improve the paper [2].

Response: We have carefully addressed the issues and revised the manuscript.

2. A more detailed exploration into the cell fusion phenomenon, focusing on the mechanisms of spontaneous fusion, could enrich the study.

Response: It is true that studying the mechanisms of spontaneous cell fusion would have enriched the study, however, that would require additional research tools such as flow cytometry to separate the two morphological cell types and to explore the nature and markers of each cell type.

3. Investigating and discussing the involvement of specific fusion proteins or cellular factors could yield deeper insights into cell fusion processes.

Response: We still keep stock of cells before cell fusion, and we hope to collaborate with specialized international research centers so as to investigate the event of spontaneous cell fusion at the molecular level.

4. By broadening the comparison to encompass additional immortal cell lines, the study could offer a more comprehensive understanding of its findings' implications.

Response: The available data about the Vero cell line allowed us to compare some of its data with these of fetal ovine heart–Saudi Arabia (FOH-SA). Future studies could include additional immortal cell lines.

5. A comparative analysis of how different cell lines undergo immortalization, their genetic integrity, and their response to viral infections could provide a more nuanced understanding.

Response: The exact mechanisms that led to spontaneous immortalization of most animal cell lines remained unexplained, and the results of this study gave an explanation of how the

Vero cell line was established, and this might encourage researchers to further investigate into this area.

6. Extending the functional characterization of the FOH-SA cell line to include its capability for differentiation and response to various external factors would add value.

Response: Such an investigation would be of great value since external factors would affect the cell density and ultimately the target product such as viruses.

7. The paper would benefit from an examination of the FOH-SA cell line's genetic stability through extended culture durations and numerous passages.

Response: In this study, we investigated the extended culture incubation at 37 °C of one passage, but we did not examine its genetic stability.

8. Detailing the cell line's potential for broader biotechnological uses, such as in gene therapy or tissue engineering, would underscore its utility.

Response: The cell line being permissive to many animal viruses, it might be a good future candidate for expression of vector viruses and plasmids and hence its possible utility in gene therapy.

9. A discussion on the safety and regulatory aspects related to the cell line's application in vaccine production, including tumorigenicity risks and quality control adherence, is essential.

Response: Depending on the fact that the Vero cell line was found to be tumorigenic at a high passage number (232), we cautiously used this to produce vaccines at passages 40-70; nonetheless, the safety of the cell line in vaccine production and its tumorigenicity should be investigated.

10. Incorporating schematic illustrations to summarize the key findings and the spontaneous cell fusion development process would enhance the paper's visual clarity.

Response: We have added a schematic summary of the spontaneous cell fusion event to the manuscript. Thank you very much.

11. Providing details on data and material accessibility, including making sequencing data and cell culture protocols available, would facilitate study replication and transparency.

Response: We have added a subsection for data and material availability to the manuscript.

Reviewer DA [3]

General Comments

The paper is well researched, innovative, and the methodologies are clear. There are some minor suggestions from my side.

1. Can you provide more details on the specific morphological characteristics observed during the fusion event that led to immobilization? Were there any distinct features or markers associated with the fused cells compared to nonfused cells?

Response: During the study, we observed the following additional morphological characteristics:

1. The population of epithelial-like cells constituted about 70%-75% of the cell population, while the fibroblast-like cells represented about 25%-30%. This may partially explain the increased proliferation rate of the fibroblast-like cells at passages 27 and 28 just before the point of fusion.
2. The size of cells after cell fusion is comparatively smaller than the size of cells before cell fusion. The figure, which was a trial to document the transformation event before we had a phase contrast microscope, clearly shows the difference in shape, size, and intensity of growth between the cells before transformation (Figure A, passage 26), the filamentous growth at passages 29, 30, 31, and 32 (Figure B), and cells after transformation (Figure C, passage 33).

2. Apart from morphological changes, were there any functional assays or markers used to confirm the immortalized phenotype of the FOH-SA cell line? How were these characteristics compared to primary heart cell cultures?

Response: Apart from the morphological changes and absence of signs of senescence after prolonged passage, we did not investigate specific markers or functional assays.

3. The paper mentions a large-scale genetic conversion leading to high homozygosity in single-nucleotide polymorphism (SNP) genotypes. What are the potential implications of this genetic conversion on the behavior and stability of the cell line, particularly in terms of its use in vaccine production and biotechnological applications?

Response: In general, the change in SNP genotype may alter the amino acid sequence (nonsynonmous SNP) or may not cause a change in the amino acid sequence (synonymous SNP). In this regard, we are particularly interested in investigating the status of the interferon gene cluster, which might partially explain the permissiveness of the cell line to animal viruses.

4. How was the FOH-SA cell line authenticated at the European Collection of Authenticated Cell Cultures? Were there any specific criteria or standards used to verify the identity and purity of the cell line, especially considering its potential for patenting and commercialization?

Response: Authentication of animal cell lines at culture collection is done by DNA barcoding of the cytochrome C oxidase gene to verify the animal species of the cell line and the test will detect the presence of any contaminating cells from other species.

The cell line deposit at ATCC (International Depository Authority) is certified as the "Original Deposit," and according to Budapest Treaty Rule 11, samples of the deposit can be furnished to a third-party by a written authorization from the depositor. An international company has already requested a nonexclusive licensing for the cell line.

5. Beyond vaccine production, what other potential applications or research areas do you envision for the FOH-SA cell line? Are there any specific experiments or collaborations planned to further explore its capabilities and characteristics?

Response: In collaboration with national or international research centers, we will look forward to studying:

- The whole genome sequence and the mutation events after cell fusion that led to immortalization,
- Comparative study of the mitochondria genome of FOH-SA and Vero cell lines, and
- The molecular events of spontaneous cell fusion.

References

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Abbreviations

FOH-SA: fetal ovine heart–Saudi Arabia
SNP: single-nucleotide polymorphism

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