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September 6th, 2019

Dear Editor,

We are very grateful for the helpful comments provided by the reviewer to our manuscript 50082 titled "Clonal isolation of endothelial colony forming cells from early gestation chorionic villi of human placenta for fetal tissue regeneration". Attached please see the revised manuscript with all the reviewer's comments addressed. The changes have also been updated in the revised manuscript with blue color. We have also included a point-to-point response to the reviewer's concerns. We wish the revised manuscript would meet the requisites for publication in World Journal of Stem Cells.

Please do not hesitate to let me know if you or the reviewers have any other questions.

Sincerely,

Aijun Wang, PhD
Associate Professor
Co-Director, Surgical Bioengineering Laboratory
University of California, Davis

Reviewers' Comments to Author

Reviewer Name: Yong-Can Huang
Review Date: 2019-07-25 05:51
Specific Comments to Authors: This is an interesting study conducted by Dr Wang's group to isolate the endothelial colony forming cells from human chorionic villi, namely CV-ECFCs. Isolation of colony forming cells from chorionic villi is not a new finding indeed and the reviewer will not feel amazing to see the good compatibility of CV-ECFCs inside SIS.
Additionally, the reviewer has the following concerns:

1) Mesenchymal stromal/stem cells have been isolated from chorionic villi, what is the difference between MSCs and ECFCs from chorionic villi?

Response: Thank you to the reviewer for the question. Both MSCs and ECFCs can be derived from placental tissues and are both important cell populations for tissue engineering applications. MSCs have unique abilities in differentiating into multiple lineages and regulating immune responses, while ECFCs have unique functions in forming vasculature and promoting angiogenesis. Our previous studies demonstrated that placenta chorionic villi derived MSCs (CV-MSCs) can be obtained from CVS tissues by explant culture, were positive for MSC markers CD105, CD90, CD73, CD44, and CD29, and negative for endothelial markers CD34 and CD31(Figure 2 in Lankford, et al., World J Stem Cells. 2015; 7(1): 195-207. DOI: 10.4252/wjsc.v7.i1.195). In this study, we found that ECFCs isolated from chorionic villi (Figure 3 in the current manuscript) highly expressed endothelial markers CD31, CD34, CD144, CD309, and did not express MSC markers such as CD90. Interestingly enough, we did find that CV-ECFCs at this stage express several conventional MSC markers such as CD105 and CD146. Further endothelial functional tube formation assay and Dil-Ac-LDL uptake assay of CV-ECFSCs in the current study confirmed that CV-ECFCs possess endothelial functions (Figure 4). More detailed functional characterization and comparison between CV-MSCs and CV-ECFCs are warranted in future studies.

2) How many placentas were used in this study? Whether CV-ECFCs can be isolated from all these specimens?

Response: Thank you for this comment. Before we determined this method, we attempted a variety of other methods, including the use of different culture media, different sorting strategies, and different criteria for selecting clones. The method described in this manuscript is the most consistent method we have identified so far. At present, attempts have been made to isolate EPCs from 5 placentas, all of which can produce ECFC-like clones. We obtained robust highly proliferative pure CV-ECFCs from 2 of the 5 placentas, by screening and culturing as described. Currently, more work is ongoing using this method. The number of specimens we tested was included in the Method section of the manuscript.

3) How about the proliferation, growth and differentiation potential of CV-ECFCs?

Response: Thank you for this comment. CV-ECFCs have good proliferative capacity and can be expanded from monoclonal cells into millions of cells. In endothelial growth medium, CV-

ECFCs gradually differentiate and mature into endothelial phenotype and exhibit endothelial cell-specific functions, such as tube formation on Matrigel and Ac-LDL uptake as shown in Figure 4 in this study.

4) Can the typical CFU assay be used to compare the CFU potential between $CD31^+$ and $CD31^-$ cells from chorionic villi?

Response: Thank you for the reviewer's comment. Yes, the typical CFU (colony-forming unit) assay be used to characterize and compare the CFU potential between $CD31^+$ and $CD31^-$ cells isolated from chorionic villi. From our experience, $CD31^+$ cells from chorionic villi represent the ECFC populations and $CD31^-$ cells from chorionic villi represent the MSC populations. As the reviewer pointed out, CFU has been a typical way of characterizing MSCs from various sources as shown in many studies such as Choi, et al., PLoS ONE 2017, 12(2): e0172642; DOI: 10.1371/journal.pone.0172642. In the current study, the method we used to isolate ECFCs is handpicking single-cell colonies, which is based on the ability of these cells to form CFUs. Therefore, CFU assay will be a good way of characterizing and comparing the CFU potential between these cells.

5) The logic in the Introduction section is not well organized, please rewrite it.

Response: We appreciate the reviewers for the suggestion. As advised, we reorganized the introduction part to make it flow better and be more fluent and clear.

6) English proof-reading is necessary.

Response: Thank you to the reviewer for this comment. Our native English speaker scientific writer modified this manuscript.

Scientific Quality: Grade C (Good)
Language Quality: Grade C (A great deal of language polishing)
Conclusion: Major revision
Specific Comments To Authors (File):