Response to Reviewer 1:

03810998
Conclusion: Minor revision
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)

This editoral is well written and readable for this journal. The proof reading and adding the newest references are necessary, especially the ones published in this journal, before it can be formally accepted.

Response:

Thanks for your suggestions and we have added some new references as well as the one published in this journal. Moreover, proof reading has been done again after revision.

Response to Reviewer 2:

02931898
Conclusion: Minor revision
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)

The authors write a review about the various possibilities to test the differentiation potentials of human pluripotent stem cells. This manuscript is clear and well written and well documented. I have two remarks: - Roughly speaking, the two major utilizations of human pluripotent cells are cell therapy on the one hand and in vitro modeling of monogenic disease on the other hand. Because monogenic disease will very probably interfere with the differentiation capacities of hiPSc lines isolated from patients, prediction of differentiation capacities of hiPSCs in such a case is directly the research subject and not a prerequisite. The authors should clarify this point, predictive differentiation makes sense in the context of cell therapy. - the authors should add comments on the expending literature about organoid formation. These new experiments should be very informative in the future about the differentiation capacities of human PSCs.

Response:

Thanks for your suggestions.

1. It is really important to predict the differentiation capability of iPSCs

derived from patients with monogenic disease. We added the following description in the revised manuscript.

"Exceptionally, the potential prediction is not a prerequisite when the iPSCs from patients with monogenic disease are utilized for the disease modeling because the differentiation capability is probably interfered by gene mutation[1]. However, the iPSCs quality control is still necessary. When cell therapy is the purpose using these patient iPSCs, it is also critical to predict their differentiation potential after some special strategies such as gene editing which could revert their defective capability."

2. We also added some literatures about the application of iPSCs in the organoid generation in the section of "INTRODUTION" as follows.

"With the development of organoids technology, hPSCs play a critical role to mimic in vivo tissues and organs at the three-dimensional level and provide a unique opportunity to model human organ development and study various diseases[2]. In the near future, integration of multiple patient-specific hPSCs-derived organoids into a dynamic four-dimensional system by organ-on-chip technology will do contribution to the study of the systematic interactions among different tissues and organs in the body[3]."

Response to Reviewer 3:

00397384
Conclusion: Minor revision
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)

The authors provide a good editorial on the topic, predicting the differentiation of human iPS. The editorial covers the quality control of iPS, differentiation potentials and malignancy potential detection. The only concern from the reviewer is that the author may include partially reprogrammed iPS, which has the potential to differentiate to specific cell types more easily and its malignancy is lower as compared to routine iPS cells.

Response:

Thanks for your comments.

About "partially reprogrammed", there are two different definitions. One is the direct reprogramming procedure such as "partial direct reprogramming

of pluripotent stem cell-derived cardiomyocytes into neurons"[4]. Since this technology involves in the conversion of two types of differentiated cells rather than iPSCs-derived differentiation, we did not include this part in our manuscript.
The other definition is the partially reprogrammed iPSCs which is also called pre-iPSCs. In these ES cell-like colonies, many somatic genes were efficiently silenced, but some endogenous pluripotency genes such as Oct4 and Nanog have not be induced [5]. These partially reprogrammed iPSCs are regarded to get trapped during the pluripotency program[6] and they might be useful in identifying the molecular mechanisms guiding the final steps of reprogramming.

There are few studies evaluating the differentiation potential of "partially reprogrammed iPSCs". Kim JS et al[7] found that partially reprogrammed iPSCs preferentially differentiated into endodermal and ectodermal lineages in Teratoma analysis when compared to the completely reprogrammed iPSCs. However, the results were collected from one cell line and there was no replication. Moreover, these iPSCs were established from mouse embryonic fibroblasts rather than human cells, so we did not include it in this manuscript.

Response to Reviewer 4:

03773730
Conclusion: Minor revision
Scientific Quality: Grade B (Very good)
Language Quality: Grade B (Minor language polishing)
The author reviewed possibilities and challenges of human pluripotent stem cells differentiation potentials, the manuscript is well-written and just minor revision need to be done before acceptance. The comments are as below: 1. Page 3, part of hPSC quality control, I think the "gold standard" should be carefully used for pluripotency of iPSC or ESCs, because the examination of pluripotency of PSCs is tetraploid complementation assay, teratoma assay is the typical of pluripotency evaluation method. 2. As we know, different culture conditions such as the culture media component, feeders or without feeders or even xeno-free culture system are still vary from different lab or the commercial product, I think this part also need to be addressed or discussed in the manuscript. 3. Is there any probes or even strategy can be used to predict the pluripotency or differentiation directly, because the sequencing and microarray is the laboring stuff, I think the author can give us more information or give use new directions to the further research.

Response:

Thanks for your comments.

1. I am sorry to make the mistake about the teratoma assay which was considered as the "gold standard" according to the previous publications. We have deleted this description in our manuscript.

2. We agree that culture condition have definite effects on the iPSCs features. We discussed some in the section of "INTRODUTION", but maybe it is not enough. We have added additional contents in our manuscript, both in "INTRODUCTION" and in "LIMITATIONS AND CHALLENGES".

3. It is a pity that we have not found any publications involved in the application of probes in the potential prediction of iPSCs. It is really a good idea and it will be the new direction in this aspect. We have added this part in "LIMITATIONS AND CHALLENGES".

   Thanks for your suggestions again and they help to improve our manuscript a lot.

Response to Reviewer 5:

00567975
Conclusion: Accept (General priority)
Scientific Quality: Grade A (Excellent)
Language Quality: Grade A (Priority publishing)
This is a nicely written mini-review, in which the questions of predicting the differentiation potential of human pluripotent stem cells are overviewed. Testing the differentiation potential of human PSCs is challenging and very important laboratory task. Modern methods of testing are both time and cost consuming and therefore the development of new testing methods is highly desirable. Present review provides compact overview of the existing methods and also gives some idea about potential new methods for testing the differentiation capacity. I have no comment and can only recommend this paper for a publication.

Response:

Thank you for accepting our manuscript.

Response to Reviewer 6:

02446120
Conclusion: Accept (General priority)
Scientific Quality: Grade B (Very good)
Language Quality: Grade A (Priority publishing)
COMMENTS TO AUTHORS The manuscript by Li-Ping Liu, Yun-Wen Zheng describe the different selection methods to choose hPSC lines for the different clinical or research applications, with the aim of saving time and costs. The authors noticed that there is a significant variation in the differentiation potential and efficiency of various human induced pluripotent stem cell (iPSC) lines and embryonic stem cells (ESCs). Also, they highlight the fact that stem cells do not uniformly differentiate into the cell linage required. To circumvent these problems the authors, propose to carefully look for specific genes which could be useful to predict the differentiation potential of the hPSC. In their manuscript, the authors also propose to check the pluripotency effectiveness of iPSC lines by performing a teratoma assay or by detecting the expression of a set of marker genes by microarray assays. Noteworthy, the authors evaluate the different occurring methods to check malignancy potential in hPSC. This issue is of maximal relevance considering the high risk of developing tumors after treating patients with stem cells. In general, the authors provide comprehensive review of the methods currently available to select the appropriate hPSC according to the intended applications required, addressing the cautions and limitations of the described methods. The manuscript is important, and, giving the growing relevance of the therapeutic use of stem cells, the present work could be useful for researchers and physicians, which must choose one or more methods.

Response:

Thank you for accepting our manuscript.

Reference

1 Shalom-Feuerstein R, Serror L, Aberdam E, Muller FJ, van Bokhoven H, Wiman KG, Zhou H, Aberdam D, Petit I. Impaired epithelial differentiation of induced pluripotent stem cells from ectodermal dysplasia-related patients is rescued by the small compound APR-246/PRIMA-1MET. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; **110**: 2152-2156 [PMID: 23355677 10.1073/pnas.1201753109]
2 Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science*. 2014; **345**: 1247125 [PMID: 25035496 10.1126/science.1247125]

3 Liu C, Oikonomopoulos A, Sayed N, Wu JC. Modeling human diseases with induced pluripotent stem cells: from 2D to 3D and beyond. *Development*. 2018; **145**[PMID: 29519889 10.1242/dev.156166]
4 Chuang W, Sharma A, Shukla P, Li G, Mall M, Rajarajan K, Abilez OJ, Hamaguchi R, Wu JC, Wernig M, Wu SM. Partial Reprogramming of Pluripotent Stem Cell-Derived Cardiomyocytes into Neurons. *Scientific reports*. 2017; **7**: 44840 [PMID: 28327614 10.1038/srep44840]
5 Plath K, Lowry WE. Progress in understanding reprogramming to the induced pluripotent state. *Nature reviews Genetics*. 2011; **12**: 253-265 [PMID: 21415849 10.1038/nrg2955]
6 Park SJ, Lee SA, Prasain N, Bae D, Kang H, Ha T, Kim JS, Hong KS, Mantel C, Moon SH, Broxmeyer HE, Lee MR. Metabolome Profiling of Partial and Fully Reprogrammed Induced Pluripotent Stem Cells. *Stem cells and development*. 2017; **26**: 734-742 [PMID: 28346802 10.1089/scd.2016.0320]
7 Kim JS, Choi HW, Choi S, Seo HG, Moon SH, Chung HM, Do JT. Conversion of partially reprogrammed cells to fully pluripotent stem cells is associated with further activation of stem cell maintenance- and gamete generation-related genes. *Stem cells and development*. 2014; **23**: 2637-2648 [PMID: 24892478 10.1089/scd.2014.0020]