**Reviews for “Transdifferentiation Meets Next-generation Biotechnologies”**

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**Reviewer 1**

Has selected to remain anonymous.

Originality, novelty and significance of results: Adequate

Technical Quality of Work: Good

Comprehensibility and Presentation of Paper: Good

What is the overall impression: Adequate

**Reviewer Recommendation Term:** Revise and resubmit pending major revisions

**Narrative (as sent to corresponding author):**

Ke et al., has selected an interesting topic for a literature review, and it would be a valuable addition to the field. However, it would be helpful to include the comments mentioned below to further improve its readability and scope.

1) The idea of the review is adequate, but it still lacks the key details.

2) Recent references should be included, for example, Mirko et al., 2019 eLife 2019;8:e41627; Cacchiarelli et al., 2018 Cell Systems 7, 258-268, September 26, 2018, and more.

3) It would be good if authors could include a summary table or a figure describing significant transdifferentiation studies & genomic technologies used to achieve transdifferentiation (discussed in the present manuscript & giving an overview of the field).

4) To further broaden the scope of review, authors could consider including reprogramming.

**Reviewer 2**

Has selected to remain anonymous.

Originality, novelty and significance of results: Inadequate

Technical Quality of Work: Inadequate

Comprehensibility and Presentation of Paper: Inadequate

What is the overall impression: Inadequate

**Reviewer Recommendation Term:** Reject outright

**Narrative (as sent to corresponding author):**

In this review, the authors summarize the major discoveries in the transdifferentiation field and describe how genome editing tools and single cell RNAseq technology could accelerate its transition toward clinical applications.
Comments:
-The authors argue that transdifferentiation is cheaper, faster and less tumorigenic than directed differentiation (following reprogramming) and therefore should offer an alternative source of cells for cell therapy. A comprehensive comparison between transdifferentiation and directed differentiation would be valuable for the reader of Stemjournal. Although the first human iPS lines were generated for the first time in 2007, there is already a handful of clinical trials using iPS derived cells for transplantation. In contrast, cells were generated through transdifferentiation for more than 20 years but none have been used in clinical applications. Evidently, there are major hurdles for the use of transdifferentiated cells for cell therapy, including cell numbers, cell function and vector integration, which should be discussed in this review.
-In the first part, the authors listed a number of transdifferentiation reports, including mouse and human cells, in vitro and in vivo and different lineages from the three germ layers. However, they did not discuss what are the limits for these cells'applications. The readers would benefit from listing a smaller number of reports (focusing on human cells) but having a greater discussion on the challenges for their applications, including clinical applications.
-Although the CRISPR activation/inhibition systems provide an alternative way to overexpress or downregulate genes to induce transdifferentiation, there are many challenges to this strategy:
1/ Overall, it is difficult to initiate a strong activation or inhibition of endogenous genes with this system. 2/ The delivery of the dCas9 fused to activators can be difficult as it is a large protein. 3/ To increase the activation of specific genes, more than one gRNA targeting the promoter should be delivered and their expression should not be transient. Therefore, the gRNA delivery method might not be suitable for clinical applications. The authors should discuss what are the advantages of using the CRISPRa rather than overexpression.

There are some inaccuracies in the manuscript:
For instance: Page 7: the Yamanaka factors are not "Oct4, Sox2, Klf2, and c-Myc" but Oct4, Sox2, Klf4 and c-Myc.
Page 8: "high pluripotency brings tumorigenicity'. What is high pluripotency?

**Author’s reply to the reviews:**

Reviewers' comments:

Reviewer #1: Ke et al., has selected an interesting topic for a literature review, and it would be a valuable addition to the field. However, it would be helpful to include the comments mentioned below to further improve its readability and scope.

1) The idea of the review is adequate, but it still lacks the key details.

Response: We appreciate the comments. We have added some details to the manuscripts, especially for the discussion of how the next-generation technologies will benefit the study of transdifferentiation process.

2) Recent references should be included, for example, Mirko et al., 2019 eLife 2019;8:e41627; Cacchiarelli et al., 2018 Cell Systems 7, 258-268, September 26, 2018, and more.

Response: We included the mentioned recent references and added more, such as Wang et al. 2020 Acta Pharm Sin B 2020; 10: 313–326; Biddy et al. 2018 Nature 2018; 564: 219–224; etc.

The paragraph we added are: “Developed by Qiu et al. in 2017, Monocle 2 is an algorithm that better identifies branch points of cell fate decisions than its previous version[100]. Revealing the conversion barriers that cells were facing during the classic MyoD-mediated myogenic conversion, the algorithm has shown its capability in “debugging” the conversion process and discovering novel key determinants[101].”
And
“More recently, by comparing the trajectories of transdifferentiation and reprogramming from pre-B cells at the single-cell level, Francesconi et al. identified distinct cell subsets in the starting cell population, among which the small pre-BII cells transdifferentiate more rapidly than the large pre-BII cells. By distinguishing and targeting the cell subset that responds better during transdifferentiation induction, we will potentially improve the efficiency of transdifferentiation process and further promote its practicability as clinical trials[103]. ”

3) It would be good if authors could include a summary table or a figure describing significant transdifferentiation studies & genomic technologies used to achieve transdifferentiation (discussed in the present manuscript & giving an overview of the field).

Response: Thanks for the excellent point. We have added the advance of technologies along with the discoveries in the Figure 3, which provided a timeline of several important studies on transdifferentiation in the past decades. We highlighted the method used for each work on the figure to emphasize them, which also include the emergence of several genomic technologies.

4) To further broaden the scope of review, authors could consider including reprogramming.

Response: Reprogramming would be a very interesting topic to discuss about and to be compared along with transdifferentiation. In the section “Directed differentiation vs. transdifferentiation” we briefly described the development of reprogramming.
In Figure 2 we also summarized and showed the difference between differentiation, reprogramming, and transdifferentiation (direct reprogramming). We were trying to emphasize on the current achievements of transdifferentiation in this manuscript, but will definitely consider reviewing the development of reprogramming in near future. That would be another attractive and informational article.

Reviewer #2: In this review, the authors summarize the major discoveries in the transdifferentiation field and describe how genome editing tools and single cell RNAseq technology could accelerate its transition toward clinical applications.
Comments:
-The authors argue that transdifferentiation is cheaper, faster and less tumorigenic than directed differentiation (following reprogramming) and therefore should offer an alternative source of cells for cell therapy. A comprehensive comparison between transdifferentiation and directed differentiation would be valuable for the reader of Stemjournal. Although the first human iPS lines were generated for the first time in 2007, there is already a handful of clinical trials using iPS derived cells for transplantation. In contrast, cells were generated through transdifferentiation for more than 20 years but none have been used in clinical applications. Evidently, there are major hurdles for the use of transdifferentiated cells for cell therapy, including cell numbers, cell function and vector integration, which should be discussed in this review.

Response: We acknowledge that transdifferentiated cells are not ready for clinical applications, even after discovered for more than 20 years. In the last paragraph of the first part, we addressed that: “A relatively lower efficiency had been one of the reasons that impeded clinical applications of transdifferentiation. There were 11 approved clinical trials to date involving human PSC-based therapies registered at the National Institutes of Health website[20], whereas no transdifferentiated cell-based therapies have appeared on the list[15]. As the fast development of direct reprogramming technologies in recent years, the shortcomings of transdifferentiation are gradually being overcame and their applications in regenerative medicine are promising.”

We believe that these shortcomings can be overcame as the next-generation technologies are rapidly developed. To support this point of view, we added more recent findings in transdifferentiation by implementing either CRISPRa system or single cell level analysis.

-In the first part, the authors listed a number of transdifferentiation reports, including mouse and human cells, in vitro and in vivo and different lineages from the three germ layers. However, they did not discuss what are the limits for these cells' applications. The readers would benefit from listing a smaller number of reports (focusing on human cells) but having a greater discussion on the challenges for their applications, including clinical applications.

Response: Thanks for the good point. We added a paragraph to the end of “Current progresses of transdifferentiation” part and discussed about the challenges: “The procedure of direct reprogramming fully differentiated cells to either multilineage or single lineage hematopoietic progenitors in vitro are solid, but the in vivo engraftment capability can be further improved and developed for clinical use. One of the major hurdles is the difficulty of delivering reagents that mediate the conversions between distinct cell types. In a 2019 study, Chang et al. presented an efficient and safe in vivo direct conversion of fibroblasts into cardiomyocytes. The critical factors of cardiac reprogramming, GMT, were loaded in cationic gold nanoparticles delivered locally into mouse hearts[83]. The nanocarriers are promising candidates for a novel treatment for all kinds of diseases.”

We would still like to introduce the history and development of transdifferentiation to the readers in the first part, and help the readers to have a more comprehensive understanding on the capability of transdifferentiation.

-Although the CRISPR activation/inhibition systems provide an alternative way to overexpress or downregulate genes to induce transdifferentiation, there are many challenges to this strategy:
1/ Overall, it is difficult to initiate a strong activation or inhibition of endogenous genes with this system. 2/ The delivery of the dCas9 fused to activators can be difficult as it is a large protein. 3/ To increase the activation of specific genes, more than one gRNA targeting the promoter should be delivered and their expression should not be transient. Therefore, the gRNA delivery method might not be suitable for clinical applications. The authors should discuss what are the advantages of using the CRISPRa rather than overexpression.

Response: Thanks for the excellent comment. CRISPRa might not be suitable for clinical applications, however, another application of CRIPSRa will greatly assist promoting transdifferentiated cells in clinical use, which is genome screening. We added the following discussion in the manuscript to summarize the advantages of using the CRISPRa:

“The major advantage of using the CRISPR/Cas9 system for direct cell reprogramming is it precisely modulates endogenous gene expression without leaving permanent genome mutation. Two 2016 studies both reported conversion from fibroblasts into neuronal cells via CRISPR/Cas9 based methods, while Rubio et al. chose to inactivate TSC2 gene[18] and Black et al. chose to activate the endogenous BAM factors by CRIPSR activation(CRISPR a)[89]. CRISPRa is a process in which the catalytically dead variant of Cas9, called dCas9, was directed to the promoter regions by fusing with transcriptional activators, affecting gene expression. In 2014, Chakraborty et al. first designed an enhanced robust CRISPRa system by fusing two transactivation domains to Cas9. This RNA-guided VP64dCas9-BFPVP64 fusion protein activated endogenous Myod1 in mouse embryonic fibroblasts and converted them into skeletal myocytes with a myogenic gene expression comparable to other Myod1-based transdifferentiation mentioned earlier[90]. Besides conversion from human fibroblasts into neuronal cells, CRISPR activators were also reported to perform reprogramming from fibroblasts into iPSCs and cardiac progenitor cells in recent studies [91, 92]. Moreover, CRISPRa system can also help locate key gene factors in cell fate changing by genome-scale screening. For example, in 2018, Liu et al. developed an approach that generates individual factor map and factor genetic interaction map after genome screening via CRISPRa. By further validations with top key genes on the maps, they discovered a novel combination of Ezh2 and Mecom, promoting transdifferentiation from fibroblasts into neuronal cells[93].”

There are some inaccuracies in the manuscript:
For instance: Page 7: the Yamanaka factors are not "Oct4, Sox2, Klf2, and c-Myc" but Oct4, Sox2, Klf4 and c-Myc.
Page 8: "high pluripotency brings tumorigenicity'. What is high pluripotency?

Response: Thanks for the good catch. The inaccuracies of Yamanaka factors are fixed.

On Page 8, by saying high pluripotency, we are indicating the plasticity of pluripotent cells compared to multipotent and bipotent. We deleted the inaccurate adjective to avoid causing confusion.

**Reviewers’ response to the revision:**

**Reviewer 1**

Originality, novelty and significance of results: Good

Technical Quality of Work: Good

Comprehensibility and Presentation of Paper: Good

What is the overall impression: Good

**Narrative (as sent to corresponding author):**

Authors have satisfactorily replied to the comments. I recommend the manuscript for publication.

**Reviewer 2**

Declined to review again.

**THE ASSOCIATE EDITOR DECIDED TO ACCEPT THE PAPER**