

Format for ANSWERING REVIEWERS

November 17, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (ESPS Manuscript NO: 21779).

Title: Generation of diverse neural cell types through direct conversion

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Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 21779

The manuscript has been improved according to the suggestions of reviewers, with revisions highlighted using track changes.

Reviewer #02446101.

We thank the reviewer for the commentary.

Reviewer #02446119

We thank the reviewer for the commentary.

Reviewer #02446120

We thank the reviewer for the commentary and suggestions.

Regarding the therapeutic use of stem cells, the following has been added to the section on Induced Pluripotent Stem Cells, outlining their approval for human macular degeneration trials in Japan:

“Significantly, the first therapeutic use of iPSC has been approved for human trials in Japan, with cells from the skin of a patient suffering from age-related macular degeneration reprogrammed into iPSC and subsequently differentiated into retinal pigment epithelium cells, prior to implantation into the eye^[50].”

Regarding neural differentiation of adult stem cells, as stated within the article, the range of chemicals including β -mercaptoethanol, butylated hydroxyanisole, dimethylsulphoxide, isobutylmethylxanthine, dibutyryl cyclic AMP, epidermal growth factor, and brain-derived neurotrophic factor have been shown to induce the differentiation of mesenchymal stem cells into neural-like cells. As the focus of this article is the generation of neural cell types through direct conversion, we believe that further information regarding neural differentiation of adult stem cells is outside the scope of this article.

Regarding insufficient cell numbers generated during neural direct conversion, this was briefly mentioned in the section on Generation of Induced Neural Stem and Progenitor Cells. To further clarify, this section has been expanded as follows:

“The generation of induced neuronal cells and subtypes is often associated with low conversion efficiencies and yields, resulting in difficulties obtaining sufficient cells for therapeutic applications. This may be in part due to the post-mitotic state of the target cell type (neuron-like cells), with the conversion procedure including a halt in proliferation, thus limiting the ability of these cells to expand once reprogrammed^[98-100]. In addition to determining methods of increasing conversion efficiency, studies have expanded into investigating whether similar methods could be utilised for generation of proliferative neural stem and progenitor cells, which are both expandable *in vitro* and capable of generating multiple neural cell types^[101], with initial studies demonstrating the generation of induced neural progenitor^[101] and crest^[102] cells.”

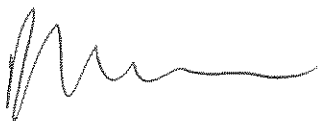
Regarding the investigation of microRNA-mediated neural direct conversion, as shown in Table 1, only microRNA-9/9* and microRNA-124 have been used in the generation of induced neuronal cells, thus only these two were discussed. To further clarify, the section on Generation of Induced Neuronal Cells has been expanded to discuss the roles of these specific microRNA in neuronal direct conversion as follows:

“Other studies have also investigated the use of microRNA in conjunction with neuronal specific transcription factors, including expression of microRNA-9/9* and microRNA-124^[83,84], as well as repression of a single RNA binding polypyrimidine-tract-binding protein, a key target and negative regulator of microRNA-124^[85]. microRNA-9/9* and microRNA-124 are known to act on critical target genes that regulate neuronal differentiation and function, with microRNA-9* and microRNA-124 found to instruct compositional changes of SWI/SNF-like BAF chromatin remodelling complexes in a process that is important for neuronal differentiation and function^[83].”

Regarding the possible regeneration of retinal neurons in patients with eye diseases, as described in the Introduction, the theme of this article is “the generation of diverse neural cell types via direct conversion of somatic cells, with comparison against stem cell-based approaches, as well as discussion of their potential research and clinical applications”. As such, we believe that the possible regeneration of retinal neurons in patients with eye disease is outside the scope of this article.

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,



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