September 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13241-review.docx).

Title: Connexin mutant embryonic stem cells and human diseases

Author: Kiyomasa Nishii, Yosaburo Shibata, Yasushi Kobayashi

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 13241

The manuscript has been edited according to the suggestions of reviewers:
1 The journal administrator requires that name of the department should be provided for Dr. Yosaburo Shibata in the cover page. He is a president of Fukuoka Prefectural University and does not belong to any department. Therefore his affiliation is correct as it is.

2 Revision has been made according to the suggestions of the reviewer. Details are described in the following pages.

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

Sincerely,

Kiyomasa Nishii, MD, PhD
Department of Anatomy and Neurobiology
National Defense Medical College
3-2 Namiki, Tokorozawa
Saitama 359-8513, Japan
Tel : 81-4-2995-1478
Fax : 81-4-2996-5186
E-mail: nishii@ndmc.ac.jp

Our responses to the reviewers' specific comments:

Overall, The review is very well written and at the same time comprehensive, But it might be a good idea that the authors summarize the findings and conclude at the end.

We added brief closing remarks at the end.
Page 12, Line 4: CONCLUSION …

1. The last sentence in the Core tip on page 3 is confusing because it gives an impression that Cx43 and Cx45 mutant mouse embryonic stem cells need to be generated in the future, and states the mutant ESCs could be a model for human iPSCs that is another model to understand human diseases. Perhaps it will be better to state that according to the studies using mutant mouse ESCs, Cx43 or Cx45-null human iPSCs may become a useful model.

Thanks for the suggestion. The sentence has been altered with a slight modification from the suggested words.
Page 3, Line 8: According to the studies using mutant mouse embryonic stem cells, *Cx* mutant human iPSCs may become a useful model.

2. On line 11, page 4, at the blastocyst stage, there are two types of trophoblasts, polar and mural trophectoderm. Perhaps the one making gap junctions with both trophoblasts and the inner cell mass cells will be the polar trophectoderm.

We agree. However, there has been no report on whether polar or mural trophectoderm cells provide the gap junctions. A short comment with an additional citation has been added.

Page 4, Line 11: …; those cells that are linked by gap junctions to both trophoblasts and cells in the inner cell mass cells probably form the polar trophectoderm [5, 6].

3. If known, the reason why no Cx23 and Cx33 knockout mice have been generated needs to be provided on line 14, page 5.

We are not aware of a specific reason. Instead, we have provided additional information about *Cx23* and *Cx33*.

Table 1, Footnote: … Notably, however, the mouse small-eye mutant *Aey12* has a point mutation in

the *Cx23* locus[101]. For *Cx33*, there is no orthologous gene in the human genome[1].

4. The statement starting on line 6, page 6, is confusing because apparently some of Cx isoforms play unique function in a specific cell type. Perhaps, it will be better to state that a specific individual Cx does not seem to possess a one-to-one association with a unique cell type in vivo.

The sentence has been altered.
Page 6, Line 6: Thus, a specific individual Cx does not seem to possess one-to-one association with a unique cell type *in vivo*.

5. The statement starting on line 15, page 6, is not accurate. Those constitutive KO mice were never born due to embryonic lethality.

The sentence has been altered.
Page 6, Line 15: Because these constitutive KO mice are embryonically lethal, …

6. Perhaps "present with" on line 2, page 7, is meant for "exhibit".

Thank you.
Page 7, Line 2: Mouse *Cx* mutants do not always exhibit the same phenotype …

7. The statement on line 2, page 9, can be more specific and informative. For example, "attempts to mutate a unique Cx isoform in a tissue specific manner have been made".

The sentence has been altered.
Page 9, Line 2: Therefore, attempts have been made to mutate a unique *Cx* isoform in a tissue-specific manner.

8. On line 14 page 9, the use of "researchers" as a subject is not advised in the review article like this. Instead of "researchers sometimes want to", "it has been of great interest to" is suggested.

Thank you for the nice suggestion.
Page 9, Line 15: …, understanding what happens at the borders between Cx-positive and -negative cells has been of great interest.

9. On line 13, page 10, "Cx45-KO mice" are supposed to carry conditional alleles. Because in this

chapter conditional KO approaches are introduced, it is advised to make a clear distinction between constitutive and conditional alleles.

&

10. Sentences starting on line 14, page 10, are redundant. In particular, the sentence starting from "Taken together" can be revised to, for example, "Taken together, the heart abnormalities are expected to be the primary defect associated with the loss of Cx45 in developing embryos."

'*Cx45*-KO mice' are 'constitutive *Cx45*-KO mice'. The subject has been altered.

Page 10, Line 13: Constitutive *Cx45*-KO mice were …

And, we found that reference to the conditional *Cx45*-KO mice was scarce in this section. We therefore added a brief sentence. Regarding the sentence starting from 'Taken together', we have followed your advice.

Page 10, Line 15: Later, as described above, the CM-specific *Cx45*-KO mice were shown to be similar to the constitutive *Cx45*-KO mice[13]. Taken together, the heart abnormalities are expected to be the primary defect associated with the loss of *Cx45* in developing embryos.

11. On line 12, page 11, why are only "several" but not "many" mouse genetic models useful to derive iPSCs from? Is the word "several" necessary in this sentence?

According to your comment, we elect to use 'many'.

Page 11, Line 13: … from many mouse genetic models.

12. On line 15, page 11, reference 58 is a review article. In this context, the original studies need to be cited.

We have revised the reference and have cited the seminal work by Hanna et al. (2007). This is shown as citation [60] in the text.

13. Table 1. What is the "partial embryonic lethality"? This needs to be defined.

Short description has been added.

Table 1, Footnote: **About 60% (*Cx31*) and 30% (*Cx31.1*) of the embryos were lost *in utero*; the surviving adult mice were observed to have no morphological defects.

14. The figure legends must be elaborated. What does the green highlight indicate? What are the

middle and right cartoons and their differences between A and B? What are the middle and right cartoons in C?

The figure legend has been overall revised.

Figure 1, Legend: Mutant cells and regions are shown in green. Mouse and heart drawings, respectively, constitute the middle and right pictures in (A) and (B). (A) In the Cre/*loxP* model shown here, the *Cx* gene, which when lost causes lethality, is deleted specifically in the CM. This results in relatively consistent delay or block in conduction[13, 49]. (B) Chimeric mice containing ESCs lacking the *Cx43* gene. The example shown here reveals multiple conduction pathways in the heart[52]. (C) ESCs can be differentiated *in vitro*. In this example, the induced CMs are subjected to planar multielectrode array analyses (middle); a typical extracellular recording data is shown in the right graph[53, 67].