May 21, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 18485-review.doc).

ESPS Manuscript NO: 18485

Title: Molecular Basis of Cleft Palates in Mice
(Previous title) **The Molecular Basis of Cleft Palates in Mice: a Minireview**

Author: Noriko Funato, Masataka Nakamura, and Hiromi Yanagisawa

We would like to thank the Editor and the Reviewers for favorable responses and constructive criticisms on our manuscript.

1 Format has been updated.

2 References and typesetting were corrected.

3 Revision has been made according to the suggestions of the reviewer.

Point-by-point responses to each Reviewer are listed below and the text has been revised and highlighted accordingly.

Reviewer 00731613

1. While I agree that the articles cited in this manuscript are useful, I suggest that the authors try and retain only those which are mandatory.

Response

We optimized the references.

2. Table 1 provides an elaborate list of molecules associated with human syndromes. Are all these molecules studied in cleft palate? If so, were they studied in mice or humans or both? Many of the syndromes associated with these molecules do not have any relation to oro-facial clefts. So in that case is it necessary to include them?

Response

As the reviewer suggested, not all of the molecules listed in Table 1 have been studied in cleft palate and have relation to oro-facial clefts in human. However, we believe a comprehensive list of genes associated with CL/P should provide insights into the genetic etiology of CL/P. We revised Table 1, and indicated the CL/P phenotype in human.

3. Table 2 and 3 provides the genetic defects associated with cleft palate in mice. Does all these have a role in humans? Expand OMIM (given in table 1)

Response

Not all the molecules listed in Tables 2 and 3 are involved in CL/P in human. We revised Tables 2 and 3, and indicated genes associated with CL/P in human. The reviewer's suggestions have been added in the manuscript (page 6, line 4-5, page 8-9).

4. Although this manuscript describes the molecular mechanism of cleft palate in male, I feel a note on genetic changes studied in human cleft palate can be added since the studies in mice are forerunners for evaluation of similar changes in humans.

Response

We agree with the reviewer's comment. Recently, we also screened the human genetic disorders/syndromes associated with cleft palate and their disease genes. The investigation revealed 370 genetic diseases/syndromes listed in as cleft palate phenotypes with genetic loci and 154 genes had one or more syndromic or nonsyndromic CL/P (N.F., unpublished data). Since the result included elaborate lists of disease genes, we think that it is appropriate for the human study to be an independent research project.

5. Quote the source/ reference for the figures.

Response

Figures 1 and 3 are our original data and Figure 2 was modified with permission from Funato *et al. Hum Mol Genet.* 21 (11): 2524-2537, 2012. We quoted the reference in Figure 2 (page 67, line 2).

Reviewer 02618027

1. What are the environmental factors that contribute to CP formation?

Response

Teratogens that cause clefts include common environmental exposures, such as alcohol, smoking, infections, dioxin, estrogens, retinoic acid, and altitude (reviewed by Murray 2002). The literature and the reviewer's suggestion have been added in the manuscript (page 4, line 11-15, page 11, line 4-6).

2. Do genetic errors "prime", or make the tissue more susceptible, to environmental factors which might induce CP formation during palatogenesis?

Response

Among the genes associated with nonsyndromic CL/P, some disease genes (*IRF6, TP63, MSX1, PVRL1, FGFR1, TBX22*) are responsible for Mendelian forms of CL/P in human. Gene–environment interactions for non-syndromic CL/P are also reported. There are strong teratogenic effects of dioxins and retinoic acid in the mouse (reviewed by Murray

2002). The literature and the reviewer's suggestion have been added in the manuscript (page 4, line 11-15, page 11, line 4-6).

3. All abbreviations, including gene and protein abbreviations, should be spelled out the first time they are used.

Response

We revised the text accordingly.

4. The complexity of "genetically engineering mice with altered miRNA functions" should be addressed.

Response

We revised the text accordingly (page 10, line 16-18).

Reviewer 02618391

1. CP is a very common yet complicated syndrome in newborn infants, the causes are both genetic and environmental. As a review, the authors should provide readers a general view, including the every factors contribute to CP. The authors should include the discussion about environmental effects in the manuscript.

Response

The reviewer's suggestions have been added in the manuscript (page 4, line 11-15, page 11, line 4-6).

2. The authors discussed many gene deletions/modulations would cause CP, but how many of these genetic defect have been found in human? And which is (are) the most common reason(s) in human CP?

Response

As we stated in the reply to the Reviewer 1's #4 comment, 370 genetic diseases/syndromes listed in as cleft palate phenotypes with genetic loci and 154 genes had one or more syndromic or nonsyndromic CL/P (N.F., unpublished data). Among the genes associated with nonsyndromic CL/P, some disease genes (*IRF6, TP63, MSX1, PVRL1, FGFR1, TBX22*) are responsible for Mendelian forms of CL/P in human. We think that it is appropriate for the human study to be an independent research project.

3. As a min-review, the manuscript is too long, especially the references (234) should be optimized.

Response

We removed "a Minireview" from the title (page 1), and optimized the references.

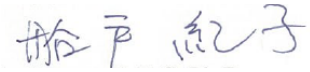
4. Abbreviations didn't give the full names at the first time.

Response

We revised the text accordingly.

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Sincerely yours,



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