

## Format for ANSWERING REVIEWERS



August 29, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 4056-review.doc).

**Title:** An inhibitor of HIF- $\alpha$  subunit expression suppresses hypoxia-induced dedifferentiation of human NSCLC cells into cancer stem cell-like cells

**Author:** Miho Akimoto, Hideko Nagasawa, Hitoshi Hori, Yoshihiro Uto, Yoshio Honma, Keizo Takenaga

**Name of Journal:** *World Journal of Medical Genetics*

**ESPS Manuscript NO:** 4056

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

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

(1) The English was edited by NPG Language Editing, LLC. Please see the attached recommendation letter from the editing company.

(2) According to the comments from the reviewers, we have shortened Introduction and Discussion. We have also reorganized the text and Figures so that the readers can understand a result by looking at one Figure. Therefore, Figs. 2, 3, 4, 8 and 9 in the previous manuscript have been reconstituted in the revised one. We have reduced the number of Figures to 9.

(3) Reviewer code: 00504391. Time-course gene expression results (Figure 4) should be shown also under normoxic conditions.

Reply: Time 0 in the time-course experiment results represents normoxia. We confirmed that there was no fluctuation in the gene expression under normoxic conditions. Therefore, we think the time-course experiment under normoxic conditions does not supply further information.

(4) Reviewer code: 00504391. Figure 5. Authors should show at least three different experiments and attempt to make statistical analysis.

Reply: According to the comment, we repeated the experiment three times and performed statistical analysis. The results are presented in Figure 5B in the revised manuscript.

(5) Reviewer code: 00504391. Authors should show more representative photos of pneumospheres (Figure 5B). They show a single pneumosphere for each case.

Reply: According to the comment, we replaced the photos to more representative ones, in which two or more spheres are present. The results are shown in Figure 5C in the revised manuscript.

(6) Reviewer code: 00504391. The “negative control” in Figure 6A shows clear fluorescence. Authors should describe in detail how was this control obtained. Is the antibody used for the other panels specific? Do they have additional information to make these results reliable?

Reply: Negative controls in the Figures 6A and B were performed by omission of primary antibody. We described this point in Materials and Methods. Immunofluorescence of negative controls is very weak and may be background staining. The immunofluorescence data coincides with Western blot data shown in Figures 6C and D.

(7) Reviewer code: 00504391. They claim that “These results indicate that at least HIF-1 is involved in the hypoxia-induced changes in expression of SP-C, CD133, and MSI1.” This statement is not necessarily true, the interpretation of the results is not correct.

Reply: According to the comment, we have changed the phrase “These results indicate that at least HIF-1 is involved in the hypoxia-induced changes in expression of SP-C, CD133, and MSI1.” to “These results indicate that HIF-1 induces changes in the expression of SP-C, CD133, and MSI1”. Accordingly, we have changed the heading.

(8) Reviewer code: 00504391. Legend to Figure 7 (A-C) makes the results confusing.

Reply: We performed these experiments under normoxic conditions and just compared the difference between with and without the compound. Therefore, we have changed the phrase to “ shown as fold change relative to control (control values set to equal 1)”

(9) Reviewer code: 02446236. As method of colony formation assay, authors describe “A549 cells were seeded in a 6-well plate at a density of  $5 \times 10^4$  cells per well in 2 ml of medium”. However, this method should be added “dissociating to single cells.”

Reply: According to the comment, we have added “dissociating to single cells.”

(10) Reviewer code: 02446236. Conclusion should be added in the text.

Reply: We have added Conclusion in the text.

(11) Reviewer code: 00225340. The work appears well-realized but perhaps should be more focused both in introduction and on data presentation.

Reply: According to the comment, we condensed Introduction and revised data presentation.

3 Typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Medical Genetics*.



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