

ESPS Peer-review Report

Name of Journal: World Journal of Medical Genetics

ESPS Manuscript NO: 4056

Title: An inhibitor of the HIF- α subunit expression suppresses hypoxia-induced dedifferentiation of human NSCLC cells into cancer stem cell-like cells

Reviewer code: 00504391

Science editor: Song, Xiu-Xia

Date sent for review: 2013-06-12 19:23

Date reviewed: 2013-07-14 09:41

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS TO AUTHORS

Akimoto et al. present an interesting paper on the expression and potential role of HIF in the hypoxia-induced dedifferentiation of lung cancer cells. Despite that some results are very interesting, the organization of the manuscript is very poor. Many organization mistakes can be found and some misinterpretations should be changed. The work requires major changes. Comments: 1. The English language should be revised by experts. 2. In addition, the authors might send the manuscript to an expert company helping them to organize the work. 3. The manuscript is too long, especially the Introduction but the whole manuscript requires major re-organization. 4. The manuscript is not properly organized. In some cases, it is necessary to look for 3 figures to observe a single result. For instance, see the next lines: "As described above, normoxic A549 cells primarily expressed SP-C as well as very small amounts of CC10 and AQP5 (Figure 1, Figure 3A). Upon hypoxic exposure, the expression level of these genes was greatly reduced in a time-dependent manner (Figure 3A, B and D, Figure 4A)." 5. Another example related to this issue is that Figure 2G is described in the section of experiments of Figure 5. 6. Time-course gene expression results (Figure 4) should be shown also under normoxic conditions. 7. Figure 5. Authors claim that gene expression results are representative of two independent experiments. Authors should show at least three different experiments and attempt to make statistical analysis. 8. Authors should show more representative photos of pneumospheres (Figure 5B). They show a single pneumosphere for each case. 9. Legend to Figure 5 is confusing. Authors describe Figure A and B as gene expression results but in the Figure panel B indicates pneumosphere photographs. Panel D is described in the text but no panel D is indicated in the Figure. 10. 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? 11. Authors transfected cells with HIF “to obtain more direct evidence of the importance of HIFs in hypoxia-induced changes”. 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. HIF expression can be induced by the presence of oncogenes and not only under hypoxic conditions. Authors should rephrase the sentence mentioning only that “these results indicate that HIF-1 induces changes in the expression of SP-C, CD133, and MSI1”. They also should change the heading “HIF mediates the hypoxia-induced changes in SP-C and stem cell-related gene expression in A549 cells”. Otherwise, authors should really demonstrate that at least HIF-1 is involved in the hypoxia-induced changes in expression of SP-C, CD133, and MSI1 by performing the gene expression experiments under hypoxic conditions in cells lacking HIF-1, for example by RNA interference. 12. Legend to Figure 7 (A-C) makes the results confusing. Authors mention that “Data are representative of two independent experiments and shown as fold change relative to normoxia (control values set equal to 1)”. Then, were these experiments performed under hypoxic conditions? If the hypoxia mimetics DFO and cobalt chloride were used, I would suppose that this was not the case. Then, why they compare with normoxic conditions instead of comparing only in the absence of the compound? Authors should make this point clear. 13. Another example of the poor organization is the following: “...treatment with 20 μ M TX-402 significantly suppressed the accumulation of both subunits while the levels of HIF-1 α and HIF-2 α mRNA were unaffected (Figure 1B and C).” Figures 1B and C do not exist. I suppose that they refer to Figures 2B and

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input checked="" type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS TO AUTHORS

Comments Authors describe an inhibitor of the HIF- α subunit expression suppresses hypoxia-induced dedifferentiation of human NSCLC cells into cancer stem cell-like cells. This article was highly evaluated because authors examine hypoxic effect on NSCLC cells in detail. However, authors had better revise it as below comments. 1)Introduction and discussion are too long. Therefore, authors' opinions are slightly vague. 2)As method of colony formation assay, authors describe "A549 cells were seeded in a 6-well plate at a density of 5×10^4 cells per well in 2 ml of medium". However, this method should be added "dissociating to single cells." or authors should refer other published articles 3)Conclusion should be added in the text.

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Title: An inhibitor of the HIF- α subunit expression suppresses hypoxia-induced dedifferentiation of human NSCLC cells into cancer stem cell-like cells

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CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input checked="" type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
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COMMENTS TO AUTHORS

This is an exhaustive experimental study about the effect of hypoxia and its molecular mediators on origin of cancer stem cells. The work appears well-realized but perhaps should be more focused both in introduction and on data presentation. Some data about pharmacological HIF inhibitors do not appear so persuasive. Some typed errors are present (i.e. Abstarct)