Reviewer's report

Title: Tanshinone IIA pretreatment renders free flaps against anoxia injury through upregulating stem cell related biomarkers in epithelial skin cells

Version: 1 Date: 30 June 2014

Reviewer: Rajkumar Tulsawani

Reviewer's report:

Major Compulsory Revisions:

Comment 1: What was the LC50 of cobalt chloride in epithelial skin cells used in the study? The LC50 data may be discussed in the manuscript.

Comment 2: In the title of their work, the authors claim that "Tanshinone 11a .... anoxia injury........". However, the authors only study effects of 50 µm CoCl2 which is hypoxia mimic and may not cause anoxia. Authors may support with the data on anoxic conditions after exposure to CoCl2. The data HIF1alpha may also be included in the study.

Comment 3: Authors must discuss rational for using 50 µm cobalt chloride to generate hypoxia/anoxia in cells in the manuscript.

Comment 4: Background section: authors say ....it is urgent to have more knowledge....solve it. Authors must add few lines on existing approved treatments for flap transplantation and their advantages and disadvantages to strongly support their hypothesis.

Comment 5: In background section, literature on role of #-catenin, GSK-3#, SOX2, OCT4, Nanog may be included highlight relation of studied markers to hypoxic/anoxia tolerance.

Comment 6: Under Materials and Methods, Real time PCR section, the primer sequences are mentioned for human genes while the study has been conducted in mice. The justification for same may be included in manuscript.

Comment 7: Under methods, immunohistochemistry of Nanong is mentioned in text but figure in missing in manuscript; VEGF in not mentioned in text but figure is included in study?.

Comment 8: Using CCK8 assay author conducted cell viability at different time points 24, 48, 72 and 96 h however trypan blue was conducted only at 72 h. Authors did not provide rational for omitting time intervals from Trypan blue assay.

Comment 9. Figure1 is highly confusing to follow. 1A three treatment groups are presented TSA- CoCl2 -, TSA- CoCl2 + and TSA+ Control+ however 1B and 1C only two groups are mentioned. Also, it is in appropriate to write hypoxia group
as Control+ Cocl2. Figure 1B graph is at different time interval however 1C shows single time point data. Uniformity, in experimental design and data presentation must be ensured or else proper justification may be provided for exclusive of data or duration. Further, significance values must be highlighted in each graph for more clarity.

Comment 10. Figure 4B significant values need to be highlighted in graph.

Comment 11. Authors have not studied CD34 in Epithelial Cells? Any specific reason for omitting CD34 from in vitro studies.

Minor Essential Revisions:

Comment 1: Controls in scientific study are baseline in studies and usually control are presented first and treatment groups are argued in comparison to controls. Authors have presented data of TSA group first followed by control in all figures. In my opinion, control data should be followed by TSA group data.

Comment 2: The manuscript is generally well written however grammatical errors need to be corrected. Some examples include:

2a. Abstract: ‘CCK8 assays’ replace with ‘CCK8 assay’ and mention ‘trypan blue assays’ as ‘trypan blue assay’.

2b. Introduction, para 1; line 6: “it is urgent....” author may consider revising to “it is important”

2c. Introduction, para 3; line 16: “different injuries including...............” consider using word various in place of different.

2d. Methods (isolation and preparation of epidermal cells): first line: dorsal skins of male BLABC mice. delete extra ‘s’ from skins.

2e. Animals and Ethics Statement: line 4: ‘All surgery was’.....replace with ‘The Surgery was’

2f. Results: Para3: line 4: ‘In the first day’...replace with 'On the first day'; line 6: 'In the third day' replace with 'on the third day'; line 8: 'In the fifth day' replace with 'On the fifth day'; line 10: 'In the seventh day' replace with 'On the seventh day'.

Comment 3: In Abstract, Author mention CCK8 assay however under methods and materials CCK8 is mentioned as kit. Is CCK8 a commercial brand name of kit, if then use method name here.

Comment 4: Authors have used ‘wild type’ to denote control group which may not be appropriate. It may recorded as untreated or control.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being
published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.