Reviewer's report

Title: Effects of Hyperbaric Oxygen on the Osteogenic Differentiation of Mesenchymal Stem Cells

Version: 4
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Reviewer: Rogeria Serakides

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The authors hypothesized that the effect of hyperbaric oxygen (HBO) on bone formation is mediated via increases in the osteogenic differentiation of mesenchymal stem cells (MSCs) which are regulated by Wnt signaling.

The effects of HBO on Wnt signaling and osteogenic differentiation of MSCs, mRNA and protein levels of Wnt3a, beta-catenin, GSK-3beta, Runx 2, as well as alkaline phosphatase activity, calcium deposition, and the intensity of von Kossa staining were analyzed.

The manuscript is interesting. But there are some methodological flaws that I consider to be important.

Major Compulsory Revisions

1) The authors do not sufficiently proved that the cells used in this study are stem cells. The phenotypic characterization of the cells is necessary. This characterization is important because the bone marrow contains mesenchymal stem cells (MSC), hematopoietic cells and fibroblasts. The International Society for Cellular Therapy stipulated that MSC must express markers such as CD105, CD73, and CD90 and others, and must not express hematopoietic cell markers.

2) The sample size (n) was not described in any of the tests. How many patients the cells were extracted? What is the age, gender and health status of each patient? The cells from each patient were separately evaluated? The cells from each patient were subjected to all treatments?

3) Why the periods of 1, 4 and 7 days were used for RT-PCR and western blot and 7, 14 and 21 days were used for the other analyzes? Seven days is sufficient for osteogenic differentiation in human cells? Authors should justify the difference between the periods studied for each test.

4) Why the groups I (induction), II (induction + HBO), III (Induction + HBO + siRNA) and IV (Induction + HBO + scrambled siRNA) were not evaluated in all tests and in all figures?

5) I did not understand why the authors in Statistical Analysis write “the results from three or four independent experiments”. Was not performed a single experiment? I suggest that the statistical analysis and statistical tests are described in detail.
Minor Essential Revisions

1) In the background, authors should explain the role of wnt proteins specifically on bone.
2) The Graph A of Figure 1 is not clear. The data are being compared to that group?
3) The asterisks in figures 5, 6 and 7 have been displaced.

Level of interest: An article whose findings are important to those with closely related research interests

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

No to all questions.