Reviewer's report

Title: The Hypoxic Microenvironment Upgrades Stem-like properties of Ovarian Cancer Cells

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Reviewer: Yuichi Hori

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Major Compulsory Revisions
(1) The authors attribute the stem-like properties to the expressions of Oct3/4 and Sox2. However, cancer stem properties indicate heterogeneity in the tumor, so authors should show CD144dim cells in the sphere and colony derived from CD144bright cells by immunohistochemistry and FACS.

(2) The authors suggested that the effects of hypoxic pretreatment on cancer cells depend on the increasing levels of HIF-1# and HIF-2#. However, the significance of these data in the present study is hard to understand since HIFs elevation under hypoxia is well-known. I believe that during the colony and sphere formation for 14 days under normoxia, hypoxic pretreated cancer cells utilize an independent pathway of HIFs.

(3) The authors should clarify the significance of hypoxic pretreatment of cancer cell lines from the viewpoints of physiological cancer biology.

(4) In vivo studies, such as tumorigenicity and metastatic ability, would strongly confirm this research.

(5) Under conventional normoxia condition, it is hard to understand that so many cells express CD44 and/or CD133, since these cell lines are probably homogeneous (In Figure 4, ES-2 data seems to be reasonable, that is all ES-2 cells are CD44 positive and CD133 negative independent of oxygen concentration).

Minor Essential Revisions
(1) To obtain accurate oxygen tensions in different incubators, it would be better to measure the oxygen tensions of medium directly because there could be a gradient of the oxygen tension in medium.

(2) In Fig.3D & Fig.5C, “colonies numbers” should be “colony numbers”.

(3) page13, line7, “are opposite than those in Fig1A” is incomprehensive expression.

(4) In Fig 4, I do not understand why isotype control shows CD44 positive and CD133 positive peak. I think FACS compensation was not performed adequately.

(5) The last paragraph in the Introduction is the same as Results in Abstract, rewrite briefly.

(6) Technically speaking, the spheres and colonies in the Figure seem to consist...
of a few thousand of single cells. How do you count 30 cells?

**Level of interest:** An article of limited interest

**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.