Reviewer's report

Title: YB-1 regulates Sox2 to coordinately sustain stemness and tumorigenic properties in a phenotypically distinct subset of breast cancer cells

Version: 1
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Reviewer: Matilde E. LLeonart

Reviewer's report:

Strengths:
This is an interesting paper that reports a novel link between Sox2 and YB-1 transcription factors.

Weaknesses:
The manuscript needs more testing of the results presented here. Most of the results are represented in form of graphs (mRNA data, reporter experiments...). In this sense, several Western-blots are missing and pictures of mamospheres and soft-agar are needed to verify the data.

Major points:

1) Describe more clearly the differences between the two cell populations RU and RR instead of refer to a previous paper from the authors (first paragraph of results section).

2) General Western-blot: Why the authors use the vinculin protein as the reference housekeeping protein instead of using b-actin?

3) Fig. 1A and 1C: Why ZR751 cell line has so different basal levels of Sox2 in the parental cells in comparison to the unsorted cells? Aparently these are the same cell populations.

4) Figure 3: Fig. 3A and 3B: Western-Blot of Sox2 is missing. Moreover, while in most Western-blots Sox2 appears as a single band: Why a double-band appear in Fig. 3C-3D?

5) Figure 4: The authors claim at the beginning of the manuscript that the siYB-1#1 is the best siRNA for inhibiting the YB-1 protein. Why they sometimes show the siYB-1#2 or even only the siYB-1#2 in some figures (Fig. 4A both, Fig. 6 both, Suppl. fig. 6 only siYB-1#2, etc...).

6) Figure 5: Fig. 5A results contrast with the results from Figure 4B. There is no details of how many experiments have been performed in figure legends or if this is the result of a single experiment done by triplicate, etc...Fig. 5B needs to show also results from ZR751 cell line as the whole figure does. Fig. 5D: ITGA6 western-blot is missing.

7) Figure 6: Pictures of mamospheres and soft-agar assays representative of the graphs are missing. Figure 6D needs to be accompanied by western-blot.
8) Akt, RSK1/2 and GSK3β kinases are multi-faceted kinases and the inhibitors used undoubtedly have a high cargo of non-specific effects (i.e. mutation of the Ser 102 aa of YB-1 can prove that the role of the inhibitors is abolished and can provide clues about its role in Sox2 expression.

9) Results section “YB-1 regulates the Sox2 reporter activity only in the RR cell subset”: The sentence “When we performed the same experiment...(Figure 4B and 4c). This is only applicable to ZR751 cells but not MCF7 cells as in MCF7 cells results are not significant.

10) Result section “YB-1 knockdown induces differential gene expression patterns in RU and RR cells”: First sentence “As we have demonstrated…” is only applicable to ZR751 cells.

11) Result section “Up-regulation of Sox2 and its downstream targets...”. Show the results from Suppl. Fig 5 in Figure 6. The manuscript compares the results of two cell lines in parallel.

12) How do you explain the controversy of your results with those from Fotovati et al., 2011? Do you only explain that by the cell type specificity? (i.e. did the authors try any glioblastoma cell line, etc...).

13) Discussion: The sentence “Specifically, following YB-1 knock-down stem cell genes NANOG and ITGA6...dramatically upregulated” is exaggerated.

14) Last paragraph of the discussion is speculative. It has to be supported by some experimental evidence or at least some references.

Minor points:

1) Describe with more detail the manospheres culture.

2) Describe more clearly in methods the differences among the cell lines used in the study.

3) Results section.“YB-1 negatively regulates Sox2 expression in breast cancer”: The last sentence of first paragraph is not clear “In both MCF7 and ZR751 cells...”

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests