Reviewer's report

Title: SLUG and Human Chorionic Gondadotropin Induce Breast Cells With "Stem Cell-Like" Properties

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Reviewer: Gianluca Storci

Reviewer's report:

In the paper of Bhat-nakshatri et al., Authors point out that SLUG plays a functional role in the breast cancer stem cell phenotype.

To support this statement, they report a series of interesting observations and experimental results. Overall, the conclusion that EMT and CSC are tightly linked, and that SLUG gene is likely to play a major role in such scenario seems to be appropriate and well supported by the data presented.

There are however some major and some minor concerns about the paper. In my opinion, their fulfilment may improve the quality of the manuscript:

Major concerns
1- a major role for HCG in CSC generation is claimed and reported even in the title, but then, throughout the ms, the data about this issue are scanty and albeit marginal. In particular, Figure 4A show a very low effect of HCG stimulation on the CD44+/CD24- phenotype.

2- In Figure 2 data presentation should be ameliorated in order to allow the reader to better understand that results. In particular, Figure 2A reports a WB analysis that is not sufficiently discussed in the text (meaning of Integrin 1, etc, see pages 11-12). In this regard, authors quote N-Cadherin but it is not clear to which data they refer to (may be those presented in Figure 3A?).

Moreover, the two panels of RT-PCR are clearly referred to and it is not clear why two panels of RT-PCR products are reported. Moreover, Authors report an increase of Notch-3 expression in CD44+/CD24- cells. Is this a result of the microarray? In any case Authors may refer to already published papers such as (Sansone et al., J Clin Invest. 2007 Dec;117(12):3988-4002) in which such gene is reported as a CSC gene. This may be also placed in the discussion section.

3- The effect of TNF alpha stimulation seems to be quite low compared to that of p65 over expression. Can the authors comment on it? Is the phenomenon due to timing or concentration? Can it be dependent on the long term exposure to TNF alpha?

4- Figure 5A. It is not clear what data the graph refers to (see Figure legend page 31 and text 16). Are these data reported already in Liu et al., 2007? Is this a
new analysis?

5- Figure 5D should report the timing of mammospheres formation and the total number of mammospheres /well not mammospheres /field;

6- Figure 6 on Gli over expression may be included as supplementary. Moreover, actin or 36b4 housekeeping gene should be included in panel C;

7- In various parts of the manuscript (eg, Introduction page 5, Results page 15, Discussion page 21) authors refer to a link between SLUG basal tumors and mammospheres. Such issue have been addressed in a previous paper (Storci et al, Journal of Pathology 2008) that may should be included and discussed throughout the text as it supports the findings reported in this paper;

Minor concerns:

1- Scale bars may be appropriate in Figure 1B and in Figure 5D;

2- Figure 2 should report the name of the cell line (MCF10A) above RT and WB panels;

3- In Figure 3B, EMSA should report the full name of the populations (since only CD44 or Cd24 is quite misleading). Though the experience of the authors in NF-kB studies is acknowledged, since that paper is likely to be read to people not very much acquainted with such techniques, the reviewer asks if the data may be presented in a more simple and detailed way;

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

**Quality of written English:** Acceptable