Author's response to reviews

Title: Sox2 suppresses the invasiveness of breast cancer cells via a mechanism that is dependent on Twist1 and the status of Sox2 transcription activity

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Author's response to reviews: see over
Authors' response to reviews (MS-1720807061945556)

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**Authors' response to reviews:** see over:
To: the BMC cancer Editorial Team

Re: Submission of MS-1720807061945556

We would like to thank the reviewers for their comments and suggestions. In response to these, we have made replies and appropriate modifications to the manuscript, as summarized below:

Reviewer: Qun Zhou

1. The manuscript by Wu et al reported that Sox2 suppressed the invasiveness of breast cancer. The authors demonstrate that downregulation of Sox2 transcription factor expression significantly increased the invasiveness of MCF7 cells. The general experiment design is reasonable.

Response: We thank the reviewer for her compliment.

2. The conclusion of this article is depended on a single cell line (estrogen receptor (ER) positive MCF7). More different ER positive breast cancer cells are necessary to confirm their conclusions.

Response: As suggested, we have examined the relationship between Sox2 and invasiveness in another ER+ cell line, namely ZR751. As shown in Figure 1A, siRNA knockdown of Sox2 in ZR751 also significantly increased their invasiveness (Figure 1A). Thus, the link between Sox2 and invasiveness is not specific to MCF7.

3. Sox2 is a key regulator for cell proliferation and maintenance of stem cell self-renewal. Published studies have demonstrated that Sox2 are expressed in ER positive and ER negative breast cancer cells. It is not clear why the authors only focus on ER positive breast cancer cells.

Response: In our previous study (Wu et al., Cell Signal 2012, 1989-1998), we had shown that Sox2 expression was detectable in 6 of 11 breast cancer cell lines (3 ER+, 1 Her2+, and 2 triple-negative). ZR751 and MCF7, both of which are ER+, expressed the highest Sox2 protein level. Thus, they served as the focus in our initial studies. As our long-term research objective is to define the roles of Sox2 in all subtypes of BC, we are currently generating various breast cancer cell clones (including those derived from ER-negative breast cancer cell lines) stably transfected with the Sox2 reporter.

4. “When the invasiveness of Sox2-active cells, Sox2-inactive cell and the unsorted Sox2R cells was compared, no significant difference was observed among these three cell population (Fig. 2A)”. It is not clear how to understand Sox2-active cells and Sox2-
inactive cells. The reviewer was confused by “Sox2 activity” and “Sox2-inactive” in this manuscript. The authors need to provide clear explanations for their experiments.

Response: We agree with reviewer that the use of the labels 'Sox2-inactive' and 'Sox2-active' can be confusing. Thus, in the revised version, we have re-labeled these two cell subsets as 'reporter un-responsive' (RU) cells and 'reporter responsive' (RR) cells, respectively. Specifically, we simply used the Sox2 reporter responsiveness as a surrogate marker, which we found correlated with different phenotypes, as detailed in our first paper in this field (Wu et.al., Cell Signal 2012, 1989-1998). The methods of how we distinguished the two cell populations based on their responsiveness to the Sox2 reporter is further elaborated in the Results section (page 7 and 8) as well as the Discussion section (Page 12).

5. Published studies already showed that Sox2 overexpression can enhance invasiveness in breast cancer. It is not clear why invasiveness remains no change in Sox2 overexpression cells in their model system.

Response: We have done our best to come up with possible explanations to this discrepancy (Page 10). We would like to stress that our results are highly consistent, as enforced Sox2 expression in MCF7 also did not result in any significant change in mammosphere formation and cell growth (Wu et.al., Cell Signal 2012, 1989-1998). In short, we have considered the possibility that the MCF7 cell clones used between the two laboratories may be different. We also have considered the possibility that the in-vitro invasiveness assays between the two laboratories have different characteristics. Last but not least, since the exact Sox2 protein level has been shown to be functionally important in ESCs, it is possible that the total Sox2 protein levels after gene transfection are substantially different between the two laboratories, and thus, leading to substantially different biological responses.

Reviewer #2: Alfonso Calvo

Authors have explored whether expression of Sox-2 in vitro (in the MCF-7 breast cancer cell line) is linked to cell invasion. They have found that siRNA targeting Sox-2 increases invasiveness in MCF-7 cells. However, when cell subpopulations were separated based on Sox- transcription activity, the invasiveness was only found in transcriptionally inactive Sox-2. In this case, Sox-2 binds to the Twist promoter.

1. Results are opposite to others described for Sox-2 in different studies (see references 37-39).

Response: We have acknowledged in the original version that our findings in contradiction with the conclusions of three published papers which correlated Sox2 expression with invasiveness in gliomas, melanomas and colorectal cancer (references 35-37 in the revised version). All of these 3 studies examined different
cancer types. Thus, cell-specificity is a possibility to explain for the discrepancy in our conclusions. Regarding the only study of Sox2 and invasiveness in breast cancer (reference 38), we have already provided a number of possible explanations (page 10-11 and replies to reviewer #1). We would like to point that at least one previously published study has similar conclusion as our (page 10, reference 39).

2. They have only used one cell line. Are these paradoxical results specific to this cell line?

Response: Please see our responses to reviewer Qun Zhou.

3. It seems very weird that Sox-2 mediated effects on invasiveness takes place precisely in cells with no detectable Sox-2 activity. What is the biological meaning of this? It makes no much sense to me.

Response: We agree with reviewer that the term 'Sox2-inactive' and 'Sox2-active' can be confusing. This point has been clarified as for reviewer #1.

4. Authors have not analyzed the expression in cells of Sox-2 by RT-PCR, so they cannot really state that Sox-2 is inactive. How much Sox-2 mRNA is present in the cells? The promoter experiment (sox-2 promoter driving GFP expression) is fine, but is there really Sox-2 expression? An RT-PCR would answer this question.

Response: The specific question regarding the Sox2 expression has been fully addressed in our previous publication (Wu et.al., Cell Signal 2012, 1989-1998). Sox2 protein is expressed in GFP-negative cells at a level comparable to that of GFP-positive cell. In addition, Sox2 is localized to the nuclei of GFP-negative as well as GFP-positive cells. As mentioned above, we have now labeled GFP-positive and GFP-negative as reporter responsive (RR) and unresponsive (RU) cells, respectively.

5. In my opinion, authors do not discuss enough the possible implications of these results, which are rather strange and in conflict with other published studies. The hypothesis of this study is not clear to me either.

Response: We would like to there is only one published paper regarding Sox2 and the invasiveness of breast cancer, which we have fully acknowledged in our manuscript. Regarding the comments about the implication of this study, we have the appropriate discussion in page 12 and 13.