Reviewer’s report

Title: The differential anti-tumour effects of zoledronic acid in breast cancer - evidence for a role of the activin signaling pathway

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Reviewer: Samaya Krishnan

Reviewer’s report:

Wilson C et al., in the present manuscript validated the differential response of ER+ and ER- breast cancer to zoledronic acid and implicate the activin signaling pathway in regulating this differential response. While the data presented in this manuscript is suggestive that the activin signaling pathway modulates response to ZOL, further evidence is needed.

Major compulsory revision:

To study the downstream signaling pathway of activin, the authors analyzed pSmad2/3C levels. However, the authors have not demonstrated that the pSmad2/3 signaling occurs in an activin-dependent manner (for which soluble activin receptor 2 could be used to inhibit the activin signaling pathway in ZOL treated cells). The authors also have not tested whether the transcriptional targets of pSmad2/3, such as p21, are regulated upon treatment with ZOL. Finally, it is also important to understand whether ZOL treatment would still sensitize the ER- cells in the presence of the activin signaling inhibitor molecule.

In figure 1, the error bars for the controls are missing. If the variations in the control sample were to be considered, would the results from the statistical test for significance still stand true? Would the depletion of follistatin (by siRNA) in the ER- cells cause an increased sensitivity to activin?

In figure 2, treatment of the cells with follistatin does not rescue the cells from activin mediated growth repression. However, the authors mention otherwise in the text. This needs to be clarified. And a possible explanation why follistatin fails to rescue the growth of the cells needs be included.

In figure 7, the method of quantification for the nuclear localization by immunofluorescence assay has not been described. If software was used, please mention.

In figure 7, while ELISA assays have been used for evaluating the signaling components, additional validation using western analysis would be highly informative and would strengthen their conclusions.

In figure 8, the authors performed a xenograft assay and show only the IHC data. Tumor volume/size data could be included.

A possible explanation for the following observations could be included in the
discussion-

a. In figure 7, the authors observed no change in total levels of pSmad2L upon ZOL treatment, however, in the xenograft assays, the authors observe a significant reduction in cells having pSmad2L upon ZOL treatment. An explanation for the discrepancy between in vivo and in vitro data could be included.

b. In figure 5B, the authors find that ZOL treatment reduced the ratio of follistatin to activin to be 4:1. However in the present study, this appears untrue.

Figure 6B could be replaced with a better quality image

Discretionary revision:

Figure 1-3 seem unessential. The focus of the manuscript could be narrowed to solving the mechanism of differential response of ZOL in ER+ and ER- breast cancers. Figures 2, 3 do not appear to add more information than what is already known in the literature or contribute to the conclusions made in this research paper.

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:

I declare that I have no competing interests.