Reviewer's report

Title: CA1 Contributes to Microcalcification and Tumorigeneic Process of Breast Cancer

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Reviewer: Rajini Rao

Reviewer's report:

This is an interesting and potentially significant study on the role of carbonic anhydrase I in breast cancer and microcalcifications. Several novel approaches are used, including a biomineralization assay. However, the link between CA1 and calcification is correlative in the absence of knockdown and inhibitor studies.

Major Compulsory Revisions:

1) Although there is a correlative link between induction of CA1 transcript and biomineralization, causality is not established. Knockdown experiments on 4T1 cells should be performed to evaluate the role of CA1 on calcium deposits. What is the effect of using the CA1 inhibitor acetazolamide on the 4T1 cells (the authors refer to the effect of this drug on a different cell line Saos-2 in a previous publication). Surprisingly, the authors only perform CA1 knockdown in MCF7 cells, which were not evaluated for calcium deposits. This disconnect is a major logical flaw in the study.

2) CA1 knockdown in MCF7 cells is largely ineffective and should be improved. How much does “considerably reduced” mean (line 375, pg 18)? Please specify in Results what positive and negative controls mean in this experiment. Also, x and y axis labels in Figure 8 need clarification and relabeling. Scientific notation may be better for y-axis, or use a log scale or % of control. In light of the weak knockdown, it is difficult to interpret significance of growth data that show no difference.

Major Essential Revisions:

1) In Results, please begin with an introduction of the SNP under evaluation. Where is it located (intron, coding region?), and what are the predicted consequences if known (expression or activity changes). How was this specific SNP chosen for analysis among the many (29) CA1 SNPs previously reported in Table 1 of Chang et al. (Arthritis Res. Ther. 14:R176; 2012).

2) Figure 9: did you forget to label the bracketed columns?

3) Figure 10: Axes need labels.

4) Figure 11: Panels have no labels (A,B, C, etc.)

5) Overall, figure quality needs to be improved. Labels should be in uniform font and legible, with correct spacing and capitalization. Typographical errors should be corrected throughout (e.g., Fig 12, replace “alternations” with “alterations” and
“relative” with “relative”.

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

**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.