Reviewer’s report

Title: Estrogen regulation of TRPM8 expression in breast cancer cells

Version: 1 Date: 21 December 2009

Reviewer: Luis A Pardo

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This manuscript describes the expression and function of TRPM8 channels in MCF7 cells and its dependence on estrogens. I feel that the article can be substantially improved if functional data after estrogen treatment is included. The observation that, in tumor samples, TRPM8 expression correlates to ER status is very interesting and I think it also deserves some more attention.

Detailed comments

Discretionary revisions
1. Are there any changes in current or Ca signals on icilin application in estrogen-treated cells with respect to starved cells? In other words, since the frequency of TRPM8 expression is moderate, does estrogen control the fraction of cells expressing the channel, or the amount of channels per cell?

2. It should be easy to perform Cox proportional hazard analysis to determine if TRPM8 expression plus ER status is more predictive than ER status alone.

Minor essential revisions
1. First line of the abstract: melastatin.
2. Is colon tumor the best positive control for TRPM8?
3. The quantification of PCR data (other than real time PCR) is not too reliable. This can play against the authors when trying to interpret, for example, how a 50% knockdown of ER justifies a reduction of the same magnitude in TRPM8 message. Is there no safety margin for ER expression? I would recommend keeping in qualitative terms.

4. 64.5% of 26 cases are 16.77 cases. Please check the figures.
5. Please give siRNA concentrations.
6. Can you be more explicit when describing immunohistochemistry protocols?
7. Please rephrase the sentence describing patch pipette production (it reads as if the starting capillaries were 3-5 M#).
8. In the description of the statistics, statistical significance (p value) and graphical indication of significance are mixed.
9. Can you reduce the line thickness in Figure 2? It looks too crowded the way it is now.
10. What do you mean by TRPM8-expressing cells? Were they selected somehow?

11. What do you mean with the last sentence of the Results section?

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests