Reviewer's report

Title: Estrogen regulation of TRPM8 expression in breast cancer cells

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Reviewer: Roland Schoenherr

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In this manuscript the authors present data on the expression of TRPM8 cation channels in breast cancer cells and the transcriptional regulation of TRPM8 by estrogen.

In general, the knowledge of ion channel expression in cancer cells and of putative roles in cell-cycle progression and potential therapeutic consequences is still at an early stage. Therefore the presented study is of value for a broad readership with interest in mechanisms of cancer formation. The authors present convincing electrophysiological evidence for the functional expression of TRPM8 channels in MCF-7 breast cancer cells. They show that icilin, as a chemical stimulator of TRPM8, induces a Ca2+ influx through the plasma membrane, whereas no release from the ER was triggered by icilin. Analysis of mRNA from MCF-7 cells after estrogen deprivation or upon stimulation with 17-beta estradiol provided evidence for a transcriptional upregulation of TRPM8 by estrogen. In line with this finding, the expression level of TRPM8 in breast cancer tumoral tissue was found to correlate with the expression of the estrogen receptor.

Despite the certainly interesting findings, Major Compulsory Revisions are required and should address the following points:

I) The expression analysis in MCF-7 cells is a key part of the presented work, but the experimental approach based on endpoint PCR and gel analysis is not really sufficient for a reliable report. In this respect, the used presentation of percent values with two decimal places for mRNA regulation appears incautious. I strongly recommend to repeat this analysis using a real-time PCR approach or other appropriate means.

II) The authors made no attempts to test whether changes in TRPM8 mRNA levels affect the observed Ca2+ currents, as must be expected. E.g. the quantitative comparison of mean currents in estrogen-treated cells versus estrogen-deprived cells should be shown.

III) The quantitative interpretation of RT-PCR signals for a cell line is almost impossible without a comparison. Unfortunately, PCR amplification with 40 cycles is prone to yield false-positive results. In other words, it is quite common that PCR signals can be amplified for a number of different ion channels, without any functional correlate. The data would be much more convincing if PCR data for other cell lines would be included for direct comparison. E.g. the prostate cancer cell line LNCaP might serve as positive control.
Level of interest: An article of importance in its field

Quality of written English: Acceptable

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