Reviewer's report

Title: Dermcidin exerts its oncogenic effects in breast cancer via modulating ERBB signaling

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Reviewer: Paraic Kenny

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Markovic and colleagues report the distribution of DCD levels in a series of breast cancer patients and perform functional studies in breast cancer cell lines which indicate that DCD may play a role in tumor growth and cell survival. The data show that altering DCD levels affects the expression and activity of various ERBB pathway components. Although the authors show that ERBB activity is modified they do not demonstrate, as claimed in the title, that the effects of DCD in breast cancer are due to its modulation of ERBB signaling.

Major compulsory revisions:

1. The novelty of the DCD-SV splice variant identified by the authors should be clarified and, if novel, the sequence should be included as supplemental data. After describing the splice variant they identified by RTPCR, they state that an isoform identified by another group, DCD-SV-2 “is similar to the DCD-SV sequence identified in our study”. The degree of similarity should be stated. If these isoforms are actually identical, then this section of the manuscript should be rewritten appropriately.

2. DCD expression was measured in 600 primary breast cancer cases by immunohistochemistry but only 26 of these (4.3%) were included in the analysis. It is difficult to understand why ~95% of the analyzed cases would be excluded from the analysis. Clearly, it is impossible to generalize when the vast majority of cases are excluded.

3. Fig 3. The numbers of tumors contributing to the observation of reduced DCD and increased CK18 levels in Fig 3D should be specified as only a single micrograph is shown. An appropriate statistical test should be used to analyze the data. Similarly, a single micrograph of one tumor from one of three shRNA lines generated is used to make the claim that EGFR levels are reduced in DCD suppressed tumors (Supp Fig 4).

4. The authors demonstrate effects of DCD on cell survival and tumor growth, and also on activity of the ERBB pathway but no mechanistic link is made between these sets of observations. In the absence of more experiments, it is not reasonable to claim that the observed effects of DCD on tumor phenotypes are mediated by the ERBB pathway.

5. The xenograft experiment with MCF7 cells and DCD overexpression
mentioned as data not shown should be included in the manuscript.

Minor essential revisions:

1. The reasons for the choice of RMA threshold cut-points for HER2, HER3 and DCD should be explicitly stated and justified (page 9).

2. Fig 2E,F,G and associated text. The effect of DCD1 on cell survival in the presence of H2O2 is much more pronounced than the experiment with staurosporine and the effect on survival in the presence of TNFα was only observed at the highest of five concentrations evaluated. The difference in the degree of protection against these stresses should be noted in the results section. Is the highest TNFα concentration used physiologically relevant?

3. The precise meaning of the phrase “Metacore software predicted higher connectivity among the genes within the EGFR signaling canonical pathway” should be clarified for those unfamiliar with this analytical approach.

4. Comparing Figs 5A and 5G, it seems that NRG3 is induced by both DCD1 suppression and by stimulation with recombinant DCD. The authors should comment on this.

5. Fig 5B is missing the x-axis labels.

6. The concentrations of DCD used in Fig 5C and the effect sizes observed at these concentrations do not match the description of these data in the results section.

7. Fig 4C contains far too much extraneous information for the point the authors wish to make about changes in the levels of AREG, EGFR and BTC. The authors should either redraw a more simple diagram, or provide a comprehensive legend to describe all of the shapes, colors and arrows in this figure.

8. The legend of Fig 2(A-D) does not match the number of panels in this figure (A-G).

9. The legend of Fig 3 describes measuring CK5 expression but this is not shown on the figure.

10. The legend of Fig 4 refers to dendrograms but no dendrograms are shown. Perhaps “Heatmap” is intended? The colors are not described correctly. A scale bar of color intensity should be added to this figure.

12. The authors claim that Fig 2A shows that MDA-MB-361 cells expressing DCD shRNA “displayed a more differentiated luminal epithelial cell phenotype compared to the control cells”. It is not clear how this particular conclusion is drawn from these data.

**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