Reviewer's report

Title: Calcitriol restores antiestrogen responsiveness in estrogen receptor negative breast cancer cells: A potential new therapeutic approach

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Reviewer: Filippo Acconcia

Reviewer's report:

In this paper the Authors studied the effect of calcitriol and one vitamin D analogue for their ability to restore ERalpha expression in ERalpha negative breast cancers and breast cancer cell lines. In this way, the Authors propose these 'novel' ERalpha positive breast cancer cells become sensitive to anti-estrogen therapies.

Although the idea proposed in this manuscript is intriguing I have many concerns that preclude me to positively judge this work.

Major Compulsory Revisions:

A-It is not clear why one should treat ERalpha negative breast cancer with molecules that will re-express ERalpha and thus, in theory, restore estradiol signalling in breast cancer.

As a matter of fact, one could argue that ERalpha positive tumours are indeed treated with drugs that induce ERalpha degradation (i.e., 4OH-tamoxifen; fulvestran). Thus a therapy that would give the opportunity to cancer cells to acquire a selective advantage in cell growth because of the ERalpha presence and than to treat them with anti-estrogens appears to be a little convoluted. Moreover, if ones put this work into a clinical perspective, women that undergo surgical removal of the breast tumour still have their ovaries functional and circulating estradiol levels. Thus, one may expect that re-expressing ERalpha in the remaining cells would have a detrimental impact for the patient, who will further undergo treatment with many drugs (i.e., calcitriol and anti-estrogens) and consequently many possible side effects.

The Authors should clarify these points addressing them with a specific experimental design that would highlight benefits of such potential therapeutic approach.

B-I found the presented data too preliminary. In this regard, my main problem is with the conclusion that calcitriol restores ERalpha signalling and functionality. In details:

1: More than one ERalpha negative breast cancer cell line is required to make a general conclusion in order to exclude that it is a type-specific effect.

2: In figure 4, the authors did not measure cell growth in response to estrogens because they used a test that assays the cellular metabolic activity. Can the
Author explain why re-expression of ERalpha does not lead to an increase in cell metabolism or in ‘cell growth’? The Authors should perform growth curves analysis as well as BrdU incorporation to directly connect with cell proliferation.

3: In figure 3, one gene is not enough to make the statement of re-acquisition of estradiol sensitivity and ERalpha re-activation. pS2/Tiff (presenelin-1) and cathepsin D could be additional candidates. ERalpha phosphorylation in the serine residue 118 is a better marker of ERalpha transcriptional activation. Furthermore, the same experiments should be performed in the presence of VDR inhibitor and siRNA for VDR to demonstrate that the effect of calcitriol on re-activation of ERalpha is via VDR.

4-In figure 1 and 2 VDR knockdown is required.

5-In general, standard deviations are very high to make any conclusion. Additional experiments should be performed to have a higher number of data to analyze.

Minor essential revision:
1-Supplemental figures should be put in the main text.
2-In figure 2 standard deviations and p values in densitometric analyses should be inserted.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.