Reviewer's report

Title: ESR1 Gene Promoter Region Methylation in Breast Cancer Patients: Correlation with Tumor Hormone Receptor Status and Luminal Phenotypes

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Reviewer: Luca Magnani

Reviewer's report:

In the submitted manuscript Joaquina J Martinez-Galan and colleagues set up an experimental plan to examine ESR1 promoter methylation in peripheral blood cells (pbc) of breast cancer patients. The goal was to determine the extent of correlation between pbc ESR1 methylation and ERalpha status in primary breast cancers.

The authors found significant correlation between methylation status and ERalpha status: when ESR1 promoter was methylated, ERalpha was not found in the primary and vice versa. In addition, the authors observed a decrease in ESR1 promoter methylation in pbc in luminal ERalpha positive breast cancer compared to ERalpha negative patients.

Although the study design is interesting and the results moderately novel, this reviewer has major concerns about this manuscript. These concerns must be fully addressed in order to reach a final decision about publication.

Major Compulsory Revisions:

1- The manuscript is poorly written. This reviewer had serious difficulties in following the prose, understand the methodology and comprehend the results. One example is found at page 10 "The correlation of ESR1 non-methylated promoter in...". The entire paragraph is convoluted and the results appear contradictory: "As such, in our series of cases, the non-methylated ESR1 promoter in pbcDNA was correlated with the presence ER(+) in tumor tissue. We did not observe correlation between non-methylated ER in pbcDNA and ER+ in tumor tissue". Similar examples are found throughout the text. Altogether, the authors should reformat the manuscript, streamline and simply the prose and communicate the results in an ambiguous way.

2- The authors fail to mention until the very end of the discussion the rationale of their design. PBC cells do not express ERalpha hence the promoter should be methylated. I assume their results imply that difference in ESR1 promoter methylation observed in pbc is accounted for by difference in the nature of tumor circulating cells. However no reference is provided and there are serious concern about the results. Indeed, the authors report that only 28% and 36% of Luminal A and Luminal B patients have pbc with ESR1 methylated. This number seems too low considering the limited amount of tumour circulating cells in the plasma.
Could the authors describe in better details how they prepare the pbc cells? Do they enrich for tumour circulating cells?

3- It is not clear what is the real advancement in the field. The author states in page 12 that “In our literature search, we did not find any studies that correlated the epigenetic profile of methylation and its relationship with ER expression status”. This statement leads this reviewer to think that the authors perform their search quite superficially. Here few examples of previous work that looked specifically at the DNA methylation profile of ESR1 promoter in breast cancer tissues:

Gaudet 2009. DNA hypermethylation of ESR1 and PGR in breast cancer: pathologic and epidemiologic associations.

Prabhu 2012. The epigenetic silencing of the estrogen receptor (ER) by hypermethylation of the ESR1 promoter is seen predominantly in triple-negative breast cancers in Indian women.

Ramos 2010 Simultaneous CXCL12 and ESR1 CpG island hypermethylation correlates with poor prognosis in sporadic breast cancer.

Maybe the author stated that there are not studies that linked pbc ESR1 methylation and ERalpha status? What is the benefit of their approach compared to normal histo-pathological staining?

4- The statement “Hence, it appears that the molecular variant of the promoter of the ESR1 gene….” Page 13 is not supported by any results. Table 5 is missing.

5- The final conclusion “The results are promising in terms of early diagnosis, monitoring of response to treatment as well as a prognostic factor predictive of response” Page 14 is a gross overstatement not supported by any data.

Overall the manuscripts result could be of relative interest in discussed in their context. However the data are poorly explained and appears to be over-interpreted. This reviewer would take into consideration to review an extensively revised version of the paper. The author should engage a professional editor or a native speaker collaborator to help revise the manuscript. At this stage I would not recommend this manuscript for publication.

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

Quality of written English: Not suitable for publication unless extensively edited.

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