Reviewer's report

Title: HER2 expression sensitizes breast cancer cells to apoptosis by PEITC in vitro and in vivo

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Reviewer: Jin-Rong Zhou

Reviewer's report:

Major compulsory revisions

1. The fundamental problem in this study is that the in vitro studies showed PEITC had potent anti-proliferative activity against the parent MCF-7 and MB231 cell lines which are HER2-negative or silenced, which clearly does not support that HER2 is an important target for PEITC. Moreover, the authors reported pretty high HER2 expression in the parent cell lines.

2. Some experiments are not well designed. In the experiments related to Fig 3 and 4, the vector control cell lines should be used in the experiments for comparison with the HER2-overexpressing cell lines. In addition, at least two colonies of the paired control and manipulated cell lines should be used for function assays.

3. Because of the lack of the appropriate control groups, most conclusions are not supported by the results. For example, the authors concluded that HER2-overexpressing cells were more sensitive to PEITC, but the results (cell proliferation data) could be simply due to cell manipulation effect.

4. Since the stable HER2-overexpressing cell lines are available, it is unclear and seems unreasonable to use transient overexpression to determine the effect of HER2 overexpression on apoptosis-inducing activity of PEITC.

5. Most results of biomarker analysis in figures 2 and 3 are based on qualitative western blot images, but not quantitative results. In addition, the comparison between figure 2 (the parent MDA-MB231 and MCF-7 cell lines) and figure 3 (the Her2-overexpressing cell lines) does not show dramatic effect of HER2 overexpression on modulation of these biomarkers, thus does not seem to support the conclusions made by the authors in the related parts in results and discussion sections.

6. Similarly, the parallel animal study using the vector control MDA-MB231 cell line should have been performed.

7. Many places in the manuscript are overstated. Most times, the results only “suggest”, but not “demonstrate”, “support” or “indicate” ... Also, “significant” should not be used if the results are not statistically analyzed or the p value is >0.05. Particularly, the last paragraph in the discussion and the conclusion are
the most overstated ones.

Minor essential revisions

1. All abbreviations need to be spelled at the first time.

2. Annexin-FITC assay: the concentrations of annexin V and propidium iodide should be provided, and the composition of binding buffer should be given.

3. Western blot analysis: the CHAPS buffer composition should be provided; the antibodies used should be given detailed information, such as the vendor, type, and dilution.

4. Was MCF-7-HER2 cell line provided by Fei (as described in cell culture) or Fei and Reeves (in acknowledgements)?

5. Fig 2D: the images of nucleus and overlay in the treatment group may be switched.

6. The statistical analysis results should be presented in a consistent way (either the letter or *).

7. The literature review is not up to date. There are several PEITC and breast cancer related publications in the past years.

8. HER2 silencing using siRNA: the sequencing of the siRNA and the dose used should be provided, and the control siRNA should be used.

9. For the combination studies, it is essential to determine the dose-dependent combination effect, and the nature of combination.

Discretionary revisions:

1. The data of PEITC and doxorubicin are very weak and very preliminary.

**Quality of written English:** Needs some language corrections before being published

**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.