Reviewer's report

Title: Protein Secretion in Human Mammary Epithelial Cells following HER Receptor Activation: Influence of HER2 and HER3 Expression

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Reviewer: Silvia Carvalho

Reviewer's report:

The acceptance of the importance of the HER family of receptors, in the development of numerous types of tumors has populated the medical literature over the last years. Although from a clinical point of view this article can bring new insights to the further knowledge of the receptors involvement in protein secretion there are some conceptual issues that require the authors’ further attention.

-To start the single use of EGF for stimulation does not allow any insight to the possible roles of the ligands family. As HER3 is also a target for scrutiny in the present paper the authors should have also addressed stimulations with HER3 specific ligands in order to validate the results encountered.

-In the continuation of the previous comment the title, abstract and introduction should be adequately changed as only when getting to the discussion part the authors explain why was EGF used (1st line of discussion). Furthermore in the results section a high relevance is given to ligand shedding and also of the involvement of PI3K and MAPK signaling pathways, and this idea is not reflected in the abstract.

-Although correct, the authors should have also included some western blots to make the paper a little bit more appealing to the reader… there are too many graphs. Some suggestions would be a WB showing the expression levels of the HER members in the different cell lines generated and also in the assessment of p-AKT and p-ERK.

-The used cell lines mimic to some extent the SKBR3 breast cancer cell line model. Did the authors test SKBR3 to determine if the some results are encountered?

-Also the authors should provide a brief explanation has to why HER1, 2 and 3 were chosen for the study and HER4 was not.

-Throughout the paper the link between HER family, cytokines and angiogenic factors is a bit confusing as both cytokines and angionenic factors expression have also been associated with several other families and are not exclusive of HER family. The relevance of the study of this for the present paper should be emphasized and better linked with HER family members.

-The authors should provide some explanation as to why HER2 and HER3 expression attenuates MMP9 secretion when induced by HER1 activation; also
what other factors besides cellular levels of TGFa might influence the ligand accumulation in CM (last paragraph of the subtitle HER2/HER3 increases AREG and TGFa expression and shedding – pag 14/15)? Also why didn’t EGF consumption occur in the parental cell line?

**Level of interest:** An article of limited interest

**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