Reviewer’s report

Title: Autocrine Regulation of Cell Proliferation by Estrogen Receptor-alpha in Estrogen Receptor-alpha-positive Breast Cancer Cell Lines

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Reviewer: Stanislaw Sulkowski

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In the interesting article ER antagonist was shown to abolish significantly Ki67 expression, which was consistent with ER downregulation and arrest of cell cycle in G1 phase in these cell lines. Growth arrest was reversed by elimination of antagonist and introduction of estrogen in cell medium. Authors presented an evident association of increased ER expression with cyclins. Thus, authors provide a detailed description of estrogen dependence of breast cancer growth on the ground of cell lines. Generally, their conclusion was not new and had been drawn pretty long time before, though.

Major Compulsory Revisions:

Authors found co-expression of ERalpha and Ki67 in breast cell lines MCF-7, T47D and ZR75-1, but the paper contains information which requires explanation, for example:

In results section of abstract: “Unlike that in the normal mammary glands and the majority of primary breast tumors, ERalpha is highly expressed throughout the cell cycle in MCF-7 cells”. On contrary to authors’ opinion, approximately 70% of primary breast cancers express ERalpha (Yamashita H. Current research topics in endocrine therapy for breast cancer. Int J Clin Oncol. 2008 Oct;13(5):380-3.). The survey was confined exclusively to breast cancer cell lines. Cellular co-localization of ER alpha and Ki-67 was assumed to suggest an autocrine mode of cell proliferation in these ERalpha-positive breast cancer cell lines. It is an interesting and highly probable supposition but certainly it is not a definite settlement as long as such co-location does not exclude the other modes of stimulation as well.

Authors claim (page 10 results) that autocrine mode in these ERalpha-positive breast cancer cell lines is an absent mechanism in normal mammary cells and most primary breast tumors. If so, they could provide references for this opinion.

Page 14, discussion: “Inferred from the paracrine model is that ERalpha-positive cells are a separate sub-population of cells that don’t proliferate, or even further that ERalpha-positive cells cannot proliferate because ERalpha inhibits the proliferation of ERalpha-positive cells.” - Can the authors refer to any report to support this astonishing opinion of discussion?

Page 7: “For estrogen stimulation after removal of ICI182780, cells treated with ICI182780 were washed briefly two times with serum-free phenol red-free
DMEM/F12, followed by replacement with medium containing 5nM estrogen. The control cells were replaced with medium with vehicle. For estrogen stimulation in the presence of ICI182780, ten fold of E2 (100nM) was added directly to MCF-7 cells in 10nM ICI182780.” If the primary concentration of estrogen is 5nM, how is it possible that, ten fold of E2 equals 100nM?

Experiments with EGFR inhibitor Gefitinib are badly described and remain unreliable in such a form. It is necessary to refer clearly to exact photos of figure 6A. Authors should explain the sequence of ICI 72hrs – gefitinib 2hrs – E2 16 hrs. Was ICI removed from the cell culture before prior to exposition to gefitinib? Was gefitinib eliminated before estrogen stimulation? If gefitinib was removed after 2 hours of stimulation, were 16 hours of estrogen stimulation enough to overcome inhibitory effect of gefitinib? What time was phosphorylation of ERK1/2 measured- was it detected directly after 2 hours of inhibition with gefitinib or maybe after 16 hours of stimulation with estrogen?

Authors do not avoid language errors e.g. co-colalized.

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

**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.