Reviewer's report

Title: Estrogen-mediated inactivation of FOXO3a by the G protein-coupled Estrogen Receptor GPER

Version: 2 Date: 28 April 2015

Reviewer: Yoichi Mizukami

Reviewer's report:

The authors have studied GPER, an estrogen membrane receptor that they discovered it, and about which are the leading researchers in the field. In this study, FOXO3a activation, as a candidate for a downstream factor involved in GPER activation, was examined, and the translocation of FOXO3a from the nucleus to the cytoplasm through GPER was observed in the presence of E2 or G-1. The inactivation of FOXO3a, a transcription factor related to pro-apoptotic genes, may be involved in the inhibition of apoptotic cell death in breast cancer cells. The manuscript includes very interesting data. However, one reviewer feels that there are several points that the authors should consider.

Major Compulsory Revisions

G-1 induced the translocation of FOXO3a at a concentration of 1 nM, which is quite low as compared with other papers. The fear is that G-1 may induce translocation through a factor other than GPER. The authors should confirm the activation of GPER at the concentration of G-1.

The translocation of FOXO3a was induced within 5 min after stimulation. The authors explain that EGF released from MCF-7 cells during GPER activation by E2 or G-1 activates PI3K and Akt through EGF-R, which induces the inhibition and translocation of FOXO3a. One reviewer feels a question as to whether these signaling molecules are really activated within 5 min. The authors should exclude the possibility that E2/G-1 and EGF work independently of FOXO3a using RNAi for EGF-R and EGF.

Minor Essential Revisions

Sisci et al show that ER# regulates the activity of FOXO3a in breast cancer MCF-7 cells, but ER# has no effect on translocation in MCF-7 cells in this study. Can the authors explain this discrepancy based on the evidence? In general, cancer cell lines often undergo changes in gene expression during cell culture. If other breast cancer cell lines give similar results, the confidence level of the data presented here would be increased. It is requested that when results are obtained that conflict with those in previous reports, the new data be carefully confirmed.

Discretionary Revisions

In the Introduction, the authors state that FOXO3a activity was examined in
“Introduction”, but there are no data for FOXO3a transcriptional activity in E2 or G-1, although translocation was observed. Please determine the transcriptional activity by G-1/E2, or confirm the degradation of FOXO3a in the cytoplasm.

Caspase activity was determined in Fig.7, but the relationship between caspase activity and FOXO3a remain unknown. Please show that the degradation of FOXO3a using RNAi leads to the inhibition of caspase activity, and that the inhibition is recovered in the presence of E2 by transfecting FOXO3a.

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

no