Reviewer's report

Title: OSU-03012 sensitizes breast cancers to lapatinib-induced cell killing: a role for Nck1 but not Nck2

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Reviewer: Chang-Fang Chiu

Reviewer's report:

In this submitted manuscript, estrogen receptor (ER) positive and ER negative breast cancer cells were genetically manipulated to up- or down-regulate either eIF2-alpha or its phospho-mutant, Nck1, Nck2, then treated with OSU-03012, lapatinib or the combination and assayed for cytotoxicity/cytostaticity using clonogenic assays. Based on the data presented, it is concluded that OSU-03012 and lapatinib act synergistically to induce cell death via the down-regulation of Nck1/PP1 and the subsequent dissociation of this complex from eIF2-#, which likely leads to a PP1-mediated enhancement of eIF2-# phosphorylation at serine51, a central event in the induction of cell death by OSU-03012/lapatinib.

Major Compulsory Revision:

1. The author concludes that eIF2-# phosphorylation is a central event in the synergistic cytotoxicity/cytostaticity induced by the combination therapy of OSU-03012 and lapatinib. However, in Fig.3C, the p-eIF2-# levels in drug treated vector or S51A over-expression cells are not significantly different, but the decrease of colony formation induced by OSU/lapatinib treatment is significantly attenuated in S51A over-expression cells (Fig. 4D). Whether it involves ER transmembrane proteins other than PERK- eIF2-#, i.e. IRE1 and ATF6, needs further clarification. Experiments using siPERK, siIRE1-#and siATF6 may be one of the possible approaches.

2. It has been widely accepted (in detail: Biochem Pharmacol, 2013, 85:653; Can Res 2012, 72:1321; Ann N.Y. Acad Sci 1271 (2012) 20-32) that, while proliferating in sub-optimal microenvironment (hypoxia, hypoglycemia, etc), chronic ER stress and permanently increased levels of GRP78 expression provide a survival advantage to tumor cells. This phenotype sets tumor cells apart from most normal cells. It shouldn’t mean that cancers cells are more resistant to ER stress. In contrast, the already engaged ER stress response system in cancer cells provides promising druggable targets. One important approach is development of pharmacological ER stress aggravators (ERSAs). OSU-03012 is one of the ERSAs that may pharmacologically aggravate the already engaged ER stress in cancer cell, which can be used to “overload” this engaged system and thus push the cell from pro-survival state to its pro-apoptotic module. I would suggest integrate this information into the discussion, such as p.11 the last paragraph.

3. In the Results and Discussion (p.12, 2nd paragraph), the author mentioned “
However, autophage ….., whereas in breast cancer cells the role of autophage seems to be more protective.” However, the cited references describe quite different study models, which is not necessarily the mechanism of sensitization of breast cancer cells to lapatinib-induced cell killing by OSU-03012. Looking into the expression of autophage related proteins, such as conversion of LC3-I to LC3-II and altered expression of p62, will be more direct evidences to support the conclusion.

Discretionary Revision:

In p.13, paragraph 2, line 2: The decrease in both clonogenic capacity and eIF2-# phosphorylation in MDA-MB-231 cells after OSU-03012 and lapatinib combination treatment was “rescued” by the ectopic expression of Nck1 (see Figure 4C), but not by ectopically expressing Nck2.

To my understanding, it should be : The increase in eIF2-# phosphorylation and decrease in clonogenic capacity in MDA-MB-231 cells after OSU-03012 and lapatinib combination treatment was “rescued” by the ectopic expression of Nck1 (see Figure 4C), but not by ectopically expressing Nck2.

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

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.