Reviewer's report

Title: HIF-1alpha activation induces doxorubicin resistance in MCF7 3-D spheroids via P-glycoprotein expression: a potential model of the chemo-resistance of invasive micropapillary carcinoma of the breast

Version: 2 Date: 25 May 2011

Reviewer: Farhad Vesuna

Reviewer's report:

Major Compulsory Revisions

1. YC-1 has a direct inhibitory effect on Pgp. See Invest New Drugs. 2010 Jul 31. YC-1, a novel potential anticancer agent, inhibit multidrug-resistant protein via cGMP-dependent pathway. Hung CC, Liou HH.

The authors must delineate the direct action of YC-1 on Pgp before they can claim it is occurring via HIF-1. One option would be to utilize siRNA or shRNA against HIF-1, instead of YC-1.

If this is not possible, then portions of the manuscript containing the YC-1 will need to be removed.

2. The authors need to explain why they claim 3% oxygen as hypoxia. Have they tried to estimate the effects of true hypoxia, less than or equal to 1% oxygen?

3. Chromatin immunoprecipitation should be quantified by real-time PCR.

4. The conclusion that the 3D spheroids are resistant to doxorubicin, if included, must be substantiated with cell death/ viability assays. This cannot be assumed from the reduced accumulation of dox in spheroids.

5. The conclusion that dox resistance is mediated via increased Pgp expression via HIF-1 is unproven unless HIF-1 is down-regulated by a means other than YC-1.

6. The Verapamil data must be shown, at least in a supplementary figure. The authors should discuss that Verapamil also blocks other efflux pumps and not only Pgp; and hence this mechanism may be acting only partly via Pgp.

7. What is the reason for not displaying an immunoblot of HIF-1 and instead using EMSA?

Minor Essential Revisions

1. Figure 2 does not indicate YC-1 lanes and labeling should be improved. Controls for either non-specific antibody or upstream region (without HIF-1 binding site) should be provided, at least in supplementary figure.

2. HIF-1 is not activated by hypoxia but stabilized by it. Authors are advised to modify their language to indicate the same.
Discretionary Revisions

1. Figure 3 A could be improved by indicating the cell line/treatment on the figure itself.

**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.