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: 12 May 2011

Reviewer: Carine Michiels

Reviewer’s report:

This work evaluated the role of HIF-1 in the respond of MCF-7 spheroid to doxorubicin treatment. It describes interesting but incomplete results.

Major Compulsory Revisions

Indeed, HIF-1 activation needs to be further confirmed for example via a western blot for HIF-1alpha and the expression of well-known target genes assessed via real-time RT-PCR. The same is true for the assessment of apoptosis: only FACS analysis was performed while caspase activity should be quantified. Finally, YC-1 is not a specific inhibitor of HIF-1, the effect of siRNA targeting HIF-1alpha needs to be evaluated.

Minor Essential Revisions

- page 7, 3rd subtitle: a TransAM assay is not an ELISA; it is a DNA binding ELISA. The same remark is also to be made for the abstract and for “results”.
- abstract and results: Pgp expression is NOT assessed by a ChIP assay. A ChIP assay assesses the binding of one transcription factor of interest on the promoter of one gene.
- Figure 4: 10% of annexin-positive cells, i.e. apoptotic cells, in the presence of doxorubicin is very low and may even correspond to basal apoptosis.
- The histological description of the spheroid (figure 5) is not part of this study.

Discretionary Revisions

- running title could be « MCF-7 3D spheroids are resistant to doxorubicin”
- abstract, 4th and 7th lines: “HIF-1alpha activation” is not correct, it is HIF-1a stabilization or HIF-1 activation
- labeling of the different lanes of Figure 2 is missing

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.