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

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Reviewer: Charles Graham

Reviewer's report:

The manuscript by Doublier, Belisario, and colleagues describes an important function of HIF-1 in doxorubicin resistance in spheroids of MCF-7 cells. The paper is well written and the study was carefully executed. Furthermore, it confirms previous studies that describe a causal role of HIF-1 in tumour cell chemoresistance. Overall, I found the paper easy to read and enjoyable. I have the following suggestions for improvement:

1. In the Introduction the authors indicate that the most common cause of MDR in cancer is over-expression of ATP-binding cassette transporters. This may be so in some cancers. However, MDR is a multifactorial phenomenon involving various intrinsic cellular mechanisms as well as extrinsic host-related factors that contribute to the sensitivity (or lack of) to chemotherapeutic agents. This should be acknowledged in the paper.

2. In the Materials and Methods section the authors state that cells were maintained in 5% CO2 and 21% O2. To be more precise, the oxygen concentration used was likely 20%. This is because the CO2 pumped in the incubator displaces some of the atmospheric oxygen present in room air. A second point they should clarify is why they chose to incubate cells in 3% oxygen. In most studies that examine the effect of hypoxia on malignant phenotypes cells are incubated in 1% oxygen (7.6 mm Hg) or less. The paper would also be improved by justifying the use of MCF-7 cells. As the authors indicate IMPC is a highly malignant form of breast cancer. However, MCF-7 cells are breast ductal carcinoma cells that for the most part are considered to be poorly metastatic and moderately differentiated.

3. The authors should consider discussing the effect of YC-1 in the context of nitric oxide. Like nitric oxide, YC-1 is a well-known activator of soluble guanylyl cyclase. It is therefore possible that both NO and YC-1 chemosensitise hypoxic tumour cells by activating cGMP-dependent signalling. We have shown in various publications that various NO mimetics, including 8-Bromo-cGMP, block hypoxia-induced resistance to various chemotherapeutic agents including doxorubicin (e.g. Matthews et al. J. Natl. Cancer Inst. 2001; Frederiksen et al. Clin. Cancer Res. 2007). In another study we also showed that NO attenuates resistance to doxorubicin in three-dimensional aggregates of human breast carcinoma cells (Muir et al. Breast Cancer Research and Treatment 2006).
role of HIF-1 in hypoxia-induced drug resistance has been published by various groups including ours (e.g. Sullivan et al. Mol. Cancer Ther. 2008, 2009; Fang et al. Exp. Cell Res. 2007). The authors should consider discussing how their findings relate to these published studies.

4. The manuscript would benefit from inclusion of scale bars in the micrographs shown in figure 5. Also, it is important that the authors describe the characteristics of the spheroids used. The method used yields spheroids of various sizes. Did the authors select spheroids of a specific diameter to generate the data, or were spheroids of various sizes pooled? Large multicellular spheroids often contain a necrotic core. What is the case in this study?

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

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

**Declaration of competing interests:**

I declare that I have no competing interests.