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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The manuscript by Doublier et al. describes that HIF-1α activation induces doxorubicin resistance in MCF7 3-D spheroids via P-glycoprotein (Pgp) expression and that MCF7 3-D spheroids could be used as a potential model of the chemo-resistance of invasive micropapillary carcinoma (IPMC) of the breast. I have some major concerns regarding this manuscript; in particular, the design of some of the experiments in this manuscript seems to me as being not persuasive.

1) The authors show that the hypoxic cultivation or 3-D growth of MCF7 cells activates HIF-1α and inhibition of HIF-1α by YC-1 abolishes the doxorubicin resistance. However, because YC-1 could exert other activities such as activation of guanylate cyclase, in addition to HIF-1α inhibition, the authors should also use another approach such as RNA interference for HIF-1α knockdown.

2) In addition, because many investigators have previously shown the regulation of the Pgp in 3-D spheroids under HIF-1α activity in several cancer cells, additional experiments are needed to provide the further mechanistic insights for this event. And, to clearly show the HIF regulation of Pgp expression, MTS could be fractionated into the outer (normoxic) region and inner (hypoxic) region, and the fractions are examined for HIF regulation of Pgp.

3) In MCF7 cells, doxorubicin is known to induce senescence but not apoptosis (if any, very small amount of apoptotic cell death). In fact, low amount of apoptosis is observed in MCF7 cells exposed to doxorubicin (<10% in Fig 4D of this manuscript). Furthermore, the chemopreventive effect observed in both MCF7 3-D spheroid and hypoxia-treated MCF7 cells is also too low; so it is difficult to assess the inhibitory effect of HIF-1α on doxorubicin-induced apoptosis in this assay system. Therefore, I recommend that they use other chemicals that could induce apoptosis in MCF7 cells.

4) Finally, the authors are implying that MCF7 3-D spheroids could be used as a potential in vitro model to investigate the role of drug resistance encountered in IMPC of the breast. Although MCF7 3-D spheroids morphologically simulate IMPC cell clusters, MCF7 cells are non-invasive, but IMPC is invasive and highly aggressive. Thus, the authors should provide additional information to insist that
the drug resistance of IMPC is associated with Pgp expression via HIF-1#.

**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:**

no