Reviewers report

Title: TMEM45A as a new biomarker of chemoresistance in cancer cells

Version: 1 Date: 12 April 2012

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Reviewers report:

Experimental and clinical studies have long suggested that tumour cell adaptation to hypoxia contributes to the development of chemoresistance and is associated with malignant progression. In this context, it is commonly believed that chemotherapy stimulates cancer cells to undergo apoptosis, inhibition of which allows the development of chemoresistance. This has led to several published studies investigating the link between hypoxia and apoptosis in tumours and is the area the paper by Flamant et al addresses. Specifically, the study uses the estrogen receptor negative, metastatic breast cancer cell line MDA-MB-231 to assess the effects of hypoxia on apoptosis mediated by paclitaxel and epirubicin and further characterise the mechanism of paclitaxel-induced apoptosis in these cells under hypoxic conditions. In doing this, a comparison is made to etoposide induced apoptosis in the HepG2 hepatocellular carcinoma cells.

Specific comments:

The manuscript by Flamant et al uses the MDA-MB-231 cell line with gene expression microarray analysis to investigate responses to epirubicin (epi) and paclitaxel (tax) under normoxia and hypoxia conditions. This has led to the finding that the TMEM45A gene is regulated by hypoxia and is involved in the epirubicin response in MDA-MB-231 cells and is also involved in the response to the DNA damaging agent etoposide in the HepG2 hepatocellular carcinoma cell line. The paper is clearly written and presented.

Major Compulsory Revisions:

1. Figure 1, parts A,B and C, all show no significant effect between normoxia and hypoxia conditions for epi at 10µM in MDA-MB-231 cells for Caspase 3 activation, LDH release and DNA fragmentation. What is the justification for using epi at 10µM in these experiments? Please clarify

2. Figure 1 C. This shows no significant effect on DNA fragmentation for epi treatment per-se in MDA-MB-231 cells. This result should be commented on.

3. Figure 1. What is the justification for using tax at 50µM for MDA-MB-231 cells? What is the optimum concentration of tax to use for these studies? Has this been established by titration?

4. Table 1. Very little validation has been presented regarding the filtered
microarray analysis (TMEM45A only). The table lists 31 probes for 27 genes, which is a sufficiently small number of genes to be validated by end point, semi-quantitative RT-PCR, or by real-time Q-RT-PCR.

5. siRNA mediated knockdown of TMEM45A in MDA-MB-231 cells has not been shown at a protein level with the antibody used for Figure 6. This would also help validate the antibody and is an important result to show.

6. Similarly, siRNA mediated knockdown of TMEM45A protein in HepG2 has not been shown.

7. Figure 6. What is the origin of the various sized proteins detected by the TMEM45A antibody? How do these relate to forms found in MDA-MB231 and HepG2 cells (additional blotting data required).

Minor Essential Revisions:

8. Page 6, paragraph 5, line 2. The increase in TMEM45A is greater than 16 fold.

9. Page 7, paragraph 2, line 2. “…was invalidated in MDA-MB-231 using siRNA….” change to . “…was knocked down in MDA-MB-231 using siRNA….”


Discretionary Revisions

11. Figure 1. A more definitive assessment of the effects of epi would come from a titration over a range of concentrations under normoxia and hypoxia conditions.

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

**Quality of written English:** Needs some language corrections before being published

**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