Reviewer’s report

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

Version: 1 Date: 8 February 2012

Reviewer: Frédéric Mazurier

Reviewer’s report:

Substantial work had been conducted previously, including Michiels’ group, to uncover the factors/mechanisms by which hypoxia promotes drug resistance in tumors. The present manuscript by Flamant et al. describes a new transmembrane protein, with unknown function, TMEM45A induced by hypoxia in breast cancer resistance to chemotherapy. In the current study, authors compare taxol and epirubicin-induced apoptosis of breast cancer cells under normoxia and hypoxia. Surprisingly, hypoxia prevents drug-induced apoptosis only in response to taxol. Transcriptomic analysis revealed overexpression of several known factors under hypoxia, and the TMEM45A that are not significantly upregulated after epirubicin treatment. The rest of the work, focused on TMEM45A, and classically led by inhibition of TMEM45A through RNA interference strategy, strongly supports a potential role of TMEM45A. Moreover, low expression in patients with breast cancer correlates with better prognosis making TMEM45A as a new prognosis marker. The authors, then, consider the effect of the inhibition of this factor in another tumor/drug model HepG2, and then look at the expression in normal epithelial cells.

Major Compulsory Revisions

1/ Authors show strong increase in apoptosis by both drugs. However, epirubicin does not cause DNA fragmentation. Why? The different should be explained.

2/ Intriguingly, apoptosis induction data are extremely inconstant between fig 1A (Ntax; 60%) and fig 4A (30%), which provides a strong difference in the Ntax/Htax ratios between experiments, 6 and 0.7 respectively.

3/ Although interesting, the work on HepG2/etoposide would be better replaced by a second breast cancer cell line (e.g. MCF7) to reinforce the assumption. Alternately a common mechanism has to be proposed.

4/ Authors previously demonstrated a role of HIF-1 and AP-1 in etoposide-induced apoptosis under hypoxic conditions (ref 29). It will be critical to show whether TMEM45A expression might be controlled by these factors.

5/ Is the keratinocyte a good epithelial cell control for breast cancer? Are there any primary mammary cells that could be used?

6/ Does increase in TMEM45A truly related to keratinocyte differentiation?

First, increased confluence might affect oxygen availability in culture. Cho et al. (BBA, 2008) showed a correlation between HIF-1 and keratinocyte confluence. Considering the point 2/ it might be interesting to make western blotting on HIF-1.
In addition, experiments can be achieved at lower confluence and differentiation induced by increased calcium concentration to avoid hypoxic effect.

Authors conclude to increased expression in more differentiated cells. However, keratinocyte progenitors that settle in the basal layer are in the more hypoxic areas. This should be comment.

Second, the immunohistochemistry is not entirely convincing. Colocalization of Keratin 14 and 10 with TMEM45A would be helpful. I would also suggest using normal mammary tissues instead and certainly recommend making a tissue array including normal and breast cancer samples.

Minor Essential Revisions

In « DNA fragmentation and nuclear fragmentation were also assessed. For the latter, the cells were fixed and nuclei were labelled with fluorescent probe DAPI and observed with fluorescent microscopy. An increase in DNA fragmentation was observed in the presence of paclitaxel under normoxia, which was significantly decreased by hypoxia (Fig. 1C). » Figure 1C does not refer to microscopy.

Figure 2 is useless. Better placed in supplementary data.

SiRNA sequences should be provided if possible. Do all sequences have the same efficacy? Could authors provide also a western blot showing the silencing efficacy?

Figure legend 4 and 5. I guess that siRNA should be anti-TMEM45A not anti-HIF1a!

Figure legend 6 (C). (b and d) d should be c.

Table 1. Please add a column showing ratio Hepi/Nepi.

Discretionary revisions

I highly suggest to remove the keratinocyte part and focus on tumor cells.

Do MDA-MB-231 cells make tumors in immunodeficient mice? The paper will really gain interest with an in vivo model, in which TMEM45A-knockdown cells are injected and mice treated with taxol.

Level of interest: An article of importance in its field

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