Reviewer's report

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

Version: 1 Date: 4 April 2012

Reviewer: Elena Favaro

Reviewer's report:

- Major Compulsory Revisions

1. The first chapter of the results and relative figure 1 (Hypoxia protects MDA-MB-231 cells against paclitaxel-induced apoptosis) should be excluded since it reproduces previous results already published by the same authors in Flamant L, Notte A, Ninane N, Raes M, Michiels C: Anti-apoptotic role of HIF-1 and AP-1 in paclitaxel exposed breast cancer cells under hypoxia. Molecular cancer 2010, 9:191.

and it’s repeated again in figure 4.

The authors should refer to this publication in the manuscript, and shouldn’t consider the investigation on the effect of hypoxia on apoptosis induced by paclitaxel and epirubicin in MDA-MB-231 as an aim of the study (see Background).

2. In the second chapter of the results, the authors should justify the choice of TMEM45A as opposed to other genes more differentially upregulated in H tax cells compared to N tax cells, as shown in the list in Table 1.

3. Third chapter of the results:
   - The authors validate the Affymetrix data for TMEM45A by quantitative PCR analysis. Analysis of TMEM45A protein levels in normoxia and hypoxia and afterspecific siRNA modulation would be advisable.
   - The authors show induction of TMEM45A in hypoxia. The authors also previously showed that HIF plays an important anti-apoptotic role in MDA-MB-231 cells exposed to paclitaxel. Importantly, TMEM45A has been previously characterized as a HIF-target gene (please reference appropriately in the manuscript). The authors should investigate the hypoxic regulation of TMEM45A by HIF in MDA-MB-231.
   - In figure 4A and B, the authors analyse the consequences of TMEM45A knockdown in MDA-MB-231 cells, and conclude that it reverses the protection conferred by hypoxia against paclitaxel.

The greater effect of siTMEM45A on N tax cells compared to H tax cells seems to suggest an additive rather then synergistic effect of TMEM45A knockdown,
which is independent of O2 concentration. Fold change increase in Caspase 3 activity and DNA fragmentation should be analysed. This result in MDA-MB-231 doesn’t indicate TMEM45A as a major player of resistance to apoptosis conferred by hypoxia, as opposed to the result obtained in HepG2 cells.

4. The authors discuss two potential mechanism of action for TMEM45A in order to explain its anti-apoptotic function. The manuscript would be greatly improved by an insight into the TMEM45A function, according to the hypotheses put forward by the authors.

- Minor Essential Revisions

Background:
2nd paragraph: replace with ‘One of the well characterized cellular factor of resistance’; ‘Other mechanisms responsible for drug resistance’ rather than ‘The mechanisms responsible for drug refractoriness’.
5th paragraph: The paper is centred on the breast cancer cell line MDA-MB-231. Please add appropriate references on hypoxia and breast cancer.
7th paragraph: please clarify first sentence.
8th paragraph: in the 2nd sentence, swap anthracyclines and taxoids.

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests