Author's response to reviews

Title: Tubulin binding cofactor C (TBCC) suppresses tumor growth and enhances chemosensitivity in human breast cancer cells

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Version: 3 Date: 24 February 2010

Author's response to reviews: see over
Dear Editor in Chief,

Thank you for giving us the opportunity to respond to the reviewers’ comments regarding our manuscript entitled: “Tubulin binding cofactor C (TBCC) suppresses tumor growth and enhances chemosensitivity in human breast cancer cells” by Rouba Hage-Sleiman, Stéphanie Herveau, Eva-Laure Matera, Jean-Fabien Laurier and Charles Dumontet. We also wish to thank the reviewers for their in-depth comments.

Detailed responses to the reviewer’s comments are given below. Explanations were added in the discussion to clarify the comments pointed out by the reviewer’s and additional results were added in some of the figures as recommended by the reviewers.

To facilitate reading, all modifications have been made in red in the revised manuscript. We hope that this revised version of our manuscript will be suitable for publication in BMC Cancer.

Sincerely,

Rouba Hage-Sleiman
Referee 1:

1) The authors have tried to explain the distinct outcomes of TBCC-involved cell growth in in vitro and in vivo models. However, they did not explain why TBCC increased the time for complete mitosis (Fig. 2D) but, on the contrary, increased the proliferative activity (Fig. 2B). These data were obtained from the same in vitro cell models.

We agree with the reviewer that we were not clear about this issue. In this study, we didn’t emphasize the difference in proliferation rates and we considered it to be minor because the differences between the two proliferation curves were modest. For this reason, in the discussion we mentioned that MC+1 have slightly higher proliferative rate than MP6.1. To assess proliferation with another method, we assayed BrdU incorporation. This assay found no significant difference in the proliferation rate between the two clones. This was confirmed on the other clones MC+2, MC+3, MP6.2 and MP6.3 (Figure 2B). The experimental procedure for BrdU assay was added in the materials and methods section of the revised manuscript.

2) Fig. 5B, the authors described that the overexpression of TBCC protein strongly decreased the PT fraction. However, it did not apply to the alpha-tubulin part of this figure.

In figure 5B, the overexpression of TBCC decreased both the alpha and beta-tubulins of PT fraction, but the decrease was more marked for beta-tubulins. We agree with the reviewer, that it is not clear for alpha-tubulins in the western blot. Unfortunately, our choice of this representative western blot caused ambiguity so in order to eliminate any doubt and to confirm this; we added in figure 5C a quantification of the protein expression of alpha and beta- tubulins in all fractions. This quantification was done using ImageJ software on three independent Western blots. In the graph, we observe an increase of expression in NPT fractions along with a decrease in both the PT and MT fractions. The differences are more marked in beta-tubulins but are also significant for alpha-tubulins.

3) The mechanisms of paclitaxel and vinorelbine are different. One is the stabilization of microtubule and the other is tubulin depolymerization. Interestingly, TBCC displayed similar activity to increase G2/M-phase population no matter what the tubulin-polymerizing or –depolymerizing agent was used. TBCC is supposed to antagonize paclitaxel-mediated effect. The readers may expect more explanation on it.

Thank you for allowing us to clarify this issue. In this study, we are using nontoxic low concentrations of both paclitaxel and vinorelbine. We mentioned this issue in the discussion by adding that even though paclitaxel and vinorelbine have different mechanisms of action with respect to microtubule, their cellular effects at low but clinically relevant concentrations are reduced microtubule dynamics inducing mitotic arrest. The cellular effect of paclitaxel at low concentrations include suppression of microtubule dynamics without affecting microtubule content. At low concentrations, vinorelbine can also stabilize microtubule dynamics and block mitosis with little or no depolymerisation of spindle microtubules.
4) Fig. 6A, the readers may also be interested in the data of apoptosis (sub-G1%).

We included the data of apoptosis in Figure 6B and we found that with both treatments the percentage of apoptotic cells increased. However this increase was not statistically significant.

Referee 2:

1) More information on the levels of expression of the considered proteins in human cancers (breast or other tissues NSCLC) should be added if available or discussed why if not. Cancer usually treated by tubulin binders.

Cancer is usually treated with tubulin binding agents and natural product drugs that target the microtubule system remain major therapies to treat many types of malignancies. Certain cancers do not respond to treatment or develop resistance. Recent studies showed that alterations in the drug target, such as tubulin mutations, altered microtubule dynamics and altered tubulin isotype expression are key mechanisms of antimicrotubule drug resistance. The response to antimicrotubule agents depends also on the composition of microtubules which is linked to tumor aggressivity. Many studies were interested in the expression levels of tubulins in cancers. For example, as mentioned in the discussion of the manuscript, detyrosinated tubulins are highly expressed in breast cancer and are linked to tumor aggressivity. Reduced expression of α and α-acetylated tubulin is associated with enhanced apoptosis in leukemia cells. High expression of class III beta tubulin by tumor cells predicts a resistance to taxane. All these studies suggest that it is important to work on development of new drugs that target specific isotypes in tumors.

To our knowledge, only one study done by Vadlamudi, Barnes et al. 2005 showed that TBCB was overexpressed and phosphorylated in breast tumors. No previous studies were done on expression levels of other TBCs in cancers. In our study, we have studied the expression of the TBCC gene in thirteen different human breast cancer cell lines. We found that the four cell lines that expressed TBCC the highest (MCF7, MDA-MB361, MDA-MB453 and UACC812) were the ones with low in vitro invasiveness capacity and the other nine cell lines that were highly invasive had low TBCC expression (Table S1).

2) The experimental model is a steroid receptor positive cell line usually using to study antihormonal therapeutic approach for breast cancer. Why the authors have selected this model rather than using steroid receptor negative cell lines (ie MDAMB231).

The MCF7 cell line has functional estrogen and EGF receptors and is dependent on estrogen and EGF for growth while MDA-MB-231 cells are hormone-independent breast cancer cells. In our study, we were interested in the role of the cytoskeleton, specifically the microtubules, in modulating the response to chemotherapy. We admit that the estrogen regulation was not a priority in our study and the choice of MCF7 cells was only based on the aggressivity of the model. MCF7 cells are less aggressive than MDA-MB-231 cells and we therefore chose to start with this model to increase our chances to observe increased aggressivity after having modified the level of expression of TBCC.

3) The change of drug sensitivity following the change of level expression should be challenged with anticancer drugs acting through a different mechanism - ie topoisomerase inhibitors or Platinum.

In our laboratory, many previous studies were done on gemcitabine, a nucleoside analog and S-phase specific antimetabolite. For this reason, we tested the response of MC+1 and MP6.1 cells to
gemcitabine and showed that MC+1 cells were less sensitive to gemcitabine than the MP6.1 cells, significantly at 1 nM (Figure 6C) and 3 nM (data not shown). This lower sensitivity to gemcitabine is linked to the fact that MC+1 cells present a lower percentage of cells in the S-phase at the basal level. The differential responses to antimicrotubule agents and gemcitabine reveal that our cell models respond to these treatments based on their distribution in the cell cycle. Therefore we suggest that other anticancer treatments that target the cell cycle could have interesting effects on our cell models.

4) Suggestion on how manipulate this new target should be proposed to identify new modulators.

In our opinion, it would be interesting to look for potential partners of TBCC by doing immunoprecipitations in cells exposed or not to antimicrotubular agents. It would also be interesting to investigate if TBCC interacts or binds to microtubule binding proteins and to synthesize a ligand that can stabilize this protein inside the cytoplasm so that it would be continuously active.