Author's response to reviews

Title: TOR complex 2 is needed for cell cycle progression and anchorage-independent growth of MCF7 and PC3 tumor cells

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Author's response to reviews:

Reviewer's report

Title: TOR complex 2 is needed for cell cycle progression and anchorage-independent growth of MCF7 and PC3 tumor cells
Version: 1 Date: 5 March 2008
Reviewer: Joseph Gera

Reviewer's report:

In the manuscript by Hietakangas et al., the authors investigate the role of mTORC2 activity in tumor cell proliferation and anchorage-independent growth in two cell lines. While a role for mTORC1 in the regulation of these processes has been clearly demonstrated, only recently has mTORC2 activity also been implicated as regulating tumor cell growth. Thus, work investigating the role of mTORC2 in tumor cell growth is of significant importance and appropriate for publication in BMC Cancer. The manuscript is well written, easy to follow and concise. The data are also of high quality and presented clearly. The authors convincingly demonstrate that Rictor expression and Akt activity can be inhibited in MCF7 and PC-3 cells via siRNAs and that knockdown of Rictor results in the inhibition of cell growth. The authors then demonstrate that inhibition of Rictor expression results in marked reductions in soft-agar colony formation relative to control treated cells. Finally, it is demonstrated that Rictor knockdown results in increased G1 arrest and downregulation of cyclin D1 expression.

(1) However, the authors should address why the basal level of Akt activity is relatively low in untreated MCF-7 cells as detected by the in vitro kinase assay, yet high levels of S473 phosphorylated Akt are detected.

Response:
The experiments presented in Figure 1 have not been performed in a way that would allow us to compare the levels of AKT hydrophobic motif phosphorylation and AKT specific activity in the different cell lines. Since we know that AKT activity is also regulated by phosphorylation of the activation loop by PDK1, this kind of comparison might not be very conclusive. The conclusion that can be made from the data in Figure 1 is that knockdown of rictor inhibits AKT hydrophobic motif phosphorylation and AKT kinase activity.

(2) As a minor point, it should be clearly indicated in figure 3 that the control cells are non-targeting siRNA transfected cells and not untreated cells.

Response:
We have indicated this as suggested by the Reviewer.

Reviewer's report
Title: TOR complex 2 is needed for cell cycle progression and anchorage-independent growth of MCF7 and PC3 tumor cells
Version: 1 Date: 15 March 2008
Reviewer: David Sabatini
Reviewer's report:
Hietakangas and Cohen attempt to show by in vitro studies that TOR complex 2 is required for two tumor cell lines to proliferate in culture. This small study aims to suggest that targeting TOR complex 2 might be a possible strategy to inhibit tumor cells. This idea is not novel and has been around for some time now, in fact the authors suggest this in their previous work in flies. However, the authors have attempted to expand here on their previous work in Drosophila to show that their findings may be applicable to human tumor cells. While this is of broad interest, this manuscript is incomplete in its current state and requires additional work.

Response:
Our aim in this report was to extend the range of cancer cells in which inhibition of TORC2 activity was assessed. We are well aware, as the reviewer knows, that this is not a new idea, nor did we present it as one. We felt that there is value in showing that inhibition of TORC2 activity might be useful in cancers other than the gliomas recently reported by the Gera laboratory. This provides further support for the idea that inhibiting TOR complex 2 might have potential as a strategy to inhibit tumor cell proliferation.

Points:
1. In the Background and Discussion, the 2006 reference to Sabatini’s mouse work is missing.

Response:
We have added this reference. Sorry for the oversight.

2. In Figure 1B, it would be nice to show the effects of knocking down rictor on AKT activity more quantitatively. This would be useful information in determining to what extent TOR complex 2 and AKT must be inhibited to effectively slow tumor growth, especially since the authors are promoting the idea of inhibiting TORC2 as a treatment strategy.

Response:
We have added quantification of the kinase assay to estimate the degree of AKT inhibition.

3. In the Background section, the author’s state “it is important to analyze which AKT functions depend on TORC2 in normal and malignant cells.” Thus the authors should also investigate the effects of inhibiting TORC2 in normal (non transformed) control cells using the same approach. One good experiment would be to put PTEN back into the PTEN-null PC3 cells and determine the effects of knocking down rictor on proliferation and apoptosis. This is important because the authors are trying to make the point that inhibiting TORC2 is more detrimental to cells with high AKT activity. The hope is that knocking down rictor will not severely affect the proliferation of cells with low AKT activity.

Response:
The effects of TORC2 on in vitro proliferation of normal cells have been addressed by two studies using genetic ablation of TORC2 components. Shiota and coworkers reported modestly reduced proliferation of Rictor-deficient MEFs compared to wild type, whereas Jacinto and coworkers reported that sin1-deficient MEFs proliferated similarly to wild type cells and displayed cell cycle distribution similar to the wild type cells. In the fly, we have shown that the effects of rictor depletion are less severe on cells with normal levels of AKT activity than on cells with elevated AKT activity. The reason for the difference between the two reports on mouse TORC2 deficient cells is unclear, but it does indicate that there are at most modest effects on proliferation of normal MEFs. Whether high AKT activity can predict the sensitivity of cancer cells to TORC2 inhibition is a question that merits further exploration in different types of cancer cells, particularly when TORC2 inhibitors are available for study. However, we feel that it is beyond the scope of this report, which aims to show that reduction of TORC2 activity can affect the proliferation other types of tumor cells, in addition to gliomas.
4. The authors point out in the Background that MEFs lacking TORC2 activity did not reduce phosphorylation of all AKT targets. Because the tools are readily available and the experiments easy to do, the authors should look at what effects knocking down rictor has on AKT target phosphorylation in the MCF7 and PC3 cells.

Response:
The effects of TORC2 on AKT targets have previously been addressed in several different mammalian cell systems and also in Drosophila. Our interpretation of the published results is that loss of TORC2 activity limits the activity of AKT in a way that phosphorylation of FOXO, but not GSK3 and TSC2, is reduced. The reviewer requests that we extend this to MCF7 and PC3 cells. We have tried to address the request. We can detect phosphorylation of endogenous GSK3, which is not affected. However, we have not been able to detect the phosphorylated forms of endogenous FOXOs in MCF7 and PC3 cells with the reagents available and therefore cannot provide the requested information. We feel that analyzing AKT target phosphorylation in MCF7 and PC3 cells is not crucial in this context, since it has been well documented in other systems and since it is not essential information to support the main conclusion of this study.

5. In their proliferation experiment, how did the authors determine that no significant cell death was observed? Considering the role of AKT in cell survival, it seems important to include these data in the paper.

Response:
Data showing the lack of increased cell death in rictor knock-down samples is provided in the new Fig. 2A.

6. In Figure 4A, it is difficult to visualize what the cell cycle stage profile looks like from a pie chart and it is also difficult to know how the authors determined stage boundaries. Could the authors include the more standard profile of cell cycle phase?

Response:
The FACS data are now shown as histograms with stage boundaries (new Fig. 4A).