Reviewer’s report

Title: Phosphatidylinositol 3’-kinase, mTOR, and Glycogen synthase kinase-3 beta mediated regulation of p21 in human bladder carcinoma cells

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Reviewer: Makito Miyake

Reviewer’s report:

Review of “Phosphatidylinositol 3’-kinase, mTOR, and Glycogen synthase kinase-3 mediated regulation of p21 in human bladder carcinoma cells”

Summary,

This report investigated the signaling pathway of PI3K, PTEN, AKT and GSK3β and their relation with p21. The authors utilized urothelial carcinoma cell lines UMUC-3 and UMUC-14, which are PTEN negative and positive, respectively. In conclusion, PI3K and AKT cause an upregulation of p21 by suppressing Gsk-3β activity and activating mTOR in both cultured human bladder carcinoma cells and mouse urothelial cancer tumors in vivo.

Comments,

1. [4th paragraph in the Background section] The word ‘the early tumors’ is used. Do the authors mean ‘low stage tumors’ or ‘high grade tumors’? The authors should define it clearly. There are also the words ‘the early bladder tumors’ and ‘late tumors’ in 1st paragraph in the section Discussion.

2. [‘Western blotting’ in the Methods section] The concentrations of extracted protein were not measured in this study. Why did not the authors measure them? In general, various modifications and treatments lead to change of cell numbers and amount of extracted protein in each dish. How much protein was loaded in each well? In the Figure 3B, amount of #-tublin, a loading control, looks significantly different among the each other.

3. [last paragraph in the Discussion section] The authors describes that “It will be helpful to examine GSK-3b activation levels…. GSK-3b inhibitor”. Why did not the authors investigate the cytotoxic assay of GSK-3b inhibitor or PI3K inhibitor using bladder cancer cell lines? It is important to assess the effect of these inhibitors for bladder cancer as well as colon cancer and ovarian cancer.

4. [Figures 1B, 1C and 2] In some experiment shown by figures 1B, 1C and 2, the authors exhibited the results only using UMUC-3. As described in 1st paragraph of the Result section, UMUC-3 is PTEN negative, whereas UMUC-14 is positive. Why did not the authors perform the experiments using UMUC-14 as similar as UMUC-3? It is essential for the authors to compare the results between UMUC-3 and UMUC-14 and add the evaluation to the Discussion section.
Moreover, in the experiments shown by figure 1A 3, and 4, there are no discussion in an aspect of comparing between UMUC-3 and UMUC14.

5. [Figure 5] In figure 5A, urothelium is exhibited unclearly, leading to make it impossible to evaluate double staining IHC correctly. That's maybe due to too much PermounTM in mounting slides. Re-mounting and re-photographing is preferred. The authors should add a scale and/or information of original magnification to these figures.

6. The authors should describe the limitation of this work more clearly.

Discretionary Revisions: 1
Minor Essential Revisions: 2
Major Compulsory Revisions: 3-6

Final decision,
I think that this manuscript is not acceptable in the present status.

Level of interest: An article of limited interest

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