Reviewer’s report

Title: Angiotensin II type 2 receptor signaling significantly attenuates growth of murine pancreatic carcinoma grafts in syngeneic mice

Version: 1 Date: 4 June 2009

Reviewer: Anson Lowe

Reviewer’s report:

Manuscript by Doi et al.

The manuscript by Doi et al. proposes that the angiotensin II receptor suppresses the growth of the pancreatic adenocarcinoma cell line, PAN02. The authors conclude that the effects on PAN02 are due to stromal derived AT2. Overall the effects on tumor growth and ERK phosphorylation are modest and not that convincing. One caveat, however, is that I was not able to interpret figure 5 at all, which is most likely due to a formatting error. Thus the data will need to be reexamined in the context of a corrected figure 5.

Major compulsory revisions:

1. The authors have previously published work on AT2 with respect to models of lung and colon cancer with apparent disparate results. The do not comment why AT2 appears to suppress tumor growth for pancreatic cancer cells and not for the previous two models where the opposite effect was found.

2. Figure 1 – How was the endpoint determined? Why is it that only the last timepoint shows a significant difference in size? The figure legend should note what is represented by the error bars. From the data, a significant difference was noted only for size and not weight at the same timepoint.

3. Figure 3 – changes in the apoptotic index were not statistically significant. Thus the authors should not refer to this result as a positive result throughout the paper.

4. Figure 4 – It is difficult to assess vascular density with the images provided. The use of an objective criteria, such as immunocytochemistry with an endothelial marker would be preferred. This would also permit some form of quantitation.

5. Figure 5 – this is an important figure that was very difficult for me understand. I believe that there may be a major formatting error in the copy I received. The figure legend states that the bars are colored in the following order, black, grey, white, and dark grey; whereas I see black, grey, striped light pink, dark grey, and dark pink striped. To my eye, the following pairs of bars are colored the same and received the same treatment but have profoundly different OD (2&3, 6&7, 10&11, 14&15). It does not make sense. I cannot see the point the authors are trying to make. In addition, it is not clear how one distinguishes the cell growth due to the fibroblasts from the cancer cells? Although it is stated earlier in figure
1. In data not shown that PAN02 cells do not express AT2, I think it would be worth showing the data for both PAN02 and the fibroblasts.

6. The effects on ERK1/2 phosphorylation are modest. Are we to assume that the increase in ERK1/2 phosphorylation is derived from the PAN02 cells? Why can’t increase ERK phosphorylation be from the fibroblast where AT2 is presumably located?

Minor Essential Revisions

1. Figure 2 – It is difficult to evaluate the immunocytochemistry. I would suggest enlarging the image so that Ki-67 positive cells can more easily be detected by the reader.

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

**Quality of written English:** Needs some language corrections before being published

**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.