Reviewer's report

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

Version: 1 Date: 5 June 2009

Reviewer: Marina Pasca di Magliano

Reviewer's report:

In the manuscript entitled “Angiotensin II type 2 receptor significantly attenuates growth of murine pancreatic carcinoma grafts in syngeneic mice” the authors explore the potential role of Angiotensin II type 2 receptor (hereby AT2) in the stroma in regulating the growth of subcutaneously transplanted pancreatic cancer cells. They use a pancreatic cancer line derived from C57/Bl6 mice, and inject it subcutaneously in syngenic mice that are either wild-type or knock-out for AT2; in addition, they conduct some in vitro experiments using the same cancer line and mouse skin fibroblasts.

The concept of tumor-associated fibroblasts promoting tumor growth is well established. We have, however, a limited understanding of how fibroblasts promote tumor growth, and therefore studies aimed at understanding the mechanism that mediate the tumor-promoting ability of components of the tumor stroma are interesting and have potential therapeutic implications.

Major Compulsory revisions:

I have some major concerns regarding the manuscript:

1) The relevance of AT2 to pancreatic cancer is hard to explain unless expression of this gene is shown in the disease. Moreover, expression of the gene should be demonstrated in the fibroblasts infiltrating the grafted tumor cells in the wild type mice. Failing that, it is hard to attribute the difference in tumor growth to knocking-out a gene that might not be expressed in the first place. This is particularly relevant since the authors mention in the introduction that the gene is expressed during embryogenesis but not in adult tissues.

2) The system used to study tumor-stroma interaction is not ideal, as the tumor cells are transplanted subcutaneously. Subcutaneous fibroblasts are very different from pancreatic ones; moreover, in a subcutaneous location pancreatic cancer cells fail to elicit a desmoplastic reaction resembling primary tumors. A more relevant approach would be constituted by orthotopic transplantation of tumor cells. A second possibility would be to transplant pancreatic fibroblasts together with the tumor cells. In any case, as per point 1, the expression of AT2 should be evaluated in the different cell populations.

Similarly, the use of mouse skin fibroblasts is not likely to be relevant to pancreatic cancer; a more advisable approach would be conducting those
experiments with pancreatic fibroblasts.

3) The authors conclude that AT2 constitutes a promising new target in pancreatic cancer. However, AT2 appears to act as a suppressor of tumor growth: the authors should explain how they would use it as a therapeutic target. Moreover, given that the findings apply to a single pancreatic cancer cell line, and that the tumor histology in the manuscript do not resemble human pancreatic cancer, I think the authors should explain the limitations of their system.

Below are specific comments on the results:

1) The changes in tumor growth reported in figure 1A are only statistically significant at the last time-point. This data set is obtained by caliper measurements that are by their own nature not very accurate. The data in figure 1B, tumor weight at dissection, reflect only a marginal difference in tumor growth that might not be statistically relevant. Therefore, I am not convinced that loss of AT2 has an effect on tumor growth.

2) The data in figure 2 can not be interpreted: the increase in proliferation cannot be attributed to a specific cell compartment in absence of a counterstaining: epithelial and fibroblast markers should be used to determine which cell population is affected.

3) The data in figure 3 is non statistically significant.

4) In order to establish vascular density of the tumors in the different animal cohorts, the authors should either use in vivo imaging systems or quantify by FACS the endothelial cells in a representative and statistically adequate number of samples. The data in figure 4 does not support the conclusion that a different vascular density is observed in the different cohorts.

5) The experimental design for this data set should be clarified, and the figure labeling should be improved to allow the reader to interpret the data. I was not able to interpret the data presented in the experiment. Are the authors showing that angiotensin 2 has a tumor promoting ability? And that AT2 is tumor-inhibiting? What is the effect of AT2 antagonist? In any case, cell proliferation is reported for both fibroblasts and tumor cells together; it might be more useful to sort the tumor cells or culture them in separate trans-wells, to be sure that the differences in proliferation can be attributed to the tumor cells.

6) The authors hypothesize that AT2 influences tumor growth by affecting the level of phospho-Erk in the tumor cells. However, the data presented does not appear to reach statistical significance, and, in the western blot, the levels of total ERK appear to change as well as phospho-Erk; therefore, it is my impression that the conclusions are not adequately supported by the data.

Minor Essential Revisions:

1) Abstract: The background section should explain why the authors hypothesize that AT2 might play a role in pancreatic cancer (is it expressed in the disease? In which cell types? Is it expressed in normal pancreas?)
The methods section should be written such that it can be interpreted even without reading the whole manuscript – reference to specific staining performed could be removed, and replaced with a description of the two experimental settings used: a syngenic transplantation model, where pancreatic cancer cells are injected subcutaneously; and an in vitro co-culture of tumor cells with fibroblasts. The conclusions section of the abstract states that AT2 would constitute a therapeutic target; this should be explained, especially since the receptor appears to be a negative regulator of tumor growth.

2) The background should be more specific, so that people not in the renin-angiotensin field can understand the relevance of the study. Page 4, end of the page: “AT2, (…), is primarily expressed in the mesenchyme of fetus limited in adult tissues” – this sentence is incomplete.

Page 5 “Our previous study revealed… deficiency”: the study should be cited or explained – which organ system? Which carcinogen? How is it affected by AT2? More or less tumor growth?

Page 5, second paragraph: This needs to be explained better: which inhibitors have a role in cancer, how do they affect tumor growth – is AT2 a positive or negative regulator of cancer growth? The sentence “This suggests… role in cancer” does not logically follow the previous statements; this paragraph should be rewritten so that non-specialists can follow the logic behind it.

3) In the Results: the use of past tenses in the paragraph titles is somewhat unusual – in general, the paper would benefit from some text editing.

4) Each panel of each figure should be cited in the text – several are missing. The figures would benefit of more extensive labeling, figure 5 in particular.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

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