Reviewer’s report

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

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Reviewer: Diego F Calvisi

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Pancreatic cancer is one of the most aggressive and lethal human malignancies, characterized by a high rate of recurrence and resistance to conventional chemotherapeutic treatments. Thus, investigation of the molecular mechanisms leading to pancreatic tumor development and progression is required. In the present manuscript, Doi et al. assessed the effect of host angiotensin II (Ang II) type 2 receptor (AT2) expression on the growth of pancreatic carcinoma. For this purpose, the Authors have investigated the growth of mouse pancreatic ductal carcinoma grafts in syngeneic wild type and AT2 receptor–deficient (AT2-KO) mice. In particular, the Authors determined the role of AT2 receptor signaling in stromal cells on the growth of murine pancreatic carcinoma cells (PAN02) via in vitro and in vivo approaches. The results show that the growth of subcutaneously transplanted syngeneic xenografts of PAN02 mouse pancreatic ductal carcinoma cells was significantly faster in AT2-KO mice than in control wild-type mice. This was associated with higher cell immunoreactivity for Ki-67 and lower apoptosis in xenografts grown in AT2–KO mice than in wild-type mice. Furthermore, the growth of PAN02 cells was significantly decreased when grown with AT2 receptor gene-transfected wild-type and AT2-KO mouse-derived fibroblasts. The Authors conclude that Ang II might play a role in the growth of pancreatic carcinoma cells through modulating functions of host stromal cells. Furthermore, the present data indicate that Ang II AT2 receptor signaling is a negative regulator of pancreatic carcinoma cell growth.

The paper by Doi et al. is novel and provides new insights on the pathogenesis of human pancreatic cancer. The techniques used and statistical methods applied are appropriate. The main strength of the paper is the demonstration of a role of the AT2 receptor in pancreatic carcinogenesis and, consequently, its possible implication as a target for pancreatic prevention and treatment. However, the work appears incomplete and not conducted in depth.

Major Compulsory Revisions:

The paper investigates only superficially the molecular mechanisms responsible for pancreatic cancer cell growth following suppression of AT2 receptor expression. Although the Authors investigated the involvement of the ERK proteins, further work is certainly required. For instance, the Authors should address whether the suppression of the AT2 receptor leads to the activation of the AT1 receptor (with an opposite, pro-oncogenic role) in their model of pancreatic cancer. This is a crucial question that requires to be answered. If the
AT1 receptor is overexpressed following inactivation of AT2 receptor, the Authors should investigate whether AT1 overexpression results in activation of protooncogenes such as c-fos, c-jun, and c-myc, which are cited in the Discussion section but not investigated. Furthermore, the study of activation of the Ras cascade is limited to ERK1/2 in the present manuscript, whereas the Authors should investigate whether other effectors of Ras involved in cell proliferation, apoptosis, and/or angiogenesis (JNK, PI3K, p38MAPK, HIF-1alpha, VEGF-A) are also triggered by AT2 receptor downregulation in pancreatic cancer.

Specific, additional concerns:
1.) The quality of Figures 2 (A,B), 3 (A,B), and 4 (A,B) is not appropriate and needs to be significantly improved. Higher magnification of the aforementioned Figures is recommended.
2.) Evaluation of microvasculature in tumors from AT2-wild type and AT2-KO mice should be performed using a more appropriate and objective (quantitative) method than the simple H&E staining. Authors should perform immunohistochemistry with an endothelial cell marker (such as CD34, for example) and count the microvessel density in AT2-wild type and AT2-KO tumors.
3.) In Figure 6, the difference in activated (phosphorylated) ERK1/2 levels between untreated and treated PAN02 cell is very small and cannot support the Authors’ conclusions.

Level of interest: An article of importance in its field

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