Reviewer's report

Title: Inhibitory effects of prostaglandin E2 on collagen synthesis and cell proliferation in human stellate cells from pancreatic head adenocarcinoma.

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Reviewer: Paul Insel

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This manuscript addresses an important topic, the desmoplastic properties of pancreatic cancer, by studying of some properties, in particular as related to COX2 and PGE2 of primary isolates of pancreatic stellate cells cultured from samples derived from patients with resectable pancreatic head adenocarcinoma. The primary use of patient samples is an admirable feature of this study. However, most of the findings are descriptive and somewhat incomplete and makes it difficult to draw definitive conclusions. The following are revisions that this review advises the authors to make:

Major compulsory revisions:

1. Further information needs to be provided on the use of cells in particular experiments shown in Results (for example in data shown Fig 1D/E, 4D, 5 and others). The methods state (pg 4) that "all experiments were performed using cell populations between passages 4 and 8." Properties of fibroblasts change during culture, potentially with conversion to a more pro-fibrotic state. It is thus possible that the authors have compared cells that are in very different biochemical and functional "states". Clearer definition of cells used in particular experiments is required. Even better would be if one or more of the variables assessed in these studies was assessed as a function of passage number. The stellate cells most relevant to the in vivo setting should be those tested at the lowest possible passage.

2. Further data related to PDGF response needs to be provided. On the last line of page 9, it states "PDGF induced COX-2 in some cell lines but not others". This may relate to my point #1. Readers are left not knowing what to conclude. Does PDGF induce COX-2 or doesn't it? If not in certain cell lines, why not?

3. Further quantitation of certain data are needed so that firmer conclusions can be made. Examples include the data in Figures 4C and 4D and in Figure 7D. Appropriate statistical analyses are required. For example, in Fig 7D, It is not clear if the decrease in response by PGE2 is statistically significant. If not, then the authors ought not state (as they do) that it "attenuated TGF#1-stimulated increase in gene expression of collagen 1A1". Consultation with a statistical expert may be needed.

4. Further studies to define the functional role of PGE2 receptors are required.
The data (Fig 3B) implicate EP2 receptors in cAMP accumulation but which PG receptors mediate the responses the authors studied (e.g., collagen synthesis, cell proliferation)? Data of effect on PG receptor blockers on those responses are needed as part of this manuscript.

Discretionary Revisions

1. The authors conclude that the cancer cells are the main source of PGE2. This conclusion would be more convincing if PGE2 produced by cancer cells were tested on PSCs. Such experiments could be conducted by pretreating BxPC-3 with/without a COX-2 antagonist and co-culturing the pretreated BxPC-3 with PSCs to assess PDGF-stimulated DNA synthesis or collagen synthesis in PSCs.

2. The authors conclude that PDGF-stimulated DNA synthesis is mediated by cAMP. Additional experiments would be helpful to support this statement; such as, addition of PGE2 and forskolin to pancreatic stellate cells in the presence of PKA or Epac inhibitors so as to confirm cAMP action and to provide evidence that one or both those downstream effectors mediate the effects of cAMP.

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

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests other than a scientific interest in this field, since my lab is working on somewhat related questions.