Author's response to reviews

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

Authors:

Ewa Pomianowska (ewa.pomianowska@medisin.uio.no)
Dagny Sandnes (d.l.sandnes@medisin.uio.no)
Krzysztof Grzyb (kgrzyb@ous-hf.no)
Aasa R Schjølberg (Aasa.R.Schjolberg@rr-research.no)
Monica Aasrum (monica.aasrum@medisin.uio.no)
Ingun H Tveteraas (i.h.tveteraas@medisin.uio.no)
Vegard Tjomsland (vegard.tjomsland@medisin.uio.no)
Thoralf Christoffersen (thoralf.christoffersen@medisin.uio.no)
Ivar P Gladhaug (i.p.gladhaug@medisin.uio.no)

Version: 5
Date: 31 January 2014

Author's response to reviews: see over
Dear Editor,

Thank you for your review and valuable comments on our manuscript “Inhibitory effects of prostaglandin E2 on collagen synthesis and cell proliferation in human stellate cells from pancreatic head adenocarcinoma”. We also much appreciated the extension of the closing time for re-submission of this paper.

We have now revised the manuscript according to the reviewers’ comments; see our detailed reply to each comment below.

Reviewer #1: Answers to comments

Major compulsory revisions:

1. Further information needs to be provided on the use of cells in particular experiments. All experiments were performed using cell populations between passage 4 and 8.

We agree that this can be a potential problem in our model system, i.e. cells in the different passages can be in different biochemical and functional states. To be able to rule out this possibility, properties of the pancreatic stellate cells in the different passages were assessed both for the presence of relevant cell markers and functional state as a function of passage number.

First, we assessed the expression of COX-2 and α-SMA by Western blotting in unstimulated preparations of pancreatic stellate cells. The results showed that the expression of COX-2 and α-SMA remained unchanged over the passages 1 to 6. We have included these results in the revised manuscript as a new Fig 1D.

Date: January 31, 2014

Email: ewa.pomianowska@medisin.uio.no
Secondly, to compare the functional properties of different cell passages, we also compared whether treatment of PSC with EGF, PGE\(_2\), TGF\(_\beta\) and PDGF had the same effect on COX-2 expression in two different passage (passage 4 and 7). The data show identical expression pattern in the two cell passages on Western blotting. These data are included in the revised manuscript as Fig 1E.

Thus, we could be able to conclude that stellate cells in the range of passages used, have stable properties regarding the biochemical and functional properties studied, allowing comparison.

2. **PDGF induced COX-2 in some cell lines but not others**

We agree that the statement regarding expression of PDGF in the original manuscript can be misleading. In most cell lines of pancreatic stellate cells, PDGF was not able to induce COX-2. However in a few cell lines, we could not rule out that there was a weak, positive effect of PDGF on COX-2 induction. Relating to this, we want to emphasize that the cell lines of pancreatic stellate cells studied in the present work are derived from different patients and it is therefore impossible to avoid some variability in biological responsiveness. In the revised manuscript we have stated that PDGF had no significant effect on COX-2 expression in the pancreatic stellate cells.

3. **Further quantitation of certain data are needed so that firmer conclusion can be made**

Figure 4C shows effects of PGE\(_2\), EGF and TGF\(_\beta\) on ERK and AKT phosphorylation as assessed by Western blotting. In the revised manuscript we have performed additional experiments and quantitated the data as the reviewer requested. Figure 4C in the revised manuscript has been enlarged and supplied with histograms and statistical analyses of data from three independent experiments.

Figure 4D shows effects of thrombin, PGE\(_2\), fluprostenol and forskolin on ERK phosphorylation. Similarly as above (Fig 4C), in the revised manuscript Fig 4D has been supplemented with histograms and statistical analysis for PGE\(_2\) and fluprostenol from four independent experiments. We agree that regarding Figure 7D in the original manuscript, our statement that PGE\(_2\) attenuated TGF\(_\beta\)-stimulated increase in gene expression of collagen 1A1 was insufficiently substantiated. We have now performed additional experiments with different concentrations of PGE\(_2\) 0.1 \(\mu\)M and 1 \(\mu\)M. These new data show clearly that PGE\(_2\) at the concentration of 1 \(\mu\)M significantly inhibited TGF\(_\beta\)-stimulated increase in gene expression of collagen 1A1. These data are included in the revised manuscript as Figure 7D and Figure 7E.

4. **Further studies to define the functional role of PGE\(_2\) receptors are required.**

Additional experiments to define the functional role of PGE\(_2\) receptors were performed. As we stated before (original manuscript page 10), preincubation with the EP4 receptor antagonist (L-1861982) did not result in inhibition of PGE\(_2\)-stimulated cAMP accumulation. We performed new experiments to assess the effect of the EP4 receptor antagonist on collagen synthesis in the stellate cells. In agreement with our previous results on cAMP accumulation, preincubation with
the EP4 receptor antagonist did not affect PGE$_2$ inhibition of TGFβ1 stimulated collagen synthesis. This is included in the revised manuscript as a new Figure 7F.

We also performed additional experiment with the EP2 receptor blocker AH 6809 on collagen synthesis and DNA synthesis. However, the results with this antagonist were not conclusive. One of the reasons for this could be that PGE$_2$ might induce its effects not only via EP2 receptors. Further studies including the use of specific siRNAs are required to explore the role of prostaglandin receptors. This is considered to be beyond of the scope of the present study.

Discretionary revisions

1  Effects of cancer cell-derived PGE$_2$

We agree that it is of considerable interest to study the effects of cancer cell-derived PGE$_2$ when added directly to stellate cells or in co-culturing model systems. These are questions we plan to address in forthcoming studies.

2. The authors conclude that PGDF - stimulated DNA synthesis is mediated by cAMP.

We believe the reviewer on this point may have misunderstood our conclusions in the original manuscript. We stated that PDGF stimulated DNA synthesis in the stellate cells (Fig 4A), and that PGE$_2$, via cAMP, exerted inhibitory effect on DNA synthesis in these cells.

Concluding remarks

The abstract in the revised manuscript has been shortened and the conclusion section in the abstract has been modified to comply with the changes in the manuscript regarding the discussion about receptors mediating the effects of PGE$_2$.

In addition, TGFβ1 has been replaced by TGFβ in the running text throughout the manuscript to comply with the text on the figures. Some minor orthographical corrections have also been made in the revised manuscript.

As stated previously, we have no competing interests to declare.

We thus hope that the manuscript will now be found suitable for publication in *BMC Cancer*.

Yours sincerely,

Ewa Pomianowska, MD.