Reviewer’s report

Title: Increase in intracellular PGE2 induces apoptosis in Bax-expressing colon cancer cells.

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Reviewer: Ivonne Loeffler

Reviewer’s report:

The study of Lalier et al. is intriguing as it looks at PGE2 as intracellular mediator of apoptosis in colon cancer cell lines. There are conflicting literature data on the regulation of apoptosis in colorectal cancer cells by PGE2. In most experiments cells are challenged with extracellular PGE2, which exerts its biological functions via binding to four types of G-protein-coupled receptors termed EP1-4. By contrast, Lalier et al. induced an increase in the intracellular PGE2 either by PGE2 microinjection or by the pharmacological inhibition of PGE2 exportation and enzymatic degradation. In general the approach is novel and revolutionary, but the amount of data presented in this study is marginal and does not warrant the conclusions drawn by the authors. Specifically, I have the following comments to the authors:

Major compulsory revisions:

1. Since the expression of COX-2 was only moderately modified by the transfection with mPGES-1 in SW1116 cells, Lalier et al. used only these cells for the rest of the experiments (Fig 2 B). To prove that mPGES-1 transfection results in an increase in intracellular PGE2 and subsequently on apoptosis, the authors should measure the intracellular PGE2 levels. It would also be important to present a similar set of data for a second colon cancer cell line.

2. Furthermore, the authors should exclude paracrine effects of mPGES-1 overexpression.

3. Lalier et al. document COX-2 protein (Fig 2 A) and mRNA (Fig 4 A) expression in HCT116 cells. In contrast, various publications reported that there is no COX-2 expression in this cell line (e.g. Sheng et al., 1998, Cancer Res.; Banu et al., 2007, Cell proliferation) The authors should at least comment on this discrepancy. Are the values in figure 4 A representative data or are they mean values?

4. Lalier et al. report that an increase in intracellular PGE2 induces apoptosis whereas stimulation with extracellular PGE2 does not. The experiment of administration of extracellular PGE2 alone was shown only with one cell line (SW1116) and a single concentration of PGE2 was used (Fig 4D). This should be extended to test a range of PGE2 concentrations.

5. Since 15-PGDH degrades PGE2 as well as other PGs and MRP4 has been shown to transport several physiological substrates (PGE1 as well as PGE2), the authors should test the effect of other prostaglandin species.
6. It could be beneficial to show the increase in intracellular PGE2 after inhibition of 15-PGDH and MRP4, to link PGE2 with induction of apoptosis directly.

7. The authors should confirm the apoptosis data with a second, unrelated apoptosis assay.

8. PGE2 activates several signal transduction pathways through activation of its different receptors. Therefore, the EP receptor expression status of the tested cell lines could be of interest.

Minor essential revisions:

1. More literature regarding the controversial issue about apoptosis induction by PGE2 would be desirable.

2. A table with information about the patient materials (mainly the stage of CRC) is missing.

3. Several methodologies (like e.g. total RNA isolation, protein extraction, etc...) are missing from the experimental section.

4. The quality of blots in figure 1 A should be improved. Furthermore, it is not clear whether some of the blots are reblots after membrane stripping. A control tissue is also missing.

5. In figure 1 B the marking of the x-axis is not clear. Are these numbers of patients and are they different from the material in figure 1 A and what is behind the mean values and standard deviations? In addition, the statistical significance is not marked.

6. Is the actin blot in figure 2 A the reblot of both COX-2 and mPGES-1 blots? Again, the statistical significances in the graph of quantified protein bands in figure 2 A are missing.

7. Figure 4b can be removed.

8. The conclusion chapter is missing.

Discretionary revisions:

1. Explanation of hMRP4 in the list of abbreviations is missing

2. Figure 4 C includes a mistake in writing of time specification (mn --> min)

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.