Reviewer's report

Title: TNF-alpha and LPA promote synergistic expression of COX-2 in human colonic myofibroblasts: role of LPA-mediated transactivation of upregulated EGFR

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Reviewer: Bernd Fiebich

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In the manuscript entitled “TNF-alpha and LPA promote synergistic expression of COX-2 in human colonic myofibroblasts: role of LPA-mediated transactivation of upregulated EGFR” Yoo et al. studied whether EGFR signalling plays a role in the synergistic COX-2 expression induced by LPA and TNF-alpha. They found that chronic exposure to TNF-alpha led to upregulation of EGFR in association with sustained LPA-mediated EGFR phosphorylation at Y1068. TNF-alpha and LPA also led to sustained p42/44 MAPK phosphorylation and synergistic COX-2 expression, effects that were partially inhibited by the EGFR tyrosine kinase inhibitor AG1478. The authors furthermore demonstrate that p42/44 MAPK phosphorylation and COX-2 expression were inhibited to the same degree by the MMP inhibitors GM6001 and BB-94, suggesting that LPA-mediated EGFR transactivation involved MMP-mediated release of EGFR ligands from the cell surface. Using the Src3 inhibitor SU6556, they found inhibition of TNF-alpha/LPA-mediated EGFR phosphorylation at Y1068, p42/44 MAPK phosphorylation, and COX-2 expression suggesting an upstream role of Src in the transactivation of EGFR.

Although, the authors show very interesting data concerning the TNF/LPA/EGF signalling in colonic fibroblasts, the following points need to be addressed:

Major points:

1) It would be very helpful for the reader of this manuscript if the data are summarized in a signalling pathway diagram showing the crosstalk between the receptors and signalling pathways.

2) COX-2 is one of the key enzymes of the prostanoid synthesis. However, there are also other enzymes involved such as COX-1 and for the synthesis of PGE2, the prostaglandin E synthases such as mPGES-1. It would be interesting to see, whether the expression of those enzyme are regulated by TNF/EGF/LPA.

3) The most interesting prostanoid in respect to gut inflammation is PGE2. The expression and regulation of COX-2, most likely but not necessarily, results in the induction PGE2. Especially if the expression, like shown via TNF, is quite low, PGe2 levels might not be affected. Data showing the release and synthesis of PGE2 after stimulation with TNF/LTA/EGF would strengthen the conclusion in respect to gut inflammation.
4) What was the statistics used in the manuscript to calculate the significance of the densitographs?

5) The statement, that SU6656 inhibited p42/44MAPK and COX-2 in the same degree then AG, GM and BB is not true by looking on the Western blots. More representative Western blots should be shown, which fit to the densitographs.

6) Alpha-SMA is not the appropriate control for phosphorylated p42/44 MAPK. An antibody recognizing total p42/44 MAPK should be used to avoid false positive effects due to increased p42/44 MAPK protein levels.

Minor points

1) Please provide the specific information about the antibodies and the dilution used for the Western blots. There are more antibodies for each target in each company and the reader needs to know, which one was used.

2) In the densitograph in Fig. 1A, the time line should be hours instead of minutes.

Level of interest: An article of importance in its field

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