Author's response to reviews

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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Author's response to reviews: see over
Dear Prof. Alberto Bardelli,

BMC Gastroenterology Manuscript 5734969227600876:

Thank you for your letter and the appended comments of the Reviewers regarding our manuscript entitled “TNF-alpha and LPA promote synergistic expression of COX-2 in human colonic myofibroblasts: role of LPA-mediated transactivation of upregulated EGFR”. We have studied the comments very carefully. We were encouraged by the fact that Reviewer #1 felt that the data was “explained clearly” and that the “findings are important to those with closely related research interests”. In turn, Reviewer #2 indicated that the manuscript showed “very interesting data”. Consequently, we have prepared a revised version of manuscript #5734969227600876. We extended the manuscript by incorporating new information to comply with the points raised by our Reviewers. The detailed description of the new information incorporated in the revised and expanded version of our manuscript is as follows:

Reviewer #1:
As pointed out by Reviewer #1, this manuscript expands on our previously published research (references #7, 8, 11) which has investigated interactions between TNF-α, G protein-coupled receptor agonists (LPA and bradykinin), and the EGF receptor (EGFR) and their effect on the regulation of COX-2 expression in colonic myofibroblasts. A graphical summary of these signaling interactions has now been included in Figure 5. We have previously reported that the increased expression of EGFR, which promotes enhanced LPA-mediated transactivation seen in the current manuscript, involves de novo protein synthesis. Similarly, we reported that the enhanced COX-2 expression seen under these experimental conditions correlates with enhanced production of PGE2 measured by ELISA, along with increases in COX-2 and mPGES-1 mRNA.

Reviewer #2:
We appreciate the insightful comments and have attempted to satisfy all 6 major points, along with the 2 minor points, made by this Reviewer, as outlined below. We believe that the inclusion of this information complies with all of the points made by Reviewer #2.

Major Points
1. A signaling pathway diagram has been added as an additional figure (Figure 5).
2. In a previous manuscript (Yoo et al, Am J Physiol Cell Physiol. 2009 Dec;297(6):C1576-87) we reported that TNF-α, in combination with a G protein coupled receptor agonist, up-regulates COX-2 and mPGES-1 mRNA levels in association with elevated PGE2 production that directly mirrors the up-regulation of COX-2 protein expression. Similar patterns of COX-2 protein expression and PGE2 production were seen when 18Co cells were treated with TNF-α and LPA (Rodriguez Perez et al, Am J Physiol Gastrointest Liver Physiol. 2011 Apr;300(4):G637-46) through a conserved pattern of activation. The focus of the
current manuscript is on COX-2, which has been strongly associated with the inflammatory response and with the development of colorectal adenomas and carcinomas. However, we agree that COX-1 is also a highly relevant enzyme. The regulation of COX-1 by TNF/EGF/LPA has not been previously described and is the focus of future investigations.

3. We agree completely. This was tested and was previously reported by our group in Rodriguez Perez et al, *Am J Physiol Gastrointest Liver Physiol.* 2011 Apr;300(4):G637-46. As suggested by Reviewer #2, treatment of 18Co cells with either TNF-α or LPA alone led to low levels of COX-2 expression and PGE2 production, as measured by ELISA. However, the combination of TNF-α and LPA not only lead to enhanced COX-2 expression, but this also corresponded to a synergistic increase in PGE2 production.

4. Statistical analysis was performed using a paired *t*-test.

5. A more representative Western blot has been added to demonstrate the effects that were quantified on densitometric analysis.

6. We have used both α-SMA (references # 8 and 11), and total ERK2 (reference #7 and #8), to confirm equal protein loading in these cells under similar experimental conditions. In fact, both α-SMA and ERK2 were used for this purpose in the same manuscript that we previously published (Reference #8), which demonstrated equivalency.

**Minor Points**

1. This information has been updated in the Methods Section.
2. The time line in Figure 1A has been appropriately changed.

**Editorial Requests:**

1. Specific details about institutional approval for the research involving human subjects were added to the Methods section of the manuscript.
2. Informed consent was obtained. This was added to the Methods section.
3. A section on Competing Interests was added.
4. A section on Author’s Contributions was added.
5. A section on Acknowledgements was added.

We believe that we have substantially modified the original version of our manuscript to comply with the points raised by the Referees. We hope that this revised version of manuscript #5734969227600876 is now suitable for publication in *BMC Gastroenterology.*

Sincerely yours,

James Yoo