**Author's response to reviews**

**Title:** The role of prostaglandin E2 (PGE2) in toll-like receptor 4 (TLR4)-mediated colitis-associated neoplasia

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**Author's response to reviews:** see over
June 7, 2010
Rachel Neilan, MSc
Assistant Scientific Editor
BMC-series Journals
BioMed Central
Floor 6, 236 Gray's Inn Road
London, WC1X 8HL

Dear Dr. Neilan,

We are submitting the second revision of our manuscript (1140468510350742) entitled “The role of prostaglandin E$_2$ (PGE$_2$) in toll-like receptor 4 (TLR4)-mediated colitis-associated neoplasia” by Yasmin Hernandez, John Sotolongo, Keith Breglio, Daisy Conduah, Anli Chen, Ruliang Xu, David Hsu, Ryan Ungaro, Lory A. Hayes, Cristhine Pastorini, Maria T. Abreu, and myself.

We have added the necessary information in the text and highlighted changes to the manuscript in blue text. All of the authors have read the manuscript and agree with its revised content.

We hope that you will find this version of our manuscript acceptable for inclusion in the Journal.

Thank you for your consideration,

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Answer to the editorial requests:

1) As you have added an additional author, Cristhine Pastorini, to the manuscript during the course of your revisions, we require you to provide some clarifications before we can proceed further with your submission.

Please can you explain why this author was not included in the first version of the manuscript and why they deserve to be included now?

Christhine Pastorini joined our laboratory in September 2009. She performed the TUNEL assay for the revision of this manuscript. We asked her to perform this experiment since she is familiar with TUNEL staining.

2. Please can you ensure that both the Competing interests and Authors? contributions sections now accurately reflect the authors of the manuscript. If you need to make any changes, please do so.

We have reviewed the competing interests section and the author’s section and are certain that they accurately reflect the authors of the manuscript. No changes were made.

3. Please ensure that all the authors appear in the correct order both in the manuscript text and online.

We have ensured that the authors appear in the correct order, both in the manuscript and online.

4. All the authors must confirm that they are happy with the changes and the addition of another author by individually emailing editorial@biomedcentral.com. This includes Cristhine Pastorini.

All authors have been contacted and will email the editor as soon as possible.

2) Please document ethical approval. Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.
All our experiments in the current study were done according to Mount Sinai School of Medicine and University of Miami Miller School of Medicine animal experimental ethics committee guidelines and the protocol has been approved by the Institutional Animal Care and Use Committee (IACUC). We added the necessary information in the text. Human subjects are not involved in the current study.

We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

Reviewer: Naofumi Mukaida
Reviewer's report:
It is widely known that COX-2 is downstream of TLR-4 and that COX-2-derived prostaglandin (PG) E2 plays a central role in colon carcinogenesis. The authors speculated that exogenous PGE2 disturb the balance between prostaglandin E2 and 15d-PGJ2, thereby accelerating colon carcinogenesis, but they failed to provide any experimental evidence. Moreover, the authors demonstrated that PGE2 could increase the amount of AR and phosphorylation of EGF receptor without any effects on proliferation and apoptosis of colon epithelial cells. In conclusion, the authors failed to provide any definitive mechanistic insights on how PGE2 can promote colon carcinogenesis, even in this revised text.

In the current manuscript, we show the role of PGE2 in TLR4-mediated colitis associated tumorigenesis. This issue has not been addressed in pre-existing research.

We have demonstrated that TLR4-/- mice have defective epithelial repair from acute mucosal damage and that this is due to defective mucosal production of Cox-2 and subsequent PGE2 synthesis (Fukata et. al., Am J Physiol 2005, Fukata et. al., Gastroenterology 2006). We have also found that the sustained activation of this process results in tumorigenesis. However, we did not know if PGE2 production is necessary or sufficient to promote tumorigenesis in the absence of TLR4 signaling. In this study, therefore, we sought to better understand the role of PGE2 in TLR4-mediated colitis-associated intestinal tumorigenesis.

In this manuscript, we show that high dose (but not low dose) PGE2 treatment (during recovery of colitis) significantly induced intestinal epithelial cell proliferation in TLR4-/- mice. This indicates that PGE2-induced epithelial proliferation in TLR4-/- mucosa is dose dependent. Corresponding to the increased epithelial proliferation, TLR4-/- mice treated with high dose (but not low dose) PGE2 have increased size and number of tumors compared with control TLR4-/- mice in our CAC model. Although both high dose and low dose PGE2 treatment induced up-regulation of AR and EGFR activation, only high dose PGE2 had increased mucosal expression of Cox-2. Since Cox-2 is the key
upstream mediator of AR and EGFR in our model, we reasoned that the positive feedback loop composed of Cox-2-AR-EGFR induced by sustained PGE2 production (during recovery from colitis) is the mechanism underlying TLR4-mediated colitis-associated tumorigenesis. This idea is the key point of the current manuscript and is demonstrated in Figure 7.

The current manuscript also shows the distinct role of PGE2 during the acute and recovery phases of colitis. Based on this study and our previous findings (Fukata et al., Gastroenterology 2006), we believe TLR4-mediated induction of PGE2 during acute colitis is indispensable for proper mucosal repair and that this induction of PGE2 (during acute colitis) is not associated with colitis-associated tumorigenesis. Altered balance of cell-proliferative PGE\textsubscript{2} and other endogenous anti-inflammatory prostanoids was suspected as the mechanism for the distinct effects of PGE\textsubscript{2} during the recovery and the acute phases of colitis. Although we do not see a difference in mucosal 15d-PGJ2 synthesis, endogenous mucosal PGE2 is significantly increased in the mice treated with PGE2 during recovery compared to those treated with PGE2 during acute colitis. By contrast, there is a significant increase of mucosal 15d-PGJ2 when PGE2 is administered during acute colitis, while no increase of endogenous PGE2 is observed. These results indicate that there is a stimuli that induces 15d-PGJ2 during active colitis but not during recovery from colitis and that the ratio of PGE2 vs. 15d-PGJ2 (cell-proliferative vs anti-inflammatory) prostaglandins are balanced only in the active phase of colitis. Thus our results indicate that without such a stimuli (to induce 15d-PGJ2 production), intestinal mucosa cannot maintain the balance between PGE2 and 15d-PGJ2 during the recovery phase.

Based on these results and support from our previous reports, we believe that the current manuscript shows the role of PGE2 in TLR4-mediated colitis associated tumorigenesis.