

MICHIGAN STATE
UNIVERSITY

January 11, 2016

Ze-Mao Gong
Scientific Editor
World Journal of Gastroenterology

Re: **ESPS manuscript NO: 31878**. Oxidative stress-induced mitochondrial dysfunction in a normal colon epithelial cell line, by Nandakumar Packiriswamy *et al.*

Dear Editor:

Please consider the revised article entitled “Oxidative stress-induced mitochondrial dysfunction in a normal colon epithelial cell line” authored by Nandakumar Packiriswamy, Kari Coulson, Susan Holcombe, and L.M. Sordillo for publication within *World Journal of Gastroenterology*.

We appreciate the opportunity to respond to the peer-review comments. Indeed, after incorporation of all the suggestions, we feel that the manuscript has been improved greatly. The following is our response to each of the reviewer’s comments:



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Reviewer 1: code 01199011

Comment 1. Fig 1, how was intracellular level of these cytokines measured? Need to show mRNA data.

AU response. Figure 1 represents ELISA data and not PCR data. The authors prefer to use actual protein expression since there could be some post-translational regulation of these cytokines, especially IL1. We did revise the document to ensure that there would be no confusion as to how cytokine concentrations were evaluated in this study.

Comment 2. Combine Figures 1 and 2.

AU response. Figure 1 represents cytokine expression whereas Figure 2 summarizes ROS production. The authors respectfully request to leave these figures separate to avoid confusion when presenting the findings. If the editors agree with the reviewer, we would of course combine as suggested.

Comment 3. Figure 2 at 6 hr and 12 hr, oxidative stress induction fold is different. In case of HKC, it was higher than that of heat killed E. coli, but 12h, it is lower. Please discuss.

AU response. Thank you for this comment. We treated the CRL.1790 cells with two different forms of microbial stimulation, HKC and heat-killed E. coli. E. coli, a gram negative bacteria, contains lipopolysaccharide (LPS) in its cell membrane which binds TLR4. The HKC is a polymicrobial stimulant that includes flagellin which binds TLR5 and TLR9 and protozoal components in addition to LPS and bacterial DNA which resulted in different magnitudes and timing of ROS generation and alterations in mitochondrial function and integrity. We modified the discussion to speculate on the differential timing of response due to the very different types of microbial stimulation as this reviewer suggested.

Comment 4. To clarify, marked treat time for HKC or E. coli somewhere in Fig E,F.

AU response. Thank you for bringing this omission to our attention. We now added the information to Figure 3 as the reviewer suggested.

Comment 5. Please check mitochondrial complex level after treatment of microbial stimulation.

AU response. We reviewed all our data relating to mitochondrial changes following microbial stimulation as the reviewer suggested to ensure it is accurate and complete.

Please let us know if there should be any other modifications to the revised text.

Sincerely,



Lorraine Sordillo
Professor and Meadow Brook Chair