Response letter to the reviewer's comments

Manuscript TOXSCI-11-0624.R1
MS title: “Chronic Nod2 Stimulation Potentiates Activating Transcription Factor 3 and Epithelial Pro-inflammatory Chemokines by Muco-active Ribotoxic Insults”

Dear Referees,

We really appreciate your kind comments on my manuscript. It was very nice chance to look deeply again into the results and scientific meaning with your precious comments and we did best to come up with the every requirements from the reviewers. We will follow up each comment and suggest responses.

Sincerely yours,

Yuseok Moon, corresponding author

Comments of reviewer 1

1. While the title is improved, it still does not capture what seems to be the unifying theme of the paper - the HuR RNA binding protein. This seems to be the mechanism that ties the ATF3 component and the cytokine expression component of the work together. I would suggest Chronic Nod2 Stimulation Potentiates Expression of ATF3 and Chemokine Expression Induced by Muco-active Ribotoxic Stressors via RNA Binding Protein Human Antigen Rh

Response: Yes, we totally agree with the opinion. Thus we added HuR protein in the title as “Chronic Nod2 Stimulation Potentiates Activating Transcription Factor 3 and Paradoxical Super-induction of Epithelial Pro-inflammatory Chemokines by Muco-active Ribotoxic Stressors via RNA Binding Protein Human Antigen Rh”

2. Along the same lines as comment 1, the involvement of HuR in the mechanism should be added to the last sentence of the introduction which presumably captures the take-home findings
of the paper.

**Response:** Yes, we totally agree with the opinion. We suggested the take-home mechanism of this study by adding “Mechanistically, elevated gene expression of ATF3 and chemokines was post-transcriptionally modulated by RNA binding protein Human antigen R (HuR).”

3. Abstract line 14: potentiated superinduction is redundant change to Nod2 also led to superinduction of □c

**Response:** Yes, we changed the expression as “Functionally, chronic stimulation of Nod2 also led to superinduction of pro-inflammatory chemokine genes by the muco-active ribotoxic stress.”

4. Page 4 second paragraph define PRR

**Response:** Yes, it was change to “modulates pattern recognition receptor-stimulated…..”

5. Last sentence of first paragraph of introduction Nod2 mediated down-regulatory control of what? Sentence is missing key words.

**Response:** Yes, it was changed to “the questions that emerge from this study begin with the hypothesis that Nod2-mediated down-regulatory control of pro-inflammatory responses to various mucosal insults is required for intestinal homeostasis.”

6. Page 5 last paragraph of introduction delete hyphen in stress-triggered

**Response:** The hyphen is required since “mucosal ribotoxic stress-triggered pro-inflammatory chemokines” is the subject in the sentence.

7. Description of RNA IP in materials and methods is still not clear on how cells were treated. Text only mentions treatment with DON while figure shows both DON and MDP treatment. Also, figure legend should explain RNA IP figure more clearly what does HuR input refer to, and what gene is RT-PCR measuring?

**Response:** Yes, we put the additional information (highlighted) in the method section and figure legend.

**Methods:** “Briefly, HCT-8 cells were seeded at 2.5 × 10^6/100-mm dish in complete RPMI and grown for 24 h. HCT-8 cells were pre-treated with vehicle control or 10 μg/ml MDP for 48 h, and then exposed to the vehicle or 500 ng/ml DON (ribotoxin) for 1 h. After DON or vehicle DMSO treatment, protein and RNA were cross-linked with 1% formaldehyde.”
**Figure legend:** “RNA-bound HuR protein was immunoprecipitated. As the input control, precipitated HuR was detected by Western blot analysis. The extracted RNA was measured using conventional and real-time RT-PCR to measure the ATF3 transcript. All results are representative of three independent experiments. A blot with ATF3 cDNA represents data using conventional RT-PCR and box data represents real time RT-PCR analysis of bound ATF transcript (box data).”

8. The manuscript requires careful editing throughout.

**Response:** Yes, we tried to do best for careful editing supported by another native speaker.

**Comments of reviewer 2**

The author made a careful effort to respond to reviewer comments