March 17 , 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 1919-review.doc).

**Title:** Refractoriness of Interferon-β Signaling through NOD1 Pathway in Mouse Respiratory Epithelial Cells using the Anticancer Xanthone Compound

**Author:** Zaifang Yu, Jarrod D. Predina and Guanjun Cheng
**Name of Journal:** World Journal of Biological Chemistry

**ESPS Manuscript NO: 1919**

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated
**Format has been updated**
2 Revision has been made according to the suggestions of the reviewer
All changes are marked in red.

**Comments To Authors** (1) This manuscript examined the role of DMXAA, a drug that is currently in clinical trials for lung cancer, in regulating the changes of IFN pathway in several mouse macrophage cell lines. Real time-RT-PCR assay was conducted to assess the mRNA levels of several genes in the IFN pathway. They found that refractoriness occurred when these cells were re-exposed to DMXAA. The drug refractoriness of anti-viral drugs is a very important topic, and the findings presented in this manuscript are generally interesting. However, the manuscript could be further improved if the authors can address some of the issues. Comments: 1. After a long exposure to DMXAA, it is interesting to find that these cells lost the response or even inhibited some of the gene expressions such as IP10, IFN-β and NOD1 (Figure 2). However, the whole study failed to determine the protein levels of any of the genes that they assessed. Is it possible that the proteins were induced at the early time points and sustained at high levels? If the protein levels were still high at 72 hrs, then the cells did not need to further induce the mRNA? It would be nice to show the changes of the protein levels for IP10, IFN-β and NOD1.

**RESPONSE (1)** We fully agree with this comment of the reviewer. Unfortunately, we were not able to find a good anti-mouse NOD1 antibody to assess protein levels in vivo or in vitro. However, we think the point brought up by reviewer is extremely important. We concur it possible that the proteins were induced at the early time points and sustained at high levels. As the first point in our response, we would like to reiterate to Reviewer that there is at least one published paper (in the JEM, 204, 1559) that provides a proof of principal for our observations. This is the carefully-done paper of Roberts et al. (with Vogel as a correspondence author) at Fig. 3A showing that DMXAA dramatically induced levels of IFN-β mRNA at 2 hrs and IFN-β protein at 24 hrs in macrophages. In one of the seminal papers (Shirey et al, JLB, 89, 351), if one looks closely at Fig. 4E, there is a significantly increase in IFN-β protein measured by ELISA in lung homogenates of DMXAA-treated mice at 3, 24, 48 and 96 hrs. Similar responses are seen in Fig. 1 and Table S1 of Cheng et al (AJRCMB, 45, 480) and Fig. 4 of Sun et al. (Biochem Pharmacol, 82, 1175). IP10 protein levels significantly induced by DMXAA was also observed in above papers and in this manuscript at Fig. 1B, Fig. 2B and Fig. 6E.

**Comments To Authors** (2) The authors indicated that results were presented as mean ± SEM in the figure legend, but it is not clear how many samples or experiments were performed for these studies. Please specify

**RESPONSE (2).** The data were presented as results representative of two to three independent experiments and three to five samples were used for each condition.

**Comments To Authors** (3) The manuscript only contained 4 figures. The supplemental figures should be included in the text.

**RESPONSE (3)** As reviewer suggested, the supplemental figures were moved into the main body of the paper so that all figures were included in the text.

3 References and typesetting were corrected
**References and typesetting were corrected**

Thank you again for publishing our manuscript in the World Journal of Biological Chemistry.

Sincerely yours,

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