ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the revised manuscript “Involvement of Cbl-b-mediated macrophage inactivation in insulin resistance.”

Manuscript NO: 25901

The manuscript has been improved according to the suggestions of reviewers:

Reviewer 02945927

General points

1. Avoid to repeat phrases in the text.

Reply: We abbreviate insulin resistance to IR and rewrite several sentences.

2. there are many self-references. Please include other evidence.

Reply: We removed some self-references and add new references instead of them.

3. Figure 1 does not provide the text.

Reply: Page 6 line 105. We added “(Fig. 1)” in revised MS.

4. Figure 2 can be fragmented during reading for better complement the idea.

Reply: Thank you for your helpful comment. We separated original Figure 2 to Fig. 2 and 3 in revised MS.

Minor points

1. Page 5 line 71. Give examples.

Reply: We have added sentences on page 5 line 71-73, as follows; The oxidative stress induced by aging causes mitochondrial dysfunction and muscle atrophy. Sarcopenia, aging-induced skeletal muscle loss, decreases energy expenditures and causes

obesity[4].

2. Page 5 line 77. Give examples.

Reply: We have added sentences on page 5 line 80-85, as follows; Resident eosinophils and regulatory CD4+ helper T cells maintain homeostasis in the adipose tissue of lean subjects[6]. In contrast to CD4+ T cells, CD8+ T cells increase in number in the adipose tissue of obese subjects and promote the inflammatory responses mediated by macrophages[7].

3. Page5 lines 80-85 the idea is not connected.

Reply: Thank you for your helpful comment. We edited sentences and add some references reported by other groups on page 6 line 85-90, as follows; Because ATMs play a critical role in obesity-associated inflammation, the suppression of ATM activation is an attractive therapeutic strategy for treating obesity-induced IR. Recently, several studies demonstrated that the ubiquitin ligase Casitas b-lineage lymphoma-b (Cbl-b) is a key regulator of macrophage activation[8-10]. Here, we review the key roles of Cbl-b in ATM activation and the pathogenesis of IR in aging-induced and HFD-induced obesity.

4. Page 6 line 89 add "There are three..."

Reply: Page 6 line 93. We added “There are”.

5. Page 7 line 113 Give examples.

Reply: We added the example for F328L mutations in *Cblb* genes on page 7 line 117-119, as follows; Yokoi et al.[22] reported that F328L is a loss-of-function mutation in T cells that was identified in Japanese subjects.

6. Page 7 line 133. explain more about fetuin A

Reply: We added sentences on page 8 line 138-142, as follows; Fetuin-A mediates

SFA-induced activation of TLR4 by directly interacting with TLR4 in macrophages and adipocytes[29]. Interestingly, treatment with the insulin sensitizer pioglitazone suppresses fetuin-A expression through peroxisome proliferator-activated receptor-γ activation in hepatoma cells[30].

7. Page 8 line 143. Give examples.

Reply: We added sentences on page 8 line 151-154, as follows; JNK is a TLR4 signaling molecule and mediates the expression of pro-inflammatory cytokines in macrophages. Hematopoietic cell-specific deletion of JNK1 ameliorated HFD-induced IR by suppressing adipose tissue inflammation in mice[34]..

8. Page 8 lines 158-159 what are Vav1 and Syk?

Reply: Page 9 line 168-169. We added “the guanine nucleotide exchange factor”.

Page 9 line 170. We added “spleen tyrosine kinase (Syk)”.

9. Page 9 lines 170-175 Rewrite.

Reply: We have rewritten on page 9 line 181-183, as follows, Several ubiquitin ligases have been identified as negative regulators of TLR4 signaling[49-52]. Triad3A is a RING finger ubiquitin ligase and directly binds to TLR4, resulting in ubiquitination and proteolytic degradation.

10. Page 10 line 193. Give reference.

Reply: We added sentences on page 10 line 201-206, as follows; Palmitate-induced JNK phosphorylation and IL-6 expression were enhanced in Cbl-b-deficient peritoneal macrophages. We also showed that TLR4 is a substrate for Cbl-b in the presence of SFAs. Overexpression of Cbl-b increased the ubiquitination and degradation of TLR4 after palmitate treatment. Consistent with this finding, the TLR4 protein expression levels on the surface of Cbl-b-deficient peritoneal macrophages were increased.

Reviewer's code: 03413692

1. A table resembling the characteristics of the considered studies should be provided.

Reply: Thank you for your helpful comment. We added the table on page 22.

2. It would be interesting to evaluate the role of prediabetes in such a condition. Please discuss the paper from Ciccone MM et al. Ciccone MM, et al. 2014;5:364. doi: 10.4172/2155-6156.1000364

Reply: We added sentences in CONCLUSIONS section on page 11 line 224-229, as follows; Obesity causes various diseases through the development of IR, which is a clinical feature of patients with type 2 diabetes. Prediabetes is defined as impaired fasting glucose, impaired glucose tolerance and/or high levels of plasma glycated hemoglobin and is a critical risk factor for cardiovascular diseases[59]. Adipose tissue inflammation is thought to be associated with the onset of prediabetes[60]. Therefore, to prevent type 2 diabetes, the development of ab effective therapeutic strategy for obesity-induced IR is urgently needed.

Reviewer's code: 02944873

This manuscript is well written; however I recommend to summarize the data in a table for better understanding of the results.

Reply: Thank you for your helpful comment. We added the table on page 22.

Reviewer's code: 02822399

Authors needs to organize their ideas and add to summarize their work in tables. Some typos and grammatical errors.

Reply: Thank you for your helpful comment. We added the table on page 22.

We have revised manuscript substantially for language, grammatical and type errors,

has been followed in this revised MS.