**AUTHORS' RESPONSES TO THE REVIEWERS' COMMENTS**

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Generation of induced secretome from adipose-derived stem cells specialized for disease-specific treatment: an experimental mouse model

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We thank the reviewers and the associate editor very much for their insightful and valuable comments. In this document, we quote the reviewers' comments in **bold type**; our replies follow in regular lettering. Moreover, we corrected a few minor improper expressions and grammatical errors that are not specifically mentioned here; we hope that this is acceptable.

**TAA-isecretome treatment in vivo mice model showed the recovering of the damaged hepatocyte by TAA treatment. This study is well organized and interesting, and further give us the possibility of isecretome therapy as a disease specific approach. Although secretome analysis was performed by LS-MAS in this study, interpretation of the data of the contents of isecretome is insufficient in the present version. Comments:**

**1) Many scientists want to know what are the essential protein/gene/enzymes in secretome from adipose-derived stem cells, and which factors detected by LS-MAS reflect inflammation, differentiation, proliferation and apoptosis. Thus, explanation and interpretation of data on secretome analysis is limited. More detail explanation and discussion of the data (Fig 6B) should be required.**

RESPONSE)

Thank you for your insightful and relevant comments. According to your kind comment, we further investigated to detect the essential protein/gene/enzymes in secretome from ASCs. Please refer to our revised figure 6 and related descriptions in the result, discussion, and figure legends. Briefly, we investigated 10 proteins that had been identified in TAA-iCM, but not in the CM. To predict affected pathway among the differentially expressed genes of the 10 proteins, gene onthology (GO) enrichment analysis were performed using DAVID (http://david.abcc.ncifcrf.gov/). GO enrichment analysis identified the 19 enriched biological networks of the 10 proteins that had been exclusively identified in TAA-isecretome. Of the 19 enriched biological networks, two most prominent biological processes were the response to reactive oxygen species and cell redox homeostasis, all of which were related with antioxidant activity. Of the 10 proteins, peroxiredoxin-1 (Prdx-1) attracted our attention because it is known to have potent antioxidant activity. We performed Prdx-1 inhibition test for the determination of the role of Prdx-1 in the hepatic reparative process. Prdx-1 inhibition test validated that Prdx-1 plays a central role in the protection of TAA-induced hepatic injury. As a result, we think that Prdx-1 could be one of the essential proteins released from ASCs that had been induced by TAA.

**2) In addition to comment 1, it is still unknown the molecular mechanism of pro-proliferative and antioxidant actions of TAA-isecretome in mouse model of TAA-induced hepatic failure (Fig.4). Molecular mechanism/pathway of them by TAA-Icm infusion should be explained.**

RESPONSE)

Thank you for your valuable comment. We performed additional experiments, and provided the possible mechanism of action of TAA-isecretome. Briefly, TAA gives harm to the liver, principally by way of increasing free radicals, and the mechanism of action of TAA-isecretome is largely dependent on its antioxidant and free radical scavenging activities. The following paragraphs are the explanation regarding the mechanism of action in the revised manuscript.

"The hepatotoxic effect of TAA is attributed to its metabolic intermediate, thioacetamide-S-oxide. It is a free radical which binds to hepatic macromolecules, subsequently leading to necrosis of hepatocytes. Silymarin is one of the best known hepatoprotective drugs, of which mechanism of action is largely dependent on its antioxidant and free radical scavenging activities. Throughout LC/MS analysis, we recognized 10 proteins that had been exclusively identified not in the CM but in the TAA-iCM. GO enrichment analysis of these proteins found that antioxidant processes were the most predominant enriched biological networks. Of the 10 proteins, Prdx-1 attracted our attention because it is known to have potent antioxidant activity.

Prdx-1 is an antioxidant enzyme belonging to the peroxiredoxin family of proteins. It has excellent antioxidant activity by catalyzing the reduction of $H_2O_2$ and alkyl

hydroperoxide, and thus protects cells from the attack of free radicals. In an experiment, Prdx1 knockdown significantly increased the cellular levels of free radicals, while Prdx1 overexpression reversed them [31]. Binding of Prx1 to TLR4 induces the release of numerous cytokines and growth factors, such as IL-6, TNF-α, and vascular endothelial growth factor (VEGF). Moreover, we found that the inhibition of TLR4 by TAK242 (a TLR4 inhibitor) led to significant reduction in the expression of p-ERK (a proliferative marker) as well as Prdx-1. Taken altogether, our results suggest that Prdx-1 is one of representative components released from ASCs that had been induced by TAA, and plays a central role in the protection of TAA-induced hepatic injury."

**3) Although 0.1mM TAA was appropriate concentration of TAA (page 11, lines 6-7 and Figure 1B-1C), figure legend of it (page 25, lines 3-5) indicated 0.25mM. Please check it.**

RESPONSE)

We apologize for the typographical error and appreciate your meticulous correction. 0.1mM TAA is wrong, and 0.25mM is right. The wording has been corrected rightly in the revised manuscript. Thanks again.

Once again we thank you for your response and hope we have been thorough in answering your comments. Your comments have aided us immensely in improving our manuscript. We hope our revision is satisfactory to your high standards and we readily await your next feed-back.

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