Author's response to reviews

Title: Sphingosine-1-phosphate attenuates proteoglycan aggrecan expression via production of prostaglandin E2 from human articular chondrocytes

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Author's response to reviews: see over
Kawasaki, January 17, 2007

Editor, *BMC Musculoskeletal Disorders*

Dear Editor,

Thank you very much for your favorable evaluation of our manuscript entitled “Sphingosine-1-phosphate attenuates proteoglycan aggrecan expression via production of prostaglandin E\(_2\) from human articular chondrocytes” by Masuko K *et al.* We greatly appreciate the reviewer’s helpful comments on the paper, and here please find our revised version, which we are resubmitting for possible publication in *BMC Musculoskeletal Disorders*.

We have addressed the reviewer’s comments point by point below. The manuscript has also been thoroughly checked for grammatical correctness by a native English speaker. We believe that the contents of our revised manuscript will be of interest to the readers of *BMC Musculoskeletal Disorders* and trust that it will now be deemed acceptable to the journal.

We hereby declare that the manuscript has not been submitted elsewhere, nor will it be simultaneously, and no portion of the data has been or will be published elsewhere. None of the authors has any other financial interests that might cause any conflict of interest with regard to the work.

We are looking forward to hearing from you soon.

Sincerely,

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Response to the reviewer #1

Thank you very much for the favorable assessment on our manuscript. As the reviewer suggested, the revised manuscript has been checked by a native English speaker. Statistical calculations were done by a software Prism4™ for Macintosh (GraphPad Software, Inc., San Diego, CA, USA), and the results were re-checked.

Response to the reviewer #2

We appreciate the reviewer’s favorable assessment and constructive comments on our manuscript.

Major points

1. 1-1) The specificity of EDG/S1PR.
   We agree with the reviewer’s point regarding specification of the type of EDG/S1PR receptor involved in the S1P-mediated aggrecan attenuation in chondrocytes. With regard to EDG5, we focused on that particular receptor at first because only anti-EDG5 receptor antibody was available to us at the start the study. As the reviewer indicated, we agree that it would be important to investigate the specificity of each EDG/S1PR receptor in the interaction with S1P in chondrocytes; for example using antibodies or antagonists for respective EDG/S1PR. On the other hand, as shown in Figure 2, it was demonstrated that each patient had a distinct pattern in regard to the EDG expression, indicating that the specificity might be different, rather than common, among the individuals tested. Because of the time limitation for the revision, we will investigate the specificity as a next step. We have brought up the issue on page 13 of the revised manuscript.

1-2) The effect of pertussis toxin in the S1P-attenuated aggrecan expression
   We have conducted an additional experiment to ascertain the effect of PTX in the aggrecan expression. Because of the short deadline for revision, we analyzed one OA chondrocyte sample for the effect of PTX, as shown in Fig. 4B in the revised manuscript. The result demonstrated that PTX could abrogate the suppressive effect of S1P and also suggested dose-dependency. This point should be further investigated using a greater number of samples and the issue has been raised on page 12-13 of the revised manuscript.

2. Dose-dependency of S1P
   Thank you for your instructions. We performed additional experiments using different concentrations of S1P. The results have been
added as new Fig. 2C in the revised manuscript.

3. Table 1
   I am afraid there was some error in the uploading of Table 1. We will make sure that the Table 1 is properly uploaded on submission of the revised manuscript.

Minor points

Thank you again for your detailed instructions. We have corrected all the errors pointed out by the reviewer, and the revised manuscript has been checked by a native English speaker.