Answers to reviewers' comments

Reviewer #1

In this Study, the Authors aimed at investigating whether thrombospondin 2 (TSP2) may induce a chondrogenic differentiation in human adipose-derived mesenchymal stem cells (hADMSCs), potentiating the therapeutic effects of hADMSCs in an in vivo model of osteoarthritis (OA) in rabbits. The chondrogenic potential of TSP2 was investigated in hADMSCs through the expression of chondrogenic markers, as well as NOTCH signaling genes in normal and TSP2 siRNA-treated hADMSCs. In vivo, anterior cruciate ligament transection (ACLT) surgery was performed as a method to induce OA in male New Zealand white rabbits, and stem cells were injected into the injured knees at high or low doses, alone, or at low dose in combination with TSP2. The Authors found that TSP2 increased the expression of chondrogenic markers (SOX9 and collagen II) as well as NOTCH signaling genes (JAGGED1 and NOTCH3) which were inhibited by TSP2 siRNA treatment. In their in vivo studies, treatment with hADMSCs or TSP2 alone resulted in lower degree of cartilage degeneration, osteophyte formation, and extracellular matrix loss. Cartilage damages and the expression of synovial inflammatory cytokines were synergistically decreased when a low dose of hADMSCs was injected together with TSP2.

On the whole, this is a well-prepared, and -conducted study. The method was carefully planned and executed. The results are of interest, especially for their potential clinical implication and application. The discussion and conclusions are in keeping with the experimental findings.

Minor points:

- In Figures 2 and 3 the immunochemical analysis of Sox 9 expression is difficult to follow through the various phases of the experiments, and it doesn't seem to match the gene

expression data in Figure 1. May be the Authors can comment on this.

Answer: Thank you for your valuable comment, and sorry for making you confused! Please see the red-stained SOX9 in central parts of the pellets, because the marginal regions exhibited false positivity due to rhodamine dye deposition.

- In the legend of Figure 4, the use of lettering A-F is confusing, as it is probably intended to refer to the various conditions: Sham, ACLT, SC (stem cells?)….., while each panel has been labeled as A, B, and C to refer to TNF alpha, IL-1 Beta, and IL-6, respectively. The Authors may even remove lettering from inside each panel, as the subject is already specified in the label of Y axis. Similarly, in the legend, listing of A through F is not necessary, as the subjected is already indicated in the body of the figure. Please, just specify that SC refers to hADMSCs. Please, also specify the concentration of TSP2 in the last set of columns labeled as TSP2.

Answer: Thank you for your correction! We removed panel labels A, B, and C, and changed the labels of X axis with A-F, respectively, to describe in a consistent manner as in Figures 6 and 7.

Reviewer #2

The original article by Shin et al., entitled: “Anti-osteoarthritis effect of a combinational treatment with human adipose tissue-derived mesenchymal stem cells and thrombospondin 2 in rabbits” evaluates the chondrogenic potential of TSP2 on hADMSCs in vitro and its synergic effect on cartilage degeneration in vivo using an OA rabbit model (ACLT+exercise). Although TSP2 was already known to promote differentiation to chodroprogenitor cells of hUBMSCs, hADSCs could be advantageous by their enhanced genetic stability in long-term culture, higher proliferation rates and reduced senescence. Therefore, the findings may be clinically

relevant.

For the assessment of the chondrogenic potential of TSP2 on hADSCs the authors evaluated molecular marker levels at the mRNA and protein levels, finding that recombinant TSP2 could rescue the inhibition of chondrogenic differentiation through the upregulation of NOTCH signaling components. In vivo inflammation markers and X-ray imaging and gross observation support a pro-chondrogenic role for TSP2.

It would have been desirable to provide evidence of the silencing of TSP2 in transfected hADMSCs cells at the mRNA and protein levels as control for the silencing levels achieved. However, a drastic effect of chondrogenic and NOTCH pathway markers under treated conditions seem clearly compensated by addition of TSP2.

The paper is clearly presented and easy to read, with the exception of Figure 3 and 4 legends which appear mislabeled A-F ??

Answer: Thank you for your comment! We changed the labels of X axis in Figure 4 with A-F, respectively, not to make readers confused and to describe in a consistent manner as in Figures 6 and 7.

The limited external validity of the obtained results due to assay of ADSCs obtained from a single individual should be added to the Discussion.

Answer: Actually, the characteristics, especially in proliferation and chondrogenic potential, of ADMSCs from individuals are different to some extent according to the age and health conditions. Thus, we selected healthy stem cells from a single individual to obtain consistent results.

Addition of catalog numbers to listed reagents should be provided, particularly for the antibodies and ELISA kits used.

Answer: We described the information.

OCT provider is missing.

Answer: We added the information.

Some minor details are recommended:

Review labeling of Figure 1 “expressin”: corrected

Remove word “of” in Figure 5 legend title: corrected

Line 92 remove “study”: corrected

Line 103, replace “efficacies” by “effects”: corrected

Lines 111 &112 use italics for “in vivo” “in vitro”: corrected

Line 131 remove word “of”: corrected

Line 174 replace “was” by “were”: corrected

Page 10: range is indicated by a hyphen “-“, not by the symbol “~”: corrected

Line 430 remove word “the”: corrected