Author's response to reviews

Title: Increased CCL19 and CCL21 levels promote fibroblast ossification in ankylosing spondylitis hip ligament tissue

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Thank you very much for reviewing and I will explain the problems as follows:

1. I have simplified the introduction part and delete irrelevant material.

2. The average age of the 29 OA patients is 43.48 years. Two reasons are considered: (1) Maximized to reduce the age gap between OA and AS group; (2) The hip ligament tissue of young OA patients are mostly normal, no lesions, no degeneration and easy to obtain. // Pathology of OA group mainly contains hip arthritis, osteonecrosis, femoral neck fracture, both of which are single incidence and don’t implicated ligament tissue. /// Because the disease duration of OA group is various and different from instantly to decades, so this data may be meaningless.

3. Generally, we choose round ligament of the femoral head for our research. If it was ossified, we then select transverse acetabular ligament and orbicularis for use.

I have changed some figures into box and whisker plots such as Fig1.B, C, D and Fig2C and D. // Non-parametric analysis may be preferable, while Mann–Whitney U-test is also possible, so can we retain the statistical methods used in this article? If not, I will revise it. // In fact, we have counted the circle-shaped vascularity. Although increased vascularity of AS ligament tissue was observed, no significant differences were found statistically. And we didn’t staining a marker of endothelial cells because of the above reasons.

4. We have verified the expression of CCR7 many times but the flow cytometry results are not satisfactory, so we choose RT-PCR and Nucleic Acid Gel Electrophoresis to test the expression. The flow cytometry results may reflect the expression of CD90. // we have delete Fig2.A for its meaningless. // 40 cycles were required to obtain each product seen in 2C. // The units of CCL19 or CCL21 is pg/ml. // I have revised Fig.2E and changed the horizontal scale.
5. Dear reviewer, I can’t find the mistakes in the units for BADAI and BASFI. Some paper use Tenth grade, while others adopt hundred mark system. So, I can’t find the errors, could you point it to me? Thank you very much.

Author’s response to reviews 2:

1. We detected the serum CCL19/21 levels in their late stage of AS, however, CCL19/21 levels shows correlation with disease duration, so we guess CCL19/21 may elevate along with the early inflammation status on the onset of AS.

2. It is possible that CCL19 and CCL21 promote ossification directly or by recruiting inflammatory cells and I think further studies are needed to verify this hypothesis.

Special comments

1# Firstly, the tissue we obtain is hip ligament anatomically and we know the main cell type is fibroblast in ligament; secondly, we provide the cell morphology under light microscope, which are probably fibroblasts; thirdly, we choose CD90 as a marker of fibroblast, and most of the cells are CD90 positive. Therefore we consider the cells are fibroblasts.

2# Although IL-17; IL-23; TNF-a are associated with ossification in AS, CCL19/21 are weak related with these cytokines, and few studies have combined these two cytokines together, if we select IL-17 and IL-23, we need another research to explain their relationship. So we choose osteogenic induced-medium as positive controls in this research.

3# In the “methods” part of “Abstract”, we noted that “the CCL19/CCL21 levels in serum and LT were measured via ELISA”. We extracted the supernatant after crushing the ligament tissue and tested CCL19/CCL21 levels. The unit of CCL19/CCL21 is pg/ml.