

**Title:** Multiple intra-articular injections with adipose-derived stem cells for knee osteoarthritis cause severe arthritis with anti-histone H2B antibody production

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**Disclosures:** The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work.

**Abstract**

Osteoarthritis (OA) is the most common form of arthritis. OA results from the breakdown of cartilage, which leads to deterioration of the entire joint and the connective tissue that holds the joint together, and gradually and irreversibly worsens over time. Adipose-derived stem/stromal cells (ADSCs) have been used in the treatment of knee OA. However, the safety and efficacy of ADSC treatment of OA remain unclear. In this study, we investigated the pathophysiology of severe knee arthritis that occurred after ADSC treatment by screening for autoantibodies in synovial fluid from patients who received ADSC treatment. **Methods:** Adult Japanese patients with OA who received ADSC treatment at Saitama Cooperative Hospital between January 2017 and January 2020 were enrolled. Antibodies (Abs) were screened using immunoprecipitation (IPP) with [<sup>35</sup>S]-methionine-labeled HeLa cell extracts. The detected protein was identified by liquid chromatography coupled with time-of-flight mass spectrometry (MS) and ion trap MS, and the corresponding proteins were confirmed as autoantigens using immunoblotting. Ab titers were measured using an enzyme-linked immunosorbent assay. **Results:** A total of 113 patients received ADSC treatment, and 75% (85/113) received ADSC injection at least twice with a 6-month interval between. No obvious abnormalities were observed in any patient after their first treatment; by contrast, 53% (45/85) of patients who received their second or third ADSC injection showed severe knee arthritis. IPP detected a common anti-15 kDa Ab in synovial fluid of 62% (8/13) of the samples analyzed from patients who showed severe arthritis. This Ab was not detected in synovial fluid obtained from the same joints before treatment. The corresponding autoantigen was identified as histone H2B. All available synovial samples from patients who tested positive for anti-histone H2B Ab were newly positive after the treatment; that is, none had been positive for anti-histone H2B Ab before treatment. **Conclusions:** Multiple ADSC injections for OA induced severe arthritis in a high percentage of patients, particularly after the second injection. Synovial fluid from some patients with knee arthritis contained Ab to histone H2B that appeared only after ADSC treatment. These findings provide new insights into the pathogenesis of ADSC treatment-induced severe arthritis.

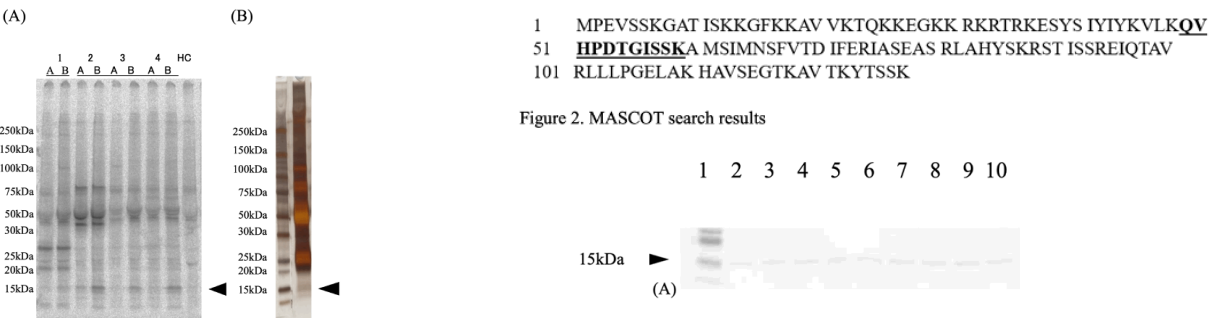


Figure 2. MASCOT search results

Figure 1. IPP of polypeptides with patient synovial fluid samples and silver staining

Figure 3. Immunoblot analysis of histone H2B protein as the corresponding autoantigen