Adipose-derived mesenchymal stem cells act on synovial cells via Gap junction

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INTRODUCTION:
The benefits of mesenchymal stem cell transplantation for treating knee osteoarthritis (OA) have been reported in numerous studies [1,2]. In particular, the secretion of joint fluid by the synovial membrane is considered an important factor in maintaining homeostasis in joints and is being investigated as a tissue that influences knee OA [3]. In addition, gap junctions are involved in stem cell administration, and the expression of gap junctions is frequently observed in knee OA [4]. Based on these findings, we hypothesized that synovial gap junctions play an important role in stem cell therapy. This study aimed to demonstrate the interaction of adipose-derived mesenchymal stem cells (ADSCs) with synovial tissues via gap junctions.

METHODS:
This study was approved by the Institutional Animal Care and Use Committee of our institution. In the in vivo study, a destabilization of the medial meniscus (DMM) model was established in 10-week-old wild-type mice. Four weeks after developing the DMM model, 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF)-labeled ADSCs were administered intraarticularly, and the knee joint was removed 10 min later. We examined the interplay between the synovial lining layer, ADSCs, and gap junctions by multifluorescent immunostaining of F4/80 and connexin 43 (Cx43) using a confocal microscope. To verify the results obtained in vivo, we performed in vitro experiments in which human synovial cells were cocultured with BCECF-labeled ADSCs and observed whether BCECF migrated. In addition, we cocultured synovial cells and BCECF-labeled ADSCs using gap junction inhibitors (1-octanol and carbenoxolone) and inserts, and the inhibition of BCECF migration was evaluated by flow cytometry.

RESULTS:
In the in vivo study, confocal microscopy at low magnification revealed an accumulation of Cx43, which indicates the presence of gap junctions, in the superfical layer of the synovial tissue. Similarly, BCECF-positive cells were observed in the peripheral area stained with F4/80, indicating the presence of the synovial lining (Figure 1a). In addition, confocal microscopy at high magnification revealed the uptake of BCECF labeling in cells surrounded by F4/80 (Figure 1b). In the in vitro study, BCECF migration from ADSCs to synovial lining macrophages was observed through the gap junctions (Cx43) (Figure 2a). Similarly, in the coculture of synovial fibroblasts and ADSCs, BCECF uptake was observed in synovial fibroblasts (synovial fibroblasts were stained with DiD to stain the cell membrane) (Figure 2b). Furthermore, in vitro experiments and analysis by flow cytometry demonstrated the inhibition of BCECF uptake in a coculture of synovial fibroblasts and ADSCs (Figure 3).

DISCUSSION: To date, there have been no reports describing the mechanism of action underlying the association between ADSCs and synovial tissue. It is believed that a vicious cycle occurs when the barrier function of the synovial tissue is disrupted. In this study, we elucidated the involvement of gap junctions in the therapeutic effect of ADSCs for treating OA. In the future, it may be possible to increase the therapeutic effect by increasing the expression of gap junctions.

SIGNIFICANCE: Our findings provide a novel understanding of the healing process in OA and suggest that ADSC transplantation enhances OA healing through gap junction-mediated cell-to-cell interactions. This insight contributes to the inhibition of OA progression and the treatment of inflammatory diseases.

REFERENCES: 1. Rizzo MG et al. *Cells* 2023. **2**. Van Lent PL et al. *Arthritis Res Ther* 2013. **3**. Scanzello CR et al. *bone* 2012 **4**. Marino AA et al. *Clin Orthop Relat Res*. 2004.

IMAGES:

Figure 1: Multiplex fluorescence immunostaining using confocal microscopy at 20× magnification and 63× magnification. The white arrows indicate BCECF-positive cells.

Figure 2: Observation of a coculture of synovial cells and ADSCs using confocal microscopy. The white arrows indicate BCECF-positive cells.

Figure 3: Flow cytometry results indicating that the gap junction inhibitors inhibited BCECF uptake.