Association of Synovial Mesenchymal Stem Cells and Synovial Microenvironment with Hip Osteoarthritis of Different Bone Morphologies

Yang Yang[a], Hideyuki Koga[a], Yusuke Nakagawa[b], Tomomasa Nakamura[a], Hiroki Katagiri[ac]
Ryohei Takada[b], Mai Katakura[a], Kunikazu Tsuji[c], Ichiro Sekiya[d], Kazumasa Miyatake[a]

[a]Department of Joint Surgery and Sports Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[b]Department of Cartilage Regeneration, Tokyo Medical and Dental University, Tokyo, Japan
[c]Department of Orthopaedic Surgery, Tokyo Medical and Dental University, Tokyo, Japan
[d]Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University, Tokyo, Japan
[e]Department of Orthopaedic Surgery, Dokkyo Medical University Saitama Medical Center, Saitama, Japan
yang.orj@tmd.ac.jp

INTRODUCTION: Variations in bone morphology in patients with hip osteoarthritis (HOA) can be broadly categorized into three types: atrophic, normotrophic, and hypertrophic. Despite investigations examining clinical elements, such as bone morphology, pain, and range of motion, our understanding of the pathogenesis of HOA remains limited, particularly with regard to differential osteophyte formation in normotrophic and hypertrophic variants. Previous studies have suggested that osteophytes typically originate at the interface of joint cartilage, periosteum, and synovium, potentially implicating synovial mesenchymal stem cells (SMSCs) in the process. Nonetheless, no thorough assessment of the gene or protein expression within the joint microenvironment has been reported. This study aimed to elucidate the mechanisms that drive the development of bone morphological features in HOA by investigating the association between SMSCs and the synovial microenvironment in different types of HOA.

METHODS: Synovial tissue and fluid were collected from 30 patients who underwent total hip arthroplasty (THA). SMSCs were isolated and cultured from normotrophic and hypertrophic synovial tissues of each hip joint in accordance with the variable bone morphology of HOA patients. Cell differentiation potential was compared using differentiation and colony-forming unit assays. RNA sequencing analysis, cytokine array, and quantitative reverse transcription-polymerase chain reaction (RT-qPCR) were performed to analyse gene and protein expression in the synovial tissue and fluid.

RESULTS: There were no significant differences in the tri-lineage differentiation potential and colony-forming capacity of SMSCs. However, RT-qPCR revealed elevated SOX9 expression levels in synovial tissues from the hypertrophic group. In the RNA sequencing analysis, 103 differentially expressed genes (DEGs) were identified, predominantly related to the interleukin 17 (IL-17) signaling pathway and osteoclast differentiation, as per the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Using a protein-protein interaction (PPI) network, 20 hub genes were identified, including MYC, CXCL8, ATF3, NR4A1 and FOSL1. Among these hub genes, four belonged to the AP-1 family. The cytokine array demonstrated significantly higher levels of CXCL8, MMP9, and VEGF in the synovial fluid of the hypertrophic group than in the normotrophic group, with CXCL8 and MMP9 being significantly expressed in the hypertrophic synovium group.

DISCUSSION: Our study revealed that morphological alterations and osteophyte development in HOA are closely associated with the upregulation of key genes, such as the AP-1 family, in the synovial fluid, along with increased concentrations of CXCL8, MMP9, and VEGF. These molecular changes were more pronounced in the hypertrophic group, suggesting their potential role in severe osteophyte formation.

We noted the expression of SOX9, a key gene involved in cartilage differentiation, was significantly increased in the hypertrophic group. This finding suggests that cartilage differentiation occurs in the synovium under these pathological conditions. Additionally, based on the genomic results, we measured the expression levels of genes in the synovium that might be involved in the IL-17 signaling pathway. The AP-1 family gene FOSL1 and its downstream pathway members, CXCL3 and CXCL8, were significantly expressed in the synovium of the hypertrophic group. Moreover, through a cytokine array analysis of synovial fluid samples from both groups, IL-17A was detectable in the synovial fluid, as predicted by the genomic data. However, no significant difference in the IL-17A levels was observed between the two groups. We hypothesized that in the late stages of HOA, cytokines from the IL-17 family are indeed involved in joint inflammation, but the family member, IL-17A, might not be the key cytokine leading to distinct outcomes. However, our study showed higher levels of MMP9, CXCL8 and VEGF in the synovial fluid of the hypertrophic group than those in the normotrophic group. This is consistent with our genomic analysis and RT-qPCR results, indicating that the expression of AP-1 family genes in synovial tissue was higher in the hypertrophic group than in the normotrophic group. Increased CXCL8 levels correspond to heightened inflammation, which could enhance the proliferative capacity of SMSCs. Notably, VEGF can directly stimulate MSCs, enhancing their osteogenic and chondrogenic differentiation abilities, and thereby causing osteophyte formation and enlargement. Enhanced levels of these markers could potentially stimulate the chondrogenic differentiation of synovium-residing MSCs, thereby exacerbating osteophyte severity.

SIGNIFICANCE/CLINICAL RELEVANCE: This study sought to elucidate the mechanisms driving the development of bone morphological features in different HOAs and offer new insights into HOA pathogenesis and potential therapeutic strategies.

IMAGES AND TABLES:

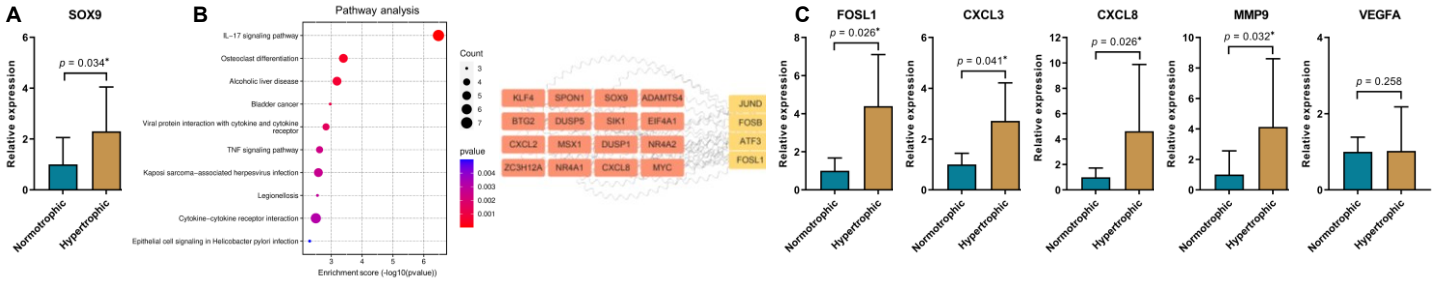

Figure A. Relative expression of *SOX9* in synovium.
Figure B. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway dot-plot analysis of 103 DEGs. Protein-protein interaction (PPI) analysis with betweenness algorithm. AP-1 family members are marked in yellow in the top 20 hub genes.
Figure C. Relative expression of *AP-1*-related genes in synovium and differentially expressed cytokines in synovium.
The data are expressed as mean ± SD, $*p < 0.05$.