HUMAN MESCIMAL STEM CELLS IN SYNOVIAL FLUID INCREASE IN THE KNEE WITH OSTEOARTHRITIS

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INTRODUCTION: The existence of mesenchymal stem cells (MSCs) in synovial fluid was first reported in 2004 [1]. The same group further reported in 2008 that MSCs in synovial fluid numerically increased in early osteoarthritis [2]. This paper is interesting because it sheds light on roles of MSCs in synovial fluid. However, the design of the study seemed to be inappropriate. All synovial fluid examined was obtained from patients with unexplained knee pain and who underwent arthroscopy. Furthermore, the non-osteoarthritis group consisted of individuals with meniscal tears in addition to individuals with no damage to the articular cartilage. Here, we improved the study design and investigated more precisely whether MSCs in synovial fluid increased in the knee with osteoarthritis. First of all, properties of MSCs in synovial fluid were compared to those of MSCs derived from bone marrow and synovium.

METHODS: Properties of MSCs in synovial fluid: The study was approved by an institutional review board, and informed consents were obtained from all study subjects. During total knee arthroplasty for osteoarthritis, synovial fluid, synovium, and bone marrow were collected. Nucleated cells were expanded for 14 days and analyzed. Surface epitopes were evaluated by flow cytometry using a FACS Calibur instrument. For chondrogenesis, 250 thousand MSCs were pelleted, and cultured in DMEM containing BMP-7, TGF-β3, dexamethasone for 21 days. For adipogenesis, MSCs were cultured in αMEM containing dexamethasone, IBMX, and indomethacin for 21 days. For osteogenesis, cells were cultured in αMEM containing dexamethasone, β-glycerophosphate and ascorbic acid for 21 days. For gene profile, 28,000 genes were analyzed with microarray (Affymetrix), and gene expressions were confirmed with quantitative real-time PCR.

Osteoarthritis grading and MSCs in synovial fluid: Synovial fluid was obtained from 6 healthy volunteers, 15 mild, and 29 severe osteoarthritis patients. Nucleated cells were expanded for 14 days, the cell colonies were stained with crystal violet, and total colony number was counted. Kellgren-Lawrence grading was evaluated by radiographs of weight bearing view at 45 degrees of flexion of the knee.

RESULTS: Morphologically, synovial fluid MSCs appeared to be closer to synovium MSCs than bone marrow MSCs, in that synovium MSCs and synovial fluid MSCs were narrower, and their nuclei were more distinct (Fig. 1). Three populations of MSCs were positive for CD44, 90, 105, and negative for CD 34, 45. These results were identical with those of distinctive MSCs, and there were no significant differences of surface epitopes among the 3 populations (Fig. 2). Three populations of MSCs could differentiate into chondrocytes, adipocytes, and osteoblasts (Fig. 3). Hierarchical clustering analysis demonstrated that gene profiles in synovial fluid MSCs were more similar to those in synovium MSCs than in bone marrow MSCs (Fig. 4). To clarify specific genes which were expressed higher in both synovium MSCs and synovial fluid MSCs than in bone marrow MSCs, 3 genes were selected from the result of microarray, and similar results were obtained in 4 other donors with osteoarthritus by RT-PCR analyses (Fig. 5). Representative culture dishes indicated that the colony number of synovial fluid MSC increased according to the radiological osteoarthritis grading (Fig. 6A). Comparing the normal, mild, and severe osteoarthritus groups, the MSC number in synovial fluid increased along with grading of osteoarthritus (Fig. 6B).

DISCUSSION: We previously reported that the number of MSC was positively correlated with the period after ACL injury [3]. Relationship between ACL injury and osteoarthritis is well known. Another study of ours showed that autologous synovial fluid enhanced expansion of MSCs in tissue culture of synovium from osteoarthritus patients [4]. Our current and previous studies suggest one particular mechanism in which synovial fluid from osteoarthritus knee promotes mobilization of synovium MSCs into synovial fluid. Among the same grade of osteoarthritus, number of MSCs in synovial fluid varied widely. To seek some factors relating to MSC number in synovial MSCs in the same grade of osteoarthritus will be important to clarify the functions of MSCs in synovial fluid of osteoarthritus knee. SIGNIFICANCE: This study clarified one aspect of pathophysiological features of osteoarthritus from the standpoint of stem cell biology.