Comparison of Self-Renewal and Differentiation Potential of Synovial Mesenchymal Stem Cells Isolated from Hip Joints Affected by FAIS and OA

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I have something to disclose.

Detailed disclosure information is available via:

- A consultant for Smith & Nephew, ConMed
- AAOS Orthopaedic Disclosure Program on the AAOS website at [http://www.aaos.org/disclosure](http://www.aaos.org/disclosure)
Background

- FAIS especially cam lesions are one of the most common causes of hip pain in the active young adult population, which predispose them to OA.

  Agricola et al, Ann Rheum Dis. 2013
  Philippon et al. KSSTA. 2007

- Despite previous reports have indicated that FAIS may be a trigger for hip OA, the molecular mechanisms have not been entirely elucidated.

- Although the expression levels of inflammatory cytokines, matrix-catabolic genes, and matrix-anabolic genes were analyzed in tissue samples, little is known about the contribution of cell dynamics to these pathologies.

Background

- MSCs are unique candidates for the use in cell therapy as they can be isolated from various types of adult mesenchymal tissue.
  
  - Sakaguchi et al. Blood. 2004

- The proliferative potential and differentiation capacity of synovial MSCs are affected by pathological conditions and depend on the location of the joint.
  

- Clarifying MSC numbers, their proliferative properties, and differentiation potential in FAIS and OA hip tissue is necessary for the development of rational MSC-based therapy of the hip.
Purpose

- To isolate MSCs from the synovium of hip joints of FAIS and OA patients and establish their characteristics.

- To determine whether any functional differences exist between MSCs obtained from these two sources.
Cultures of Colony-Forming Cells in Synovium

Nucleated cells were dispersed from synovial samples by collagenase digestion for 2 hours at 37°C. After 14 days, 3 dishes for each concentration were stained with 0.5% crystal violet.
In Vitro Expandability and Cell Viability Assay
Passage 1 cells were replated at 50 cells/cm² in 150-cm² culture dishes every 7 days until their expansion potential was exhausted.
To determine the live/dead status of cells in each passage, we evaluated with the LIVE/DEAD Viability/Cytotoxicity Kit for mammalian cells.

Epitope Profile
One million passage 3 cells were resuspended in PBS containing a FITC or PE coupled antibody.

Differentiation Assays
Adipogenesis in a Colony-Forming Assay.
Osteogenesis in a Colony-Forming Assay.
In Vitro Chondrogenesis With Pellet Culture Assay.

Quantitative Real-time Polymerase Chain Reaction
Cells that entered adipogenesis or osteogenesis were trypsinized, and total RNA was obtained with use of the RNeasy Mini Plus Kit according to the manufacturer’s protocol.
Results

Comparison of cells derived from FAIS and OA synovial samples.

(A, B) Histological analysis of tissues stained with hematoxylin and eosin.
(C) Representative image of cell colonies derived from donor synovium stained with crystal violet.
(D) Fibroblastic spindle cell morphology of cells at 14 days (passage 0)
Proliferation potential and cell viability

(A) The cells were replated at 50 cells/cm² every 14.
(B) Representative examples of the assay with the LIVE/DEAD Viability/Cytotoxicity Kit
(C) Results of the Cell Counting Kit-8 assay for passage-4 cells. *P < .05.

Flow cytometry analysis
Adipogenic Differentiation potential

(A) Positive staining of colonies with Oil Red-O/von Kossa & ALP and crystal violet.
(B) Fractions of Oil Red-O/von Kossa & ALP positive colonies in relation to the total number of colonies obtained from FAIS and OA synovial cells.
(C) Quantitative real-time polymerase chain reaction results. *P < .05.

Osteogenic differentiation potential
Chondrogenic Differentiation potential

(A) Microscopy view of the pellets. Histological appearance of osteogenic cells stained with (B) toluidine blue, (C) safranin O and immunohistochemical detection of (D) type II collagen, (E) type X collagen staining.

Quantitative real-time polymerase chain reaction results *P < .05.
Discussion

- Intra-articular injections of MSCs into the areas of osteoarthritic or cartilage defect of the knee improved patient self-reported outcome, MRI score, and qualitative histology.
  
  *Jo et al Stem Cells. 2014*
  
  *Sekiya et al. Clin Orthop Relat Res. 2015*

- Although the larger number of more MSCs in OA joint is good for healing the generalized cartilage loss, the proliferative and chondrogenesis ability of these cells might advance OA progression.

- The next step is to investigate the differentiation mechanism in OA-affected joint. If the mechanisms that prompt cells to undergo osteogenesis rather than chondrogenesis in OA joint are established, drugs preventing OA progression may be created more rationally.
Conclusion

- Our findings indicate that synovial MSCs harvested from hip joints of patients with FAIS and OA have different self-renewal properties and multi-lineage differentiation potential.

- Synovial tissue collection must be carried out with maximal efficiency if synovium-derived MSCs are to be harvested from FAIS patients.