Inner meniscus cells maintain higher chondrogenic phenotype compared with outer meniscus cells

INTRODUCTION
Meniscal injuries induce functional impairments of the knee. The key factor in the process of meniscal healing is vascularization[1]. In human meniscus, perimeningual capillary plexus supplies the outer 10-25% of meniscus. Inner 70-80% of meniscus is avascular tissue and hence inferior in healing[2]. Meniscal injury located in the avascular zone has poor healing potential even after meniscal repair[3]. Porcine meniscus cells in avascular inner two-thirds have more chondrocytic phenotype, and the cells in vascularized outer one-third have more fibroblastic phenotype[4]. However, the chondrogenic property of each meniscus cell still remains unclear in human menisci.

Menisci play an important role in controlling complex biomechanics of the knee. The region-specific function of meniscus depends on the composition and organization of its extracellular matrix (ECM)[5]. To understand the specific distribution of cartilaginous ECM molecules in human menisci, we investigated the difference between inner and outer meniscal tissues in ECM deposition, and compared the chondrogenic potential of inner meniscus-derived cells with that of outer meniscus cells.

MATERIALS AND METHODS

Cells and cell culture: Institutional Review Board Approval was obtained before all experimental studies. Meniscus cells were isolated from macroscopically intact lateral menisci obtained at total knee arthroplasties in patients with osteoarthritis of the knee (n=6). Inner and outer meniscus cells were prepared from inner and outer halves of menisci, respectively, by collagenase treatments. Attached cells (passage 0) were subcultured. Meniscus cells between passage 2 and 4 were used.

Cell proliferation assay: Meniscus cells were seeded at 10,000 cells/well on 96-well plates. A serum-free DMEM containing 0.1% BSA was used as a control. Recombinant human TGF-β3 was added into serum-free DMEM at indicated concentrations. Cells were incubated for 48 h prior to addition of WST-1. The optical density (OD, 450-630 nm) was measured. The mean value derived from 5 wells was evaluated.

In vitro differentiation and histological analyses: For chondrogenic induction, pellet-cultured meniscus cells (500,000 cells/pellet) were maintained in chondrogenic induction media for 2 weeks[6]. Mid-posterior section of menisci and chondrogenic pellets were observed with safranin O staining. Immunohistological analyses were performed using anti-collagen type II antibodies. To induce adipogenesis, confluent meniscus cells were cultured with adipogenesis induction media and adipogenic lipid vacuoles were observed by phase-contrast microscope.

RT-PCR and quantitative real-time PCR: For all the RT-PCR fragments, the reaction was allowed to proceed for 28-32 cycles. Differentiation was assessed using the following primers: Sry-type HMG box (SOX) 5′/6′; Scleraxis (SCX), peroxisome proliferators-activated receptor (PPAR)γ, COL1A1, COL2A1, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Relative expression levels were normalized with the G3PDH level of each meniscal cell.

RESULTS

Inner meniscus cells showed chondrocytic morphology compared with outer meniscus cells: Safranin O-stained proteoglycans were not observed in the inner half of lateral meniscus (1A). On the other hand, outer half of lateral meniscus was stained by safranin O dye (1B). Cells packaged in meniscal ECM had round and chondrocytic morphologies in both inner and outer half of meniscus (1A, B). Type II collagen deposition was observed in inner half of meniscus (1C). Inner meniscus cells showed small and ovoid shapes (1E). However, slender and fibroblastic cells were obtained from outer half of meniscus (1F).

DISCUSSION
The present study demonstrated that chondrogenic property was higher in inner meniscus cells than in outer meniscus cells. Human meniscus cells showed the distinct gene expression pattern under chondrogenic condition compared with previous studies that have investigated animal menisci[7]. Our results suggest that the difference between inner and outer meniscus cells in chondrogenic property might have a fundamental role in preserving a zone-specific meniscal feature.

SIGNIFICANCE
Our results indicated that TGF-β stimulated the proliferation of inner meniscus cells and chondrogenic treatments reinforced the chondrocytic property of inner meniscus cells. This study would contribute to achieve a new clinical technique that elevates the healing rate of meniscal injury in inner avascular zones.

REFERENCES