INTRODUCTION

Anterior cruciate ligament (ACL) and medial collateral ligament (MCL) are major stabilizers of the knee. Several authors have reported the difference in cellular potential between ACL and MCL. ACL includes more chondrocytic cells, identified as fusiform, ovoid, and spheroid cells, compared with MCL. We have previously demonstrated that growth factor-stimulated cellular migration is slower in ACL than in MCL fibroblasts. In addition, we have identified chondroblastic interface cells derived from the ACL-to-bone insertion. However, the critical difference between ACL- and MCL-derived cells remains unclear. We hypothesized that the higher chondrocytic feature of ACL could cause disadvantage of ligament repair compared with MCL. In this study, we investigated the property of mesenchymal differentiation in ACL- and MCL-derived cells. The present study demonstrated that ligament-derived cells differentiated into mesenchymal lineages, and that chondrogenic property was higher in ACL-derived cells than in MCL-derived cells.

MATERIALS AND METHODS

Cells and cell culture: Institutional Review Board Approval was obtained before beginning all animal studies. Ligament-derived cells were isolated from ACL and MCL of 10-week-old Japanese white rabbits (n=4). The synovial sheath and fat tissue of ligaments were removed. The midsubstance cores of ACL and MCL were digested using collagenase. Attached cells (passage 0) were subcultured at a density of 5,000 cells/cm² to avoid colony formation. Ligament-derived cells between passage 3 and 6 were used.

In vitro mesenchymal differentiation and histology: To induce adipogenic differentiation, confluent cells were cultured with adipogenesis induction and maintenance media for 3 weeks. Lipid vacuoles were stained with oil red O solution. Osteogenic induction was performed using Mesenchymal Stem Cell Osteogenesis Kit and 1 ng/ml of bone morphogenetic protein (BMP)-2 for 3 weeks. Calcium deposition was visualized by von Kossa staining. For chondrogenesis, pellet-cultured cells (5 x 10⁶ cells/pellet) were maintained in the chondrogenic induction medium (CIM) supplemented with 10 ng/ml of BMP-2 and/or transforming growth factor (TGF)-β3. Pellets were observed with safranin O staining.

RT-PCR and quantitative real-time PCR: For all the RT-PCR fragments, the reaction was allowed to proceed for 28-32 cycles. Mesenchymal differentiation was assessed using the following primers: Sry-type HMG box 9 (Sox9) and α(II) collagen (Col2a1) for chondrogenesis, peroxisome proliferators-activated receptor γ (Ppar γ) for adipogenesis, alkaline phosphatase (Alp) for osteogenesis, and glyceraldehyde-3-phosphate dehydrogenase (G3pdh). Relative expression levels were normalized with the level of each CIM-treated controls. Expression of Sox9, Col2a1, and G3pdh were normalized with the level of each CIM-treated controls.

RESULTS

Ligament-derived cells have a potential to differentiate into mesenchymal lineages: ACL-derived cells differentiated into adipogenic, osteogenic, and chondrogenic lineages (Fig. 1, 2). MCL-derived cells also had a multilineage potential (Fig. 1, 2). Chondrogenic-differentiated pellets in the presence of TGF-β3 (10 ng/ml) were stained by safranin O dye after 3 weeks (Fig. 1, B and F). The intensity of safranin O-stained proteoglycans was higher in ACL-derived pellets than in pellet-cultured cells of MCL origin. The inductions of chondrogenic genes, Sox9 and Col2a1, were faster in ACL-derived cells than in MCL-derived cells. However, the expression of Alp was also detected in both osteogenic pellets of MCL origin. The expression of Alp was also detected in both osteogenic pellets of MCL origin. However, the expression of Alp was also detected in both osteogenic pellets of MCL origin.

DISCUSSION

In this study, we prepared ligament-derived cells without forming mesenchymal stem cell (MSC)-originated colonies. These ligament-derived cells might contain undifferentiated progenitors or MSCs. However, there is no appropriate method to discriminate ligament cells and MSCs, except for cell electrophoresis. Our results suggest that the interaction between ligament cells and undifferentiated MSCs might have a key role in chondrogenic redifferentiation of ligament-derived cells.

REFERENCES