HUMAN RUPTURED ANTERIOR CRUCIATE LIGAMENT-DERIVED CELLS HAVE MULTILINAGE DIFFERENTIATION POTENTIAL TO OSTEOSTATIC CELLS, ADIPOCYTES AND CHONDROCYTES.

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Introduction
Anterior cruciate ligament (ACL) ruptures are difficult to heal and surgical replacement is often required. Although there is a great deal of clinical interest in ligaments, very little has been done to study the healing process and precursor cells participated in repair of the ligament. It is reported that myoblasts proliferated at the end of repaired ACL (1). Other investigators suggested that these cells participated in healing process of the ligament. However, the character and differentiation potency of these cells are unclear.

Recently, Pittenger et al. have demonstrated that adult human mesenchymal stem cells (MSCs) from bone marrow are able to differentiate into osteoblasts, chondrocytes, adipocytes and myoblasts. Successively, muscle-derived cells, adipose tissue-derived cells, trabecular bone-derived cells and synovial membrane-derived cells are reported to differentiate into osteoblastic cells, adipocytes, chondrocytes, and myoblasts. These cells could contribute to tissue homeostasis by replacing differentiated cells lost by physiologic turnover and injury.

The main objective of this study was to define the characteristics of human ruptured ACL-derived cells following rupture.

Materials & Methods
Cells culture
The ruptured ACL tissues were obtained from 5 donors (three male and two female patients, average age, 25) undergoing reconstruction of anterior cruciate ligament after informed consent. The ruptured ligaments were obtained from 10 weeks to 12 weeks after rupture. The tissue was cut into smaller fragments and digested with 0.125% collagenase at 37°C for 24 hours. Culture medium consisted of Dulbecco’s modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin. The cells were incubated in a humidified atmosphere (95% air, 5% CO2) at 37°C. All the experiments were performed using the cells between three and four passages.

Osteogenesis
The cells were plated into 6 well plates at a density of 1.0 × 104 cells/well. After twenty-four hours of cells seeding and incubation, To induce osteogenic differentiation, the cells were cultured in DMEM supplemented with 10% serum, 50 µg/ml 2-phosphate ascorbate, 10 mM β-glycerophosphate and 100 mM dexamethasone. The osteogenic medium was changed twice a week. Alkaline phosphatase (ALP) histochemistry was performed using Sigma Diagnostic Kit 85. For staining calcium deposits, cultured ACL-derived cells fixed with 10% neutral buffered formalin at room temperature, and stained with the Arizarin red S solution for 3 minutes.

Adipogenesis
For adipogenic differentiation, ACL-derived cells were allowed to become confluent. The cells were then cultured in adipogenic induction medium, which was DMEM high glucose (4.5 g/l) containing 10% serum, 1 µM dexamethasone, 0.5 mM isobutylmethylxanthine, 10 µg/ml insulin. After 72 hours, the medium was changed to adipogenic maintenance medium (10 µg/ml insulin in DMEM high glucose (4.5 g/l) containing 10% serum) for 24 hours. The cells were treated 4 times with adipogenic induction medium. After 4 weeks, the cells were rinsed twice with PBS, fixed with 4% para-formaldehyde for 5 minutes, washed with distilled water. For histochemical analysis, cytoplasmic lipid droplets in cell culture treated with adipogenic mediums were stained with Oil red O solution for 30 minutes.

Chondrogenesis
For chondrogenic differentiation, 2.5 x 105 cells pelleted by centrifugation in 15 ml conical tubes were cultured for 4 weeks as high-density cell pellet in a serum-free, chemically defined medium supplemented with 10 ng/ml TGF-β3. After 4 weeks, the cells were histochemically stained and immunostained.

Results
Our studies showed that the cells isolated from human ruptured ACL possess potential to be differentiated into osteogenic, adipogenic and chondrogenic lineages similar to bone marrow-derived mesenchymal stem cells (Fig. 1).

Discussion
Although it is unclear the origin of the ruptured ACL-derived cells used in this study, we may indicate a few possibilities as follows: First, these cells might migrate by inflammatory response series of cytokines and growth factors following rupture of ACL. Secondly, these cells might be tissue-specific stem cells. Tissue-specific stem cells have been detected in several tissues of adult mammals. They are thought to be responsible for tissue regeneration after injury. For instance, it has been reported that tissue-specific multipotent mesenchymal stem cells could be isolated from the synovial membrane of knee joints (2). It might support this hypothesis that gradual growth of epiligamentous and synovial tissue was noted over the ruptured end of the ligament, between three and eight weeks after rupture (1). Finally, these cells might be differentiated by ACL rupture and trans-differentiated into multipotent cells.

These cells may be subsequently used for the regeneration or repair of the ACL. In addition, because we demonstrated that ACL-derived cells could be differentiated into chondrocytes, cartilage tissue could be produced by adequate induction of differentiation of these cells. These cells might be available cell source for tissue engineering of meniscus and articular cartilage, when ACL was ruptured synchronously with damage of meniscus and/or articular cartilage.

References