Cellular and Extracellular Matrix Changes in Human ACL Aging and Degeneration

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INTRODUCTION:
Anterior cruciate ligament (ACL) degeneration can lead to mechanical failure, cartilage damage and the development of osteoarthritis (OA). ACL degeneration is observed in the majority of OA-affected knee joints. Our recent observations indicate that ACL changes, in particular chondroid metaplasia, collagen fiber disorganization and mucoid degeneration can occur prior to the onset of significant cartilage degeneration in individuals without history of knee trauma and already at relatively young age. ACL aging and degeneration are associated with ACL cell death, cell proliferation or recruitment and changes in ACL differentiation and activation. However, the specific spatial and temporal relationship of these changes and their association with extracellular matrix (ECM) degeneration are not well understood. The objective of this study was to characterize cellular and ECM changes in human ACL in aging and OA from a large number of donors across the entire adult age spectrum at all stages of OA development.

MATERIALS AND METHODS:
Human knee joints were obtained at autopsy with approval of the Scripps Human Subjects Committee. In this study 185 human knee joints were analyzed and none of the donors had a history of knee joint trauma. There were 50 male donors and 57 female donors with mean age of 64.4 years (range 23-92 years). Cartilage and ACL were graded macroscopically and histologically. Macroscopic grading of all cartilages was performed using a modified Outerbridge scoring system and the ICRS knee map. The macroscopic appearance of ACL degeneration was classified as normal, abnormal, and ruptured [1]. For histological analysis, ACLs were resected at the insertion sites on the femur and tibia. Transverse and longitudinal sections were harvested from the ACL midsubstance. ACL sections were stained with hematoxylin and eosin and graded histologically using a modification of previously reported scoring systems [2]. To characterize cellular and ECM changes in the ACL in aging and OA, the donor population was divided into 3 groups; young (< 45 years old with normal cartilage), aging (> 60 years old with minimal cartilage degeneration), and OA (> 60 years old with moderate to severe cartilage degeneration). Immunohistochemistry was performed with antibodies to stem cell and differentiation markers, ECM components and MMP-3.

Statistical analysis: Student’s t test was used to determine the significant differences between two groups. The results are presented as mean ± SD (standard deviation). P values of less than 0.05 were considered statistically significant.

RESULTS:
Relationship between ACL pathology, aging and OA
Total cell number in normal ACL decreased with aging. However, in degenerated ACL, cell density increased due to the formation of perivascular cell aggregates and islands of chondrocyte-like cells (Figure 1). MMP-3 expression also was reduced in the normal aging ACL, but increased in degenerated ACL, mainly in the chondrocyte-like cells. Collagen I was expressed throughout normal and degenerated ACL. Collagen II was only detected in the areas with chondroid metaplasia. In normal ACL, Collagen I was expressed throughout normal and degenerated ACL. Collagen III was also detected in regions with disorganized collagen fibers and in the loose connective tissues separates collagen bundles. In degenerated ACL, collagen III was also observed in dense collagenous tissue, perivascular areas, as well as synovial sheath. In degenerated ACL, both chondrocyte-like cells and fibroblast-like cells expressed tenomodulin. Alpha-smooth muscle actin (α-SMA), a marker of myofibroblast subpopulations was detected in normal ACL and the number of α-SMA positive cells decreased with aging in normal ACL. In degenerated ACL the majority of cells in the new cell aggregates were positive for α-SMA. The pattern of progenitor cell marker STR0-1 was similar to α-SMA (Figure 3).

DISCUSSION:
ACL aging is characterized by reduced cell density and activation. In contrast, ACL degeneration is associated with cell recruitment or proliferation, including progenitor cells or myofibroblasts. These cells are activated and abnormally differentiated. This cellular response to ACL tissue damage illustrates regenerative capacity of ACL cells that may contribute to tissue damage as the cells are not programmed towards normal ligament cell differentiation.

SIGNIFICANCE:
This is the first study to address cellular and ECM changes in human ACL and their relationship with cartilage degradation in human knee joints across the entire adult age spectrum and at all stages of OA development.

REFERENCES:

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