Fibulin-3 In Joint Aging And Osteoarthritis Pathogenesis

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Disclosures:

Introduction: Osteoarthritis (OA) is the most common joint disease. Among the earliest lesions during OA development is disruption of the superficial zone (SZ) of articular cartilage. The SZ of articular cartilage contains adult mesenchymal progenitor cells and is unique in extracellular matrix composition. Recently, we found that the EFEMP1 gene encoding fibulin-3 is specifically expressed in SZ. However, the expression pattern of fibulin-3 in articular cartilage and its role is unknown. The objectives of this study were to examine fibulin-3 expression patterns in joint aging and OA and to investigate the role of fibulin-3 in OA pathogenesis.

Methods: Human knee joints were obtained at autopsy with approval of the Scripps Human Subjects Committee. Joints were processed within 72 hours post-mortem. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee at The Scripps Research Institute. Fibulin-3⁻/⁻ mice were generated by McLaughlin et al [1]. Immunohistochemical analysis was performed on human and mouse knee cartilage to determine changes in fibulin-3 expression during joint aging and in OA. To examine the role of fibulin-3 in cartilage homeostasis, experimental OA was induced in wild type and fibulin-3⁻/⁻-mice. The mice were euthanized 10 weeks after the knee surgery for histological analysis [2]. To address whether fibulin-3 is involved in regulating chondrocyte differentiation, human articular chondrocytes were transfected with fibulin-3-specific siRNA. Cells were harvested at 48 hours and 72 hours for quantitative PCR and Western blot analyses. To further address the role of fibulin-3 during chondrogenesis, bone marrow mesenchymal stem cells (MSC) were transduced with lentivirus (LV) encoding EFEMP1 or control LV expressing LacZ, then MSC pellets were prepared and analyzed for chondrogenesis.

Results: Fibulin-3 was specifically expressed in the SZ of normal cartilage in human and mouse knee joints. Fibulin-3 expression was intracellular and in the extracellular matrix (ECM) and declined with aging (Fig.1). Both aging-related OA and experimental OA were significantly more severe in fibulin-3⁻/⁻-mice compared with wild type mice (Fig2). Fibulin-3 expression was high in MSC and decreased during chondrogenesis (Fig.3). Suppression of fibulin-3 by siRNA in MSC significantly increased SOX9, collagen II and aggrecan in articular chondrocytes, while the overexpression of fibulin-3 inhibited chondrogenesis in MSC (Fig.4).

Discussion: We found that fibulin-3 is specifically expressed in the SZ of articular cartilage and its expression is reduced in aging. Fibulin-3 regulates survival and differentiation of adult progenitor cells and its aging-related decline is an early event in OA pathogenesis. Preventing or restoring aging-associated loss of fibulin-3 in SZ chondrocytes has potential to delay or prevent onset of OA.

Significance: This is the first study to address fibulin-3 expression in joint aging and osteoarthritis and indicate that preventing or restoring aging-associated loss of fibulin-3 in SZ chondrocytes has potential to delay or prevent onset of OA.

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Figure 1. Fibulin-3 expression in human and mouse articular cartilage aging.

Patterns of fibulin-3 expression in human articular cartilage were characterized by immunochemistry. In normal cartilage from young donors (n=5, age 27.1±5.4 years), fibulin-3 expression was localized to the superficial zone (SZ) and upper middle zone (Fig. 1A, C). In contrast, expression of fibulin-3 was much lower in human cartilage from old donors (n=6, age 73.5±5.0 years) with non-cartilaginous cartilage (Fig. 1B, C).

Fibulin-3 was localized to the superficial and upper middle zones in mouse articular cartilage. Fibulin-3-positive cells are decreased with aging and almost completely absent in articular cartilage at 12 months (Fig. 1D-G).
Figure 2. Increased severity of surgical OA and aging-related OA in fibulin-3 deficient mice.

Two-month-old C57Bl/6J mice were subjected to OA surgery in the right knee. The left knee was not subjected to surgery and was used as a control. Panel A shows representative images of 2-month old WT and fibulin-3/- mice 10 weeks after surgery. Fibulin-3/- mice had significantly higher histological scores than WT mice when OA surgery was performed at 2 or 6 months (B). Panel C shows representative images of 6- and 9-month old WT and fibulin-3/- mice. Knees from WT mice were almost normal at 6 months and mild Safranin O staining reduction was observed at 9 months. In contrast, fibulin-3/- mice on the same background showed reduced Safranin O staining at 6 months. At 9 months cell death in the cartilage layer was observed (arrow heads). Mankin scores were significantly different between WT and fibulin-3/- mice at 9 and 12 months (D). Values are mean ± SEM. *p<0.05.
Figure 3. Suppression of Fibulin-3 increased chondrogenic markers in cultured chondrocytes.
A) Western blotting showing knock down of fibulin-3.
B) Quantitative PCR shows significant increase of Sox9 and ACAN in human chondrocytes at 48 hours after transfection.
C) Quantitative shows significant increase of ACAN and Col2a1 PCR in human chondrocytes at 72 hours after transfection.

Figure 4. Lentiviral fibulin-3 transduction inhibits chondrogenesis in human MSC.
A) Exogenous lacZ, fibulin-3, and endogenous fibulin-3 expression in human MSC monolayer culture at 72 hours after transduction is confirmed by Western blotting.
B) Safranin-O staining and immunohistochemistry for fibulin-3. At 21 days upon chondrogenesis induction, LVfLacZ pellets shows high Safranin O staining, while the LVfEFEMP1 pellet is negative.
C) Quantitative PCR shows that EFEMP1 is overexpressed, and chondrogenic markers, Sox9, ACAN, and Col2a1 expression are suppressed in LVfEFEMP1 MSC pellets compared with control LVfLacZ pellets at 21 days upon chondrogenesis induction. Values are mean ± SEM. *p<0.05.