Fibronectin Splice Variation in Human Knee Cartilage, Meniscus and Synovial Membrane: Association with Cartilage Degeneration

INTRODUCTION: Knee osteoarthritis (OA) involves pathologic changes in multiple joint tissues, including cartilage, synovial membrane (SM), and meniscus. Extracellular matrix (ECM) reorganization and turnover occurs, along with a low-grade synovial inflammation. One widely expressed molecule that can participate in all these processes is Fibronectin (FN). FN is a glycoprotein with important roles in ECM organization, cell adhesion, migration, and differentiation. Alternative splicing of FN occurs at three major sites: the Extra Domain A (EDA), Extra Domain B (EDB) and Variable (V) regions. Splice variation is associated with different tissue distributions and developmental phases, resulting in functional consequences. EDB+ FN is a marker of angiogenesis. EDA+ FN participates in inflammation, and both EDA+ and V+ forms promote cellular adhesion. Adult cartilage expresses a unique splice variant lacking the entire V region and two neighboring domains (V[C]-FN). EDA+ isoforms are found in OA and RA synovial fluid (SF), and may predict progression in RA. V+ and EDB+ forms are present in OA and normal cartilage, but variation with stage of disease has not been described. Little is known about splice variation in other tissues such as SM. The present studies were carried out to define FN splice variation in cartilage, SM and meniscus, and determine if splicing varies with degree of underlying cartilage degeneration.

METHODS: Cartilage, meniscus, and SM specimens: Knee joint tissues were collected from one organ donor with no history of arthritic disease, but with moderate cartilage fibrillation and fissuring observed during dissection. Additional SM biopsies were obtained from patients undergoing arthroscopic procedures enrolled in an IRB approved tissue repository at Rush University Medical Center. Cartilage integrity was graded intra-operatively using the Outerbridge score. Total RNA was isolated from tissues by homogenization in Trizol (Life Technologies, Rockville, MD). One microgram of RNA was reverse transcribed with Superscript III and oligo-dT primers (Invitrogen, Carlsbad, CA). PCR was performed using primers flanking EDA, EDB and V regions. 30 cycles of denaturing at 94°C for 30 sec, annealing at 55°C-60°C for 30 sec, and extension at 72°C for 30 sec were carried out. PCR products were separated in 1.5-2% agarose gel.

RESULTS: Figure 1: Tissue expression of FN splice variants: Joint tissues were obtained from a single donor with both normal and degenerative areas of cartilage. Lane 1 = meniscus, Lane 2 = SM, Lane 3 = normal cartilage, Lane 4 = esional cartilage. Top: EDB+ FN (390 bp band) species were found in all three tissues. EDA+ transcripts (410 bp band) were weakly detectable only in SM. Bottom: V region RT-PCR demonstrated multiple variants, with bands corresponding to both V+ (1243 to 1076 bp bands) and V- (884 and 471 bp bands) forms in all tissues. The 417 bp [V+C]- cartilage-specific variant was detectable in meniscus and cartilage.

DISCUSSION: We investigated tissue distribution and OA stage dependent expression of FN splice variants. We found that i) EDA+ transcripts were only detected in SM; ii) EDB+ transcripts were detected in all tissues including meniscus; and iii) SM from patients demonstrated transcription of the “cartilage-specific” [V+C]- variant. Most interestingly, both EDB+ and V+ FN expression in SM varied with degree of underlying cartilage degeneration in these arthroscopy patients. Future work should investigate the predictive value of these isoforms on OA disease progression. In the future, SM from patients undergoing total knee arthroplasty will be examined to determine if splice variation differs significantly in end-stage disease compared to this arthroscopy patient population.

SIGNIFICANCE: The lack of biomarkers predictive of disease progression or diagnostic of early-stage OA is a significant barrier to development of disease-modifying treatments. Given our observations, EDB+ and V+ FN should be investigated as potential biomarkers of disease stage or progression in a larger OA patient population.

ACKNOWLEDGEMENTS: * Both DZ Markova and CR Scanzello contributed equally to this work. Y. Zhang is supported by NICHD, 1K08 HD049598-01. C. Scanzello is supported by NIAMS, 1K08 AR057859-02. A portion of this work was also supported by a Career Development Award from the American College of Rheumatology and Association of Specialty Professors (CRS). We thank Drs. Charles Bush-Joseph, Nikhil Verma, and Arkady Margulis for collection of joint tissues, and Anjali Nair for technical assistance.

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