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Characterization of degenerative human facet joints and facet joint capsular tissues

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SUMMARY

Objective: Lumbar facet joint degeneration (FJD) may be an important cause of low back pain (LBP) and sciatica. The goal of this study was to characterize cellular alterations of inflammatory factor expression and neovascularization in human degenerative facet joint capsular (FJC) tissue. These alterations in FJC tissues in pain stimulation were also assessed.

Design: FJs were obtained from consented patients undergoing spinal reconstruction surgery and cadaveric donors with no history of back pain. Histological analyses of the FJs were performed. Cytokine antibody array and quantitative real-time polymerase chain reaction (qPCR) were used to determine the production of inflammatory cytokines, and western blotting analyses (WB) were used to assay for cartilage-degrading enzymes and pain mediators. Ex vivo rat dorsal root ganglion (DRG) co-culture with human FJC tissues was also performed.

Results: Increased neovascularization, inflammatory cell infiltration, and pain-related axonal-promoting factors were observed in degenerative FJCs surgically obtained from symptomatic subjects. Increased VEGF, (NGF/TrkA), and sensory neuronal distribution were also detected in degenerative FJC tissues from subjects with LBP. qPCR and WB results demonstrated highly upregulated inflammatory cytokines, pain mediators, and cartilage-degrading enzymes in degenerative FJCs. Results from ex vivo co-culture of the DRG and FJC tissue demonstrated that degenerative FJCs increased the expression of inflammatory pain molecules in the sensory neurons.

Conclusion: Degenerative FJCs possess greatly increased inflammatory and angiogenic features, suggesting that these factors play an important role in the progression of FJD and serve as a link between joint degeneration and neurological stimulation of afferent pain fibers.

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Introduction

Back pain ranks among the most common medical conditions afflicting American adults. The lifetime prevalence is about 70–85%, with 10–20% experiencing chronic low back pain (LBP)[1]. Facet joint degeneration (FJD) as a source of LBP is often called 'facet joint syndrome'. FJD arising from osteoarthritic changes, which is a common cause of LBP with an incidence of 15–45% among patients with chronic LBP[2–6], has been extensively reviewed in recent articles[7,8].

Facet joints (FJs) are paired zygophyseal joints between two consecutive vertebrae. The paired FJs and their corresponding intervertebral disc comprise a 'three-joint complex' constituting a 'spinal motion segment'[7]. FJs are subject to compressive, shear, and axial loading at the spinal motion segment. FJD may be associated with intervertebral disc degeneration[9–12]. The FJ consists of hyaline cartilage, menisci, synovium, and joint capsule. The medial branch of the dorsal primary ramus courses through the FJ and distributes sensory innervation. The human facet joint capsular (FJC) tissue, defined as the fibrous connective tissue lined with synovium surrounding the joint, is richly innervated with nociceptive nerve fibers, autonomic nerve fibers and mechanoreceptors[13–15]. Additionally, the FJ lies in close proximity to the dorsal root ganglion (DRG)[16].

Cytokine mediators of inflammation, angiogenesis, sensory neuron ingrowth and pain have been implicated as participants in joint degeneration[17–20]. Pathologic loading, which has been associated with the production of cytokines associated with inflammation, angiogenesis, sensory neuron ingrowth and pain in joint tissues[21–23], is associated with FJD[24,25]. Studies have shown that LBP may result from facet hypertrophy, inflammation, or degradation; however, the precise relationship between FJC tissues and LBP is not completely understood[26–29].

Previous studies have demonstrated cytokine release from degenerative FJs and that the levels released are proportional to the LBP symptoms of patients[30,31]. Other joints, such as the knee, have been shown to exhibit neovascularization in association with degeneration. Angiogenesis and associated nerve growth are linked to joint degeneration and pain[17–20]; its underlying mechanisms contributing to FJD require further study. The mechanisms by which FJD progresses to pain formation remain unclear and are the subject of continued investigation.

The goal of this study is to investigate the inflammation, angiogenesis, neuronal ingrowth and pain mediators that occur in FJC tissues obtained from subjects with chronic LBP with degenerative FJs. Additionally, to study functional mechanisms and potential cellular communication between peripheral tissues and sensory neurons, an *ex vivo* organ co-culture system using degenerative FJC tissues and rat lumbar DRGs was developed.

Methods

Human spine tissue acquisition

Donor tissues: Consented asymptomatic organ donor tissue samples were obtained from the Gift of Hope Tissue Network (Elmhurst, Illinois) within 24 h of death. The Gift of Hope Tissue Network provided clinical information about the organ donors from hospital charts and personal history from next of kin. Lumbar spine segments from those donors with no reported clinical back pain symptoms were harvested for our experiments. Each lumbar segment was examined by magnetic resonance imaging (MRI). Intact FJs were removed and processed aseptically. FJC tissues were harvested and the cartilage was visually graded for degeneration from grade 0 (normal), 1–2 (early degeneration) to 3–4 (advanced degeneration) according to the scale developed by Collins *et al.*[32] in conjunction with an established MRI grading system for FJD[10].

Surgical tissues: After obtaining Institutional Review Board (IRB) approval and patient consent, intact FJs were removed from patients with LBP undergoing routine spinal fusion and supplied to us by the Orthopedic Tissue Repository. The FJs were then graded as described above. Tissue sources and detailed tissue information are listed in Table I.

Western blotting (WB)

Total protein from human FJC tissues was extracted using cell lysis buffer (Cell Signaling, Danvers, MA, USA), following the instructions provided by the manufacturer. Protein concentrations of human FJC tissues were determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA). Equal amounts of protein (30 μg protein/well) were separated by 10% SDS-PAGE and then electroblotted onto nitrocellulose membranes for western blot analyses. Immunoreactivity was visualized using the ECL system (Amersham Biosciences, Piscataway, NJ, USA).

Reverse transcription (RT) and real-time polymerase chain reaction

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA), following the instructions provided by the manufacturer. RT was carried out with 1 μg total RNA, using the ThermoScript™ RT-PCR system (Invitrogen) for first strand cDNA synthesis. For real-time PCR, cDNA was amplified using the MyiQ Real-Time PCR Detection System (Bio-Rad Hercules, CA, USA). A threshold cycle (Ct value) was obtained from each amplification curve using iQ5 Optical System Software provided by the manufacturer (Bio-Rad). Relative mRNA expression was determined using the ΔΔCT method, as detailed by manufacturer (Bio-Rad). The primer sequences and their conditions will be provided upon request.

Cytokine antibody array and quantification

An array for cytokine proteins (Cytokine Array, RayBio, Norcross, GA, USA) was used to determine alterations in cytokine levels. For the microarray assay, the directions provided by the manufacturer were precisely followed. Briefly, the membranes were incubated with 2 mL of a 1X blocking buffer at room temperature for 30 min to block membranes. After decanting the blocking buffer, the membranes were incubated overnight at 4°C with either 500 μg total protein extracted from asymptomatic donor controls (FJ grade 0 or 1 with no sign of capsular hypertrophy) or surgical FJC tissues from subjects with symptomatic LBP, followed by biotin-conjugated antibodies. After decanting, all membranes were washed three times with 2 mL of 1X wash buffer I at room temperature for 5 min, followed by washing twice more with 1X wash buffer II at room temperature. Then, all membranes were incubated with a dilution (1:250) of biotin-conjugated antibodies at room temperature for 2 h, and the washing steps were repeated. To visualize and measure the immunoreactivity, these membranes were further incubated with horseradish-peroxidase (HRP)-conjugated streptavidin. Immunoreactivity was visualized using the ECL system (Amersham Biosciences, Piscataway, NJ, USA) and the Signal Visual Enhancer system (Pierce) to magnify the signal intensity. Densitometric measurements were performed by calculating the integrated density values (area relative intensity) for each spot using the Molecular Imager Versadoc MP 4000 System (Bio-Rad) and Quantity One-4.5.0 Basic 1-D Analysis Software (Bio-Rad). The positive control signals of each membrane were used to normalize signal intensity.

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