Published in final edited form as:

Mol Biol Rep. 2011 November ; 38(8): 5335–5339. doi:10.1007/s11033-011-0684-7.

Osteoarthritic Tissues Modulate Functional Properties of Sensory Neurons Associated with Symptomatic OA Pain

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Abstract

Osteoarthritis (OA) is an age-related degenerative disease of cartilaginous tissues that is accompanied by hyperalgesia. Molecular cause and effect relationships between OA and pain remain to be elucidated. In this study, we have developed an experimental ex vivo organ co-culture system with dorsal root ganglia (DRGs) and knee synovial tissues from OA patients or unaffected human subjects. Our results suggest that tissues may generate symptomatic pain by altering the functional properties of sensory neurons. Specifically, we find that the expression levels of genes associated with neuronal pathways (e.g., SP, NK1, NK2, NPYR1, NPYR2, $\alpha 2\delta 1$) or inflammation (COX2/PTGS2 and IL6/interferon $\beta 2$) are clearly elevated in DRG explants cultured in the presence of OA derived synovial tissues. These findings are consistent with a model in which cytokines and pain molecules produced by knee synovium sensitize nociceptive neurons in tissues peripheral to joint cartilage.

INTRODUCTION

Pain is a prominent and disabling symptom of OA, and arthritic pain is associated with inferior functional outcomes and reduced quality of life when compared with a range of other chronic conditions[1]. To develop new therapies to prevent disease progression and relieve pain [2], it is necessary to understand the pathological mechanisms leading to joint degeneration and pain symptoms. Our laboratory and others have mechanistically linked OA to pathological changes in the metabolism of extracellular matrix (ECM) proteins[3–15] that may be controlled by epigenetic [16], epigenomic [17] and systemic [18–20] processes. However, the cause of pain in knee OA remains elusive because the primary site of pathology in OA (i.e., cartilage) does not have neuronal pain receptors that can directly detect tissue injury due to mechanical damage. Nociceptors are located throughout the joint in tissues peripheral to cartilage, including capsule ligaments, menisci, periosteum and subchondral bone[21]. Nociceptive input from the joint is processed in different types of spinal cord neurons, and inflammation could reduce the pain threshold for nociception.

How painful mechanical stimuli from knee OA are converted into electrical signals that propagate along sensory nerves to the central nervous system remains to be fully explored.

One attractive theory is that movement of the joint generates shear stresses on the axolemma of the “free” nerve endings, resulting in the opening of neuroimmune pain pathways and/or gated ion channels, such as calcium or sodium channel causing ectopic neuronal firing in injured tissue. Painful stimuli are exaggerated (hyperalgesia) by changes in the dorsal root ganglion (DRG) that cause in-growth of normally non-pain transmitting nerve terminals into the superficial pain transmitting areas of the Dorsal Horn (‘nerve sprouting’). DRG neurons also express mRNAs that cause atrophy of normal C-fiber nociceptors in these same areas. Nerve sprouting causes non-painful sensory input to be interpreted and passed to the brain as a pain producing signal [22]. Defining the origins and blocking neuropathic pathways is a critical strategy for joint pain management in OA. For example, some pain mediators such as substance P(SP)/tachykinin 1 (TAC1) and Neuropeptide Y (NPY) and their cognate receptors, as well as cyclooxygenase 2 (COX2 or PTGS2) and the calcium channel $\alpha 2\delta 1$ subunit play important roles in the transmission of pain in peripheral and central nervous system [3, 23]. In this study, we have investigated whether synovium from OA patients can modulate the expression of genes associated with neuronal pathways (e.g., SP/TAC1, NK1, NK2, NPYR1, NPYR2, $\alpha 2\delta 1$) or inflammation (COX2/PTGS2 and IL6/interferon $\beta 2$) in DRG using an explant culture system.

MATERIALS AND METHODS

Human tissue acquisition and preparation of knee joint explants.

Normal (grade 0, 1)[24] cartilage, synovial fluid, and synovial tissues, and knee joint capsules were obtained through the tissue repository systems of either the gift of Hope Tissue Donor Network or the Department of Orthopedic Surgery tissue repository system in which patients with no knee symptoms and no history of previous knee injury were recruited from the Rush University Medical Center with full consent. The tissue donors were strictly screened and are negative for HIV and hepatitis. Exclusion criteria also include individuals with presence or history of malignant diseases, a history of connective tissue disease, or with conditions or diseases of unknown etiology. The tissues were collected within 24 hours of death by a pathologist. All donor tissues are assessed and graded using Collin’s Score system[24]

The Collin’s Score involves careful examination of the surface of the articular cartilage. Abnormalities in the gross appearance of the cartilage including fibrillations, full-thickness defects and osteophytes of joint are scored from 0 to 4 based on the gross morphological scale of Collins. Grade 0 shows no cartilage lesions. Grade 1 shows only minor disruptions of cartilage surface, minor fibrillations, flaking, shallow pits or grooves or small blisters without affecting surface geometry. Grade 0 and 1 are considered as “an almost perfect normal” joint. Grade 2 shows deep fibrillation and fissuring, flaking, and/or blistering, early marginal hyperplasia and possibly small osteophytes. Grade 3 shows extensive fibrillation and fissuring and 30% or less of the articular surface is eroded down to the subchondral bone, and osteophytes. Grade 4 shows lips or shelves at the articular margin, greater than 30% of the articular surface eroded down to the subchondral bone, as well as gross geometric changes and osteophytes.

Homogenously-sized cartilage, synovium and joint capsule explants with a 4 mm diameter were isolated from synovial tissues by applying a biopsy punch as previously described¹. The biopsy punch ensures that the layer of synovium tissue remains intact within the tissue structure. The tissues were aseptically weighed before use and each explant has comparable thickness and size to minimize data variability.

DRG co-culture experiments.

The DRGs were harvested from adult Sprague-Dawley rats (250–300 g). Bilateral lumbar DRGs from L1 to L5 (10 DRGs per rat) were carefully removed by aseptical dissection using a stereoscopic microscope. DRGs were co-cultured with knee joint synovium tissue isolated from OA patients with painful joint patients or control tissue from asymptomatic human subjects. DRGs were cultured 5 days with human joint synovial tissue (Fig. 1).

Total RNA extraction, Reverse Transcription, Real-Time Polymerase Chain Reaction: For total RNA extraction, 500 mg to 1 g of joint capsule explants or DRGs (pulverized under liquid nitrogen) were homogenized in Trizol reagent (3 ml) and the samples were subjected to real-time quantitative reverse transcriptase PCR (RT-qPCR) experiments. Total RNA isolated from either tissue or cells was subjected to reverse transcription using ThermoScript RT kit (Invitrogen). For RT-qPCR, cDNA preparations were amplified using the MyiQ Real-Time PCR Detection System (Bio-Rad). Relative mRNA expression was determined using the $\Delta\Delta C_T$ method, as detailed by the manufacturer (Bio-Rad). The standard deviations in samples are based on results from different donors in three separate experiments.

RESULTS

Results from our group and others suggest that pain-related neuropeptides released from synovial fibroblasts, such as substance P (SP/TAC1), induce inflammatory processes the neurokinin/tachykinin receptors (NK1, NK2). These G-protein-coupled receptors are pathophysiologically linked to joint degeneration[25]. Because synovium in OA is heavily enriched with newly formed blood vessels but also nerves [26], we examined whether OA synovial tissues influence sensory neurons in DRGs. Before we initiated our experimental *ex vivo* co-culture of rat DRGs and human OA synovial tissues, we first ensured that basal gene expression of bilateral DRGs harvested from different lumbar levels (L3-L5) are equal. Harvested bilateral rat DRGs were mechanically homogenized to prepare total RNA for RT-qPCR analyses. There were no significant differences in gene expression among the levels of DRGs (L3-L5) for the housekeeping gene GAPDH or other selected genes (e.g., $\alpha 2\delta 1$), (Fig. 2).

To determine the length of incubation time of DRGs, we performed a time-course experiments of DRGs by harvesting explants after 1 to 7 days in culture. Each DRG was cultured in the presence or absence IL-1 β stimulation at 10 ng/mL and analyzed by qPCR. The results indicate that, gene expression levels are steady from day 1 up to day 7, but that gene expression gradually decreases after day 7 (data not shown). Therefore, we selected an incubation period of five days for the explant co-culture experiments.

We co-cultured the DRGs together with human synovial explant tissues prepared from normal or surgically removed OA synovial tissues from patients who have a history of symptomatic knee joint pain. After 5 days of incubation, the DRGs were subjected to total RNA extraction, and analyzed by RT-qPCR using rat-specific primer sets. We observed markedly increased gene expression levels of substance P and its cognate receptors NK1 and NK2 from the DRGs that were co-cultured with OA synovium. In contrast, DRGs that were co-cultured with normal synovium (isolated from knee joints with Colin's Grade G0) demonstrated gene expression levels similar to those observed in controls (DRGs co-cultured with media) (Fig. 3A). We also examined expression of neuropeptide receptors that are associated with chronic pain. For example, neuropeptide Y which transduces the nociceptive signals *via* neuropeptide Y receptors has been shown to be highly expressed in peripheral sensory neurons in chronic pain animal models [27]. Our RT-qPCR analyses clearly demonstrate a striking induction of both receptors (NPY1R, NPY2R) that may lead to enhanced nociceptive signals by neuropeptide Y (Fig. 3B). Transgenic mice that constitutively over-express the $\alpha 2\delta-1$ subunit exhibit enhanced electrical currents that alter voltage-gated calcium channel activation in sensory neurons. These changes exaggerate and prolong neuronal responses in the dorsal horn to peripheral mechanical stimulation and pain behaviors[28]. In addition, a previous behavioral study using the $\alpha 2\delta$ ligand gabapentin, an effective for neuropathic pain, showed alleviation of hyperalgesia in the monosodium iodoacetate (MIA)-induced model for knee joint OA pain[29]. Based on these previous findings, we tested the potential influence of synovial tissues on sensory neurons in DRGs in our *ex vivo* co-culture system. Indeed, the DRGs co-cultured with OA synovial tissues highly upregulated $\alpha 2\delta 1$ expression, whereas DRGs that were co-cultured with either synovium from G0 or G1 knee joints showed no significant alterations in gene expression levels compared to media (control) (Fig. 3C).

We have previously established the pathophysiologic links between prostaglandin E₂ (PGE₂), a downstream product of COX2, and OA [7]. Our results indicate that the COX2-PGE₂ axis combined with IL-1 β synergistically accelerates expression of pain-associated molecules such as inducible nitric oxide synthase (iNOS) and IL-6. Thus, we examined if co-culture of OA synovium tissues changes inflammatory pain modulators such as COX2 or IL-6 in the sensory neurons in DRGs. Our qPCR results demonstrated markedly induced COX2 (Fig. 4A; 7-fold induction, $p < 0.05$) and IL-6 (Fig. 4B; $p < 0.05$) which is less dramatic in terms of the fold-induction. Collectively, our results suggest that synovium tissues in OA potentially influence activation of the sensory neurons in DRGs which may lead to sensitization of knee joint in OA condition. Furthermore, these results may provide initial evidence of nociceptive “cross-talk” between peripheral joint tissues with the peripheral sensory neurons in DRGs for pathological pain pathway in OA.

DISCUSSION

In this we assessed whether there are biological changes in neuronal tissues in response to factors secreted by knee joint explants that are co-cultured *ex vivo*. The key finding of our study is that DRGs respond to factors secreted by synovial tissue from OA patients. The resulting major changes in the expression of neuronal and inflammatory proteins in DRGs

is consistent with in vivo studies [26] and our conceptual model that there are mutual cause and effect relationships between OA associated pain and inflammation.

Joint cartilage and synovium injury influences DRG neurons and sensitizes symptomatic pain perception through the dynamic interactions between neuropathic pathways and OA tissues. Our results suggest that alterations in the functional properties of sensory neurons are a key determinant for the severity of symptomatic OA pain. Early intervention in OA associated pain at the level of the central nerve system (CNS) may represent a novel therapeutic paradigm for the treatment of chronic OA pain

ACKNOWLEDGMENTS

This work was supported by grants from NIH R01AR053220 (HJ Im), the Arthritis Foundation (HJ Im), and the National Arthritis Research Foundation (HJ Im).

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Fig 1.

Diagram of the co-culture model for assessing the effects of OA synovium on neuronal modifications in human knee joint tissue.

Fig 2.

Similar level of basal gene expression in bilateral DRGs harvested from different lumbar levels (L3-L5). Harvested bilateral rat DRGs were mechanically homogenized to prepare total RNA and $\alpha 2\delta 1$ RT-qPCR.

Fig 3.

[A] DRGs that were co-cultured with normal synovium and OA synovium. After 5 days of incubation, the DRGs were subjected to total RNA extraction, and analyzed by RT-qPCR. Our data showed the gene expression of Substance P and tachykinin receptor (NK1, NK2) increased in rat DRGs which were co-cultured with human OA synovium. [B] the gene expression of NPY receptor NPY1R and NPY2R increased in DRGs which were co-cultured with OA synovium.

[C] the gene expression of α2δ1 increased in DRGs which were co-cultured with OA synovium

P<0.05 compare to normal synovium

Fig 4.

[A] DRGs that were co-cultured with normal synovium and OA synovium. After 5 days of incubation, the DRGs were subjected to total RNA extraction, and analyzed by RT-qPCR. Our data showed the gene expression of COX2 increased in DRGs which were co-cultured with OA synovium.

[B] IL-6 in inflamed tissue contributes both to the inflammation itself and to pain hypersensitivity. Our RT-qPCR data showed the gene expression of IL-6 increased in DRGs which were co-cultured with OA synovium.