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Identification of shoulder osteoarthritis biomarkers: comparison between shoulders with and without osteoarthritis

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Background: The biologic factors associated with shoulder osteoarthritis (OA) have not been elucidated. The purpose of this study was to investigate osteoarthritic biomarkers of the shoulder. To our knowledge, this is the first study to analyze shoulder cartilage for OA-associated genes and to examine human shoulder cartilage for a possible biomarker, connexin 43 (Cx43).

Materials and methods: Cartilage from 16 osteoarthritic and 10 nonosteoarthritic humeral heads was assessed for expression of the following genes by real-time polymerase chain reaction: types I, II, and X collagen; matrix metalloproteinases (MMPs); tissue inhibitors of MMP (TIMPs); interleukins; versican; cyclooxygenase 2 (Cox-2); inducible nitric oxide synthase (iNOS); tumor necrosis factor α (TNF-α); aggrecanase 2 (ADAMTS5); and Cx43.

Results: In osteoarthritic shoulders, Cx43, Cox-2, versican, collagen type I, ADAMTS5, MMP-3, and TNF-α expressions were significantly increased compared with controls. TIMP-3 and iNOS trended toward significance, with robust expression in osteoarthritic shoulders and low expression in nonosteoarthritic shoulders. In osteoarthritic shoulders, gene expression of Cx43, ADAMTS5, collagen type I, Cox-2, versican, and TIMP-3 showed predominance (85-, 33-, 13-, 12-, 11.5-, and 3-fold increases, respectively) relative to nonosteoarthritic controls. Spearman correlation analysis showed significant correlations between Cx43 and collagen (types I, II, and X), MMP-9, TIMP-2 and TIMP-3, versican, Cox-2, iNOS, and ADAMTS5.

Conclusions: Certain genes are markedly upregulated in osteoarthritic shoulders compared with nonosteoarthritic shoulders, with Cx43, Cox-2, versican, collagen type I, ADAMTS5, MMP-3, and TNF-α expression being significantly increased. These genes might be useful biomarkers for examining shoulder OA.

Clinical relevance: Identification of osteoarthritic biomarkers can help us better understand shoulder OA and build the foundation for future research on disease progression and treatments.

Level of evidence: Basic Science, Molecular and Cell Biology.

Keywords: Shoulder; osteoarthritis; biomarkers; connexin 43

Osteoarthritis (OA) of the shoulder is both common and debilitating but is far less studied than OA of other joints. In contrast, knee OA has been widely explored regarding biomarkers, risk factors, natural history, and treatments,

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including total joint replacements. Extensive studies have elucidated markers of knee OA, including interleukin 1 (IL-1), tumor necrosis factor α (TNF-α), nitric oxide, prostaglandin E_2, matrix metalloproteinases (MMPs), and aggrecanases (ADAMTS4 and ADAMTS5), factors that control the breakdown of cartilage.[4,6,18,22,39,52]

The shoulder joint, unlike the more widely studied knee and hip joints, is not a weight-bearing joint. Considering that shoulders experience a mechanical environment different from that of knee joints, it is unclear whether the same catabolic biomarkers found in knee OA are ubiquitous among all joints with OA. Studies of OA biomarkers within the glenohumeral joint, or shoulder joint, not only are far sparser but are limited to the synovial fluid or subacromial bursa.[53,54] These studies elucidated the biomarkers in patients with rotator cuff tears and suggested that such biomarkers might be related to shoulder OA. This indirect association was postulated because many patients with rotator cuff tears develop OA.[13,32] One study[40] compared those with and without OA in which the condition of the cartilage was intraoperatively determined on the basis of a grading system.[2,37,40] After the cartilage was graded, synovial fluid was analyzed, but the cartilage, which plays a significant role in the development of OA, was not analyzed.[40]

The aim of this study was to determine the markers associated with osteoarthritic cartilage obtained directly from the cartilage of glenohumeral joints. The chondrocyte markers investigated include well-known biomarkers that have been found in studies of other joints, such as the knee and hip joints. This study also investigated a possible biomarker of joint cartilage, connexin 43 (Cx43), a gap junction protein. Gap junctions are specialized communicative cell structures present in the plasma membrane of cells. They are made up of connexin monomers that assemble to form a hemichannel. Hemichannels provide a pathway for direct intercellular communication when they are paired with a hemichannel on an adjacent cell. The resultant gap junction channel permits the direct exchange of second messengers, metabolites, ions, and other small molecules among coupled cells. Gap junctions aggregate into large gap junction plaques at the interface of adjacent cells, forming a functional syncytium for the coordinated function of a tissue.[30] Several studies have implicated Cx43 in the etiology of OA.[19,25,49,50] Synovial biopsy specimens from patients with OA were shown to have an increase in Cx43 expression and an increase in the size and number of gap junction plaques.[25] Ex vivo analysis of these cells revealed that pharmacologic inhibition of Cx43 function could reduce the basal and IL-1β-stimulated production of collagenase activity.[19,25] In addition, the inflammatory cytokine IL-1β has been shown to increase the expression of Cx43 in articular chondrocytes[49,50] and synovial fibroblasts.[34] Recently, a study by Mayan et al[30] investigated cartilage from osteoarthritic knees and femoral heads and found significantly elevated levels of Cx43 compared with nonosteoarthritic cartilage.

Cx43 can affect the responsiveness of cells to extracellular cues by modulating signal transduction and gene transcription.[23,33,35,44,45] In addition to the impact of inflammation on Cx43 expression, Cx43 has long been linked to mechanical load.[5] Cx43 expression is dramatically increased by mechanical loading in skeletal tissues, and its function has been implicated in the production of prostaglandins in response to mechanical perturbation in bone cells.[15] Both mechanical load and prostaglandin production are also factors that influence joint destruction in OA, circumstantially providing additional implication of a role for Cx43 in OA. Further, the upregulation of Cx43 is implicated in the production of both proinflammatory and catabolic factors by synovial fibroblasts.[19,25] No study has investigated OA-associated catabolic factors from cartilage of the glenohumeral joint, and Cx43 has not been studied in human humeral head cartilage.

Materials and methods

Participant selection

The study was conducted prospectively at a single institution. Patients with and without OA of the shoulder who were undergoing either arthroscopic or open shoulder surgery were recruited. Twenty-six patients were selected from the clinical practice of one orthopedic surgeon who specializes in shoulder and elbow surgery. All patients provided consent according to Institutional Review Board protocol at the surgeon's institution. Patients were defined as having OA whether they developed OA from a degenerative wear-and-tear process or from a secondary process, such as rotator cuff arthropathy or instability, in accordance with a study by Ratcliffe et al.[40] Exclusion criteria included inability to provide consent and presence of fracture. All patients undergoing either arthroscopic or open surgery had their cartilage intraoperatively graded by a single orthopedic surgeon. The cartilage was graded according to a previously described grading system for OA[2,37,40]: grade I (normal), the articular cartilage is smooth and shiny with an intact surface that is firm when probed; grade II, the articular surface has localized softening of the surface and fibrillation; grade III, the articular cartilage has extensive softening, pervasive fibrillation or fissuring, and clefts; and grade IV, eburnated bone and osteophyte formation are present, and the articular cartilage shows pitting, with tufts and fronds.[40]

Ten patients with grade I cartilage composed the nonosteoarthritic (control) group. The average age of this group of 4 men and 6 women was 59 years (age range, 29-72 years). This group underwent arthroscopic surgery for rotator cuff repairs (9 patients) and instability (1 patient). Sixteen patients with grade IV cartilage composed the osteoarthritic group. The average age of this group of 5 men and 11 women was 66 years (45-82 years). This group underwent total or reverse shoulder arthroplasty for OA (10 patients), avascular necrosis (3 patients), cuff tear arthropathy (2 patients), and rheumatoid arthritis (1 patient). No patients recruited were found to have grade II or grade III cartilage intraoperatively. Patients who underwent arthroscopic surgery required surgical suture anchors for tissue repair. A small piece of cartilage at the edge of the suture anchors was obtained for