RELEVANCE TO MUSCULOSKELETAL CONDITIONS:
The remodeling of the extracellular matrix (ECM) of cartilage is taking place under physiological and pathological conditions. There is more and more evidence that members of a newly discovered family of proteins with disintegrin and metalloproteinase domains express proteolytic activity against ECM components and thus could be involved in the process of cartilage degeneration seen in osteoarthritis (OA).

INTRODUCTION: ADAMs (metalloproteinases with disintegrin domains) were recently discovered as membrane-anchored cell-surface proteins structurally related to replosyns (1,2). Novel studies have demonstrated that human articular cartilage expresses mRNA for at least three members of this family, ADAM-10, -12, and -15 (3,4). Previously, we have demonstrated an up-regulation of mRNA expression for ADAM-10 in cartilages with higher metabolic rate (e.g. newborn and osteoarthritic tissues). Importantly, mRNA expression of ADAM-10 in OA cartilage was higher than in the age-matched normal tissue (4). In addition, it has been shown that ADAM-10 possesses the Tumor Necrosis Factor processing activity (5,6). These findings suggest the possible involvement of at least one member of ADAM family (ADAM-10) in the process of cartilage remodeling. To test the hypothesis that ADAM-10 could degrade the ECM components, we intended to prove that the protein ADAM-10 is synthesized by chondrocytes and that it can be detected in the ECM of articular cartilage. The purpose of the current study was to evaluate whether ADAM-10 protein is present in human articular cartilage and to describe its localization and its distribution via immunohistochemical methods in normal (newborn and adult) and OA cartilage.

METHODS: Full thickness human articular cartilage was dissected from the load-bearing regions of the femoral condyles of donors with no symptoms of joint disease within 24 hours of death. Samples (male and female, ranging from newborn to 80years) were obtained through the Regional Organ Bank of Illinois. Human OA cartilage was collected through the Department of Orthopaedic Surgery as surgical specimens from patients (aged 50 to 80 years, both men and women) undergoing total knee arthroplasty due to advanced OA. Two adjacent pieces of the cartilage from all donors and patients were processed either for histology and immunohistochemistry or used for cartilage extraction for western blot analyses. For immunohistochemistry cartilage sections (6 μm) were fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin. Prior to incubation with primary antibodies tissue sections were digested with keratanase (0.01 U/ml), keratanase II (0.0001 U/ml) and chondroitinase ABC (0.01 U/ml) in 100mM Tris/50 mM NaAc buffer (pH 6.5) at 37o C for 90 min to increase the penetration of antibodies chondroitinase ABC (0.01 U/ml) in 100mM Tris/50 mM NaAc buffer and keratanase (0.01 U/ml), keratanase II (0.0001 U/ml) and keratanase II (0.0001 U/ml) and incubation with primary antibodies. As an additional control for the anti-ADAM-10 antibody (blocking control), the synthetic peptide was preabsorbed into cartilage tissue. Extraction was performed on 1mm³ pieces of cartilage in 1 M guanidine HCl, 10 mM CaCl₂, 50 mM Tris-HCl, pH 7.5 buffer for 3 hours in the presence of protease inhibitors. Both, western blot and immunohistochemistry were performed with two types of anti-ADAM-10 monoclonal antibodies (provided by Procter & Gamble Pharmaceuticals) raised against peptides either in the disintegrin or in the metalloproteinase domains. As a negative control for immunohistochemistry, cartilage sections were incubated with no primary antibodies. As an additional control for the anti-ADAM-10 antibody (blocking control), the synthetic peptide was preabsorbed with primary antibody prior to incubation with the tissue. As a standard for western blots, human recombinant ADAM-10 was used. To increase the sensitivity of western blots, Enhanced Chemiluminescent kits (Amersham) were applied.

RESULTS: In the current study we were able to prove that human articular chondrocytes express ADAM-10 protein. Immunostaining of articular cartilage sections revealed different patterns of staining in newborn, normal adult and OA cartilage. In newborn growth plate cartilage, anti-ADAM-10 staining was detected throughout the whole tissue section within chondrocytes from the proliferative and hypertrophic regions. The strongest cell-associated staining was found around cartilage canals and in the upper part of the newborn cartilage. Some matrix staining was evident in the hypertrophic zone of the cartilage. In normal adult cartilage, the staining intensity was lower than in newborn cartilage and was primarily detected in chondrocytes localized in the superficial and upper/middle zones of the tissue. Chondrocytes and the matrix of the deep zone of normal adult cartilage revealed no detectable staining with the anti-ADAM-10 antibodies. In articular cartilage from OA patients, the strongest immunoreactivity was detected in the fibrillated areas of the remaining upper part of OA cartilage. Both, chondrocytes and interterritorial matrix, were positively stained with ADAM-10 antibodies. Anti-ADAM-10 staining was predominantly localized in cell clusters. In the deep zone of OA cartilage only single cells showed some immunopositivity and there was nearly no detectable anti-ADAM-10 staining in the matrix. On western blots, the extracts from all human articular cartilages revealed immunoreactive ADAM-10 bands detected by the two types of antibodies. The distribution of bands was similar for both antibodies. At least five major bands were found in extracts from normal and OA cartilage, representing, possibly, some degradation products. Under reduced conditions, recombinant ADAM-10 also showed immunoreactive bands with the strongest at about 60 kDa, the estimated molecular weight of this recombinant protein.

DISCUSSION: The results of the current study demonstrate for the first time that, in parallel with the message data (4), human articular chondrocytes express ADAM-10 as protein. Moreover, ADAM-10 message and protein are co-localized within the same areas of articular cartilage. The strongest mRNA expression was previously found in newborn and OA cartilage, tissues with higher metabolic activity than normal adult cartilage. Similar to these findings, the most intensive immunostaining with anti-ADAM-10 antibodies was detected in the same type of tissues and in the same areas: growth plate newborn cartilage and clusters of chondrocytes in the fibrillated areas of OA cartilage. Localization of ADAM-10 in the vicinity of cells as well as in the matrix of metabolically active tissue suggests a potential role of this molecule in the physiology and pathophysiology of articular cartilage.


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