Introduction - Although the involvement of matrix degrading enzymes in the progression of osteoarthritis (OA) is well characterized, the molecular mechanisms causing onset of this disease and regulating the expression of these enzymes are largely unknown. Interestingly, the changes in chondrocyte gene expression and cartilage matrix remodeling that occur during the progression of OA are similar to the changes that occur during chondrocyte hypertrophy in normal developing growth plates. The initiation of chondrocyte hypertrophy is regulated by the transcription factor Cbfa1 (Runx2). Knock-out of the Runx2 gene blocks hypertrophy in most skeletal elements and its over expression in cartilage induces precocious and ectopic chondrocyte hypertrophy (1,2). Hypertrophic chondrocytes and OA chondrocytes express collagen type X, alkaline phosphatase, and the matrix remodeling enzyme MMP-13 (collagenase 3) (3-6). Over expression of MMP-13 induces joint cartilage erosion, and Cbfa1 is known to bind to the MMP-13 promoter and regulate its expression (7,8). Therefore, we hypothesize that increased Cbfa1 activity in articular chondrocytes contributes to the onset or progression of OA.

Methods - Human OA articular cartilage was obtained from five patients undergoing joint replacement. Control cartilage was obtained from three patients with no history of joint disease undergoing hip replacement as a result of injury. All patients provided informed consent under institutional review board approval. Cartilage specimens were dissected from subchondral bone, frozen, sectioned, and histochemically or immunostained using standard protocols. Anti-Cbfa1 (Santa Cruz) and anti-MMP-13 (R&D Systems) were used as recommended. The articular cartilage was histologically graded as intact or fibrillated based on toluidine blue staining, and the percentage of Cbfa1 immunoreactive chondrocytes was determined on three to six sections per patient. Data was analyzed using the two-sample Student’s t-test assuming equal variances.

Results - Chondrocytes in the articular cartilage of OA patients exhibited increased staining for Cbfa1 as compared to control cartilage. The percentage of chondrocytes immunoreactive for Cbfa1 in fibrillated OA cartilage was higher than control (75.6% vs. 32.0%, p<0.01). To confirm specificity of the staining, the antibody was incubated with cartilage from three patients with no history of joint disease undergoing hip replacement as a result of injury. All patients provided informed consent under institutional review board approval. Cartilage specimens were dissected from subchondral bone, frozen, sectioned, and histochemically or immunostained using standard protocols. Anti-Cbfa1 (Santa Cruz) and anti-MMP-13 (R&D Systems) were used as recommended. The articular cartilage was histologically graded as intact or fibrillated based on toluidine blue staining, and the percentage of Cbfa1 immunoreactive chondrocytes was determined on three to six sections per patient. Data was analyzed using the two-sample Student’s t-test assuming equal variances.

Expression of Cbfa1 in OA cartilage was confirmed by RT-PCR.

To determine whether chondrocytes expressing Cbfa1 exhibited abnormal gene expression, we stained adjacent sections for Cbfa1 and either alkaline phosphatase or MMP-13. Alkaline phosphatase was not detected in control cartilage, and only a few cells in non-fibrillated OA cartilage exhibited this enzymatic activity. Alkaline phosphatase was present in cell clusters in fibrillated OA cartilage (Fig. 1 D), which overlapped with the expression of Cbfa1. MMP-13 was expressed in a few cells in control cartilage while most cells in fibrillated OA cartilage expressed this enzyme, especially in larger cell clusters. Cell clusters that expressed Cbfa1 also expressed MMP-13 (Fig. 1 H) in a similar distribution.

Discussion - Increased Cbfa1 expression in OA cartilage correlated with the severity of cartilage erosion and the expansion of cell clusters. Since Cbfa1 is known to be a direct, positive regulator of MMP-13 promoter activity, the co-expression of Cbfa1 and MMP-13 in these cell clusters suggests that Cbfa1 drives the increased expression of MMP-13 associated with OA collagen degradation (9). Increased Cbfa1 expression may be a relatively early marker for OA since we observed increased staining in the superficial zone of relatively intact cartilage of OA joints. Because of its significant role in regulating hypertrophic differentiation of chondrocytes during normal endochondral ossification, the increased expression of Cbfa1 in articular cartilage suggests that Cbfa1 activity may contribute to the onset or progression of OA by regulating matrix remodeling genes. However, since Cbfa1 is also involved in chondrogenesis, its increased expression in OA tissue may reflect an attempted cartilage repair mechanism. Existing information and continuing characterization of the regulation of Cbfa1 expression and activity by growth factor signaling during endochondral ossification may contribute to an understanding of the mechanisms regulating the onset and early progression of OA.