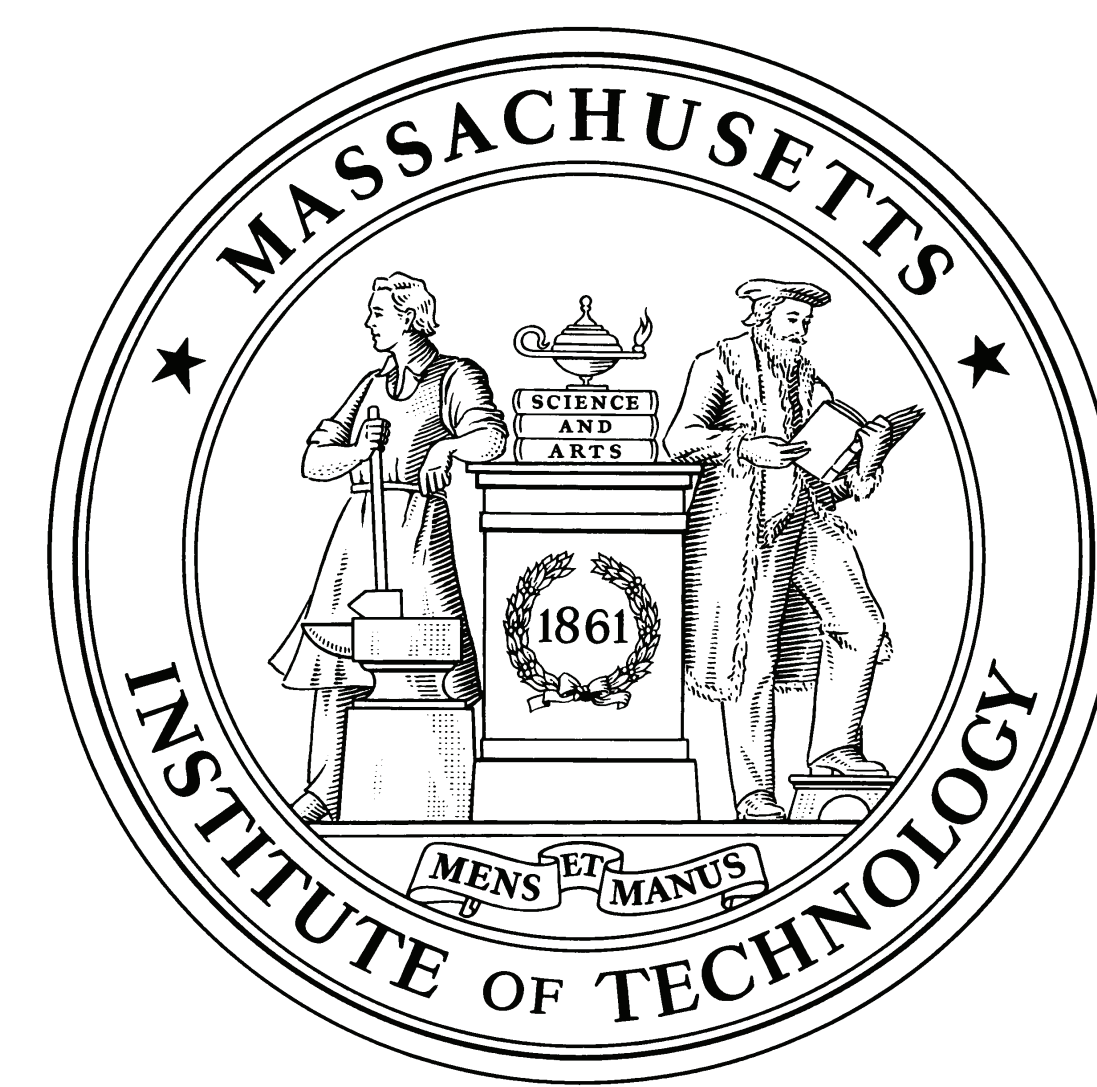


Mechanical loading-induced TGF- β 1 mediates cartilage degradation caused by upregulation of HTRA1/DDR2

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INTRODUCTION: The goal of this study is to understand the molecular basis underlying articular cartilage degeneration. Results from our recent investigations suggest that HTRA1 (high temperature requirement A1, a serine protease) degrades the pericellular matrix of chondrocytes, resulting in enhanced exposure of chondrocytes to collagen type II. Interaction of chondrocytes with collagen type II activates DDR2 (discoidin domain receptor 2, a cell membrane receptor tyrosine kinase for native collagen type II). This, in turn, induces expression of MMP-13 (matrix metalloproteinase 13). The end result is osteoarthritis. In this proposed molecular sequence of events underneath articular cartilage degeneration, a critical question concerns which factor(s) cause induction of HTRA1 in chondrocytes. In this study, we tested whether biomechanical factors (hydrostatic pressure and mechanical injury) and/or biochemical factors (TNF α , and TGF- β 1) are implicated in the induction of HTRA1/DDR2.

METHODS and RESULTS:

HTRA1 and TGF- β 1 expressions in chondrocytes

Human primary chondrocytes were cultured with recombinant human TNF α (10ng/ml), recombinant human TGF- β 1 (10ng/ml) and, heating to 42 °C for time periods of 0, 6, 12 and 24 hrs. For hydrostatic pressure, chondrocytes were exposed to hydrostatic pressure (5,000 psi = 34 MPa) for 2 and 4 hrs. The mRNA levels of the genes were examined by real-time qPCR.

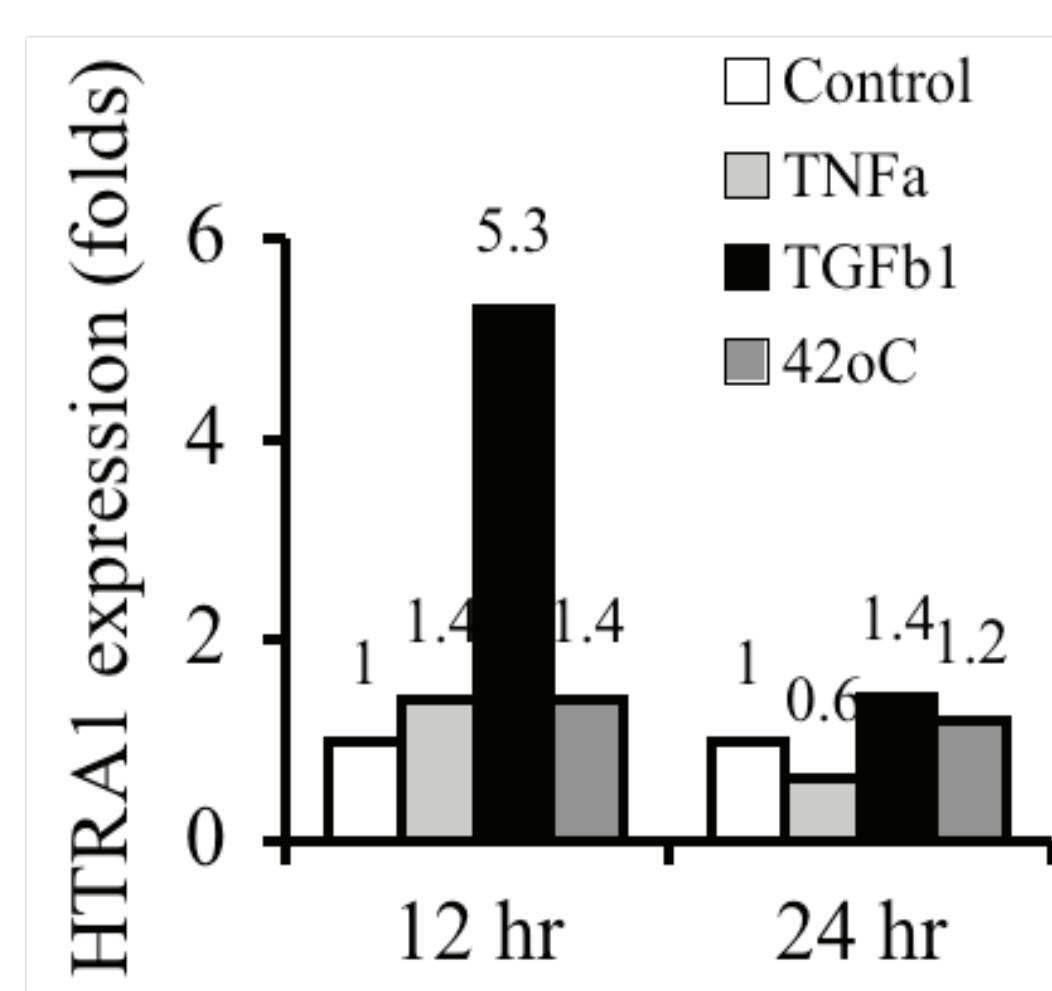


Figure 1

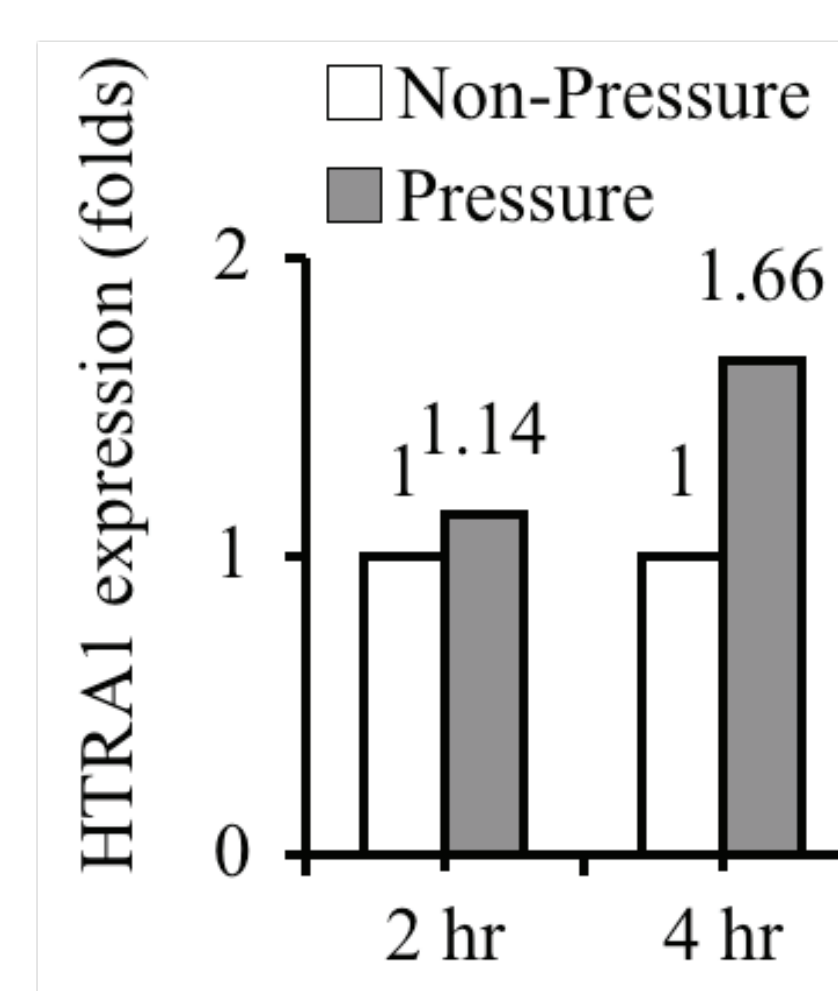


Figure 2

HTRA1 expression increased by ~5-fold in chondrocytes treated with TGF- β 1 (Figure 1). However, HTRA1 mRNA levels in chondrocytes were similar in response to heating, TNF α treatment, hydrostatic pressure and controls (Figures 1 & 2).

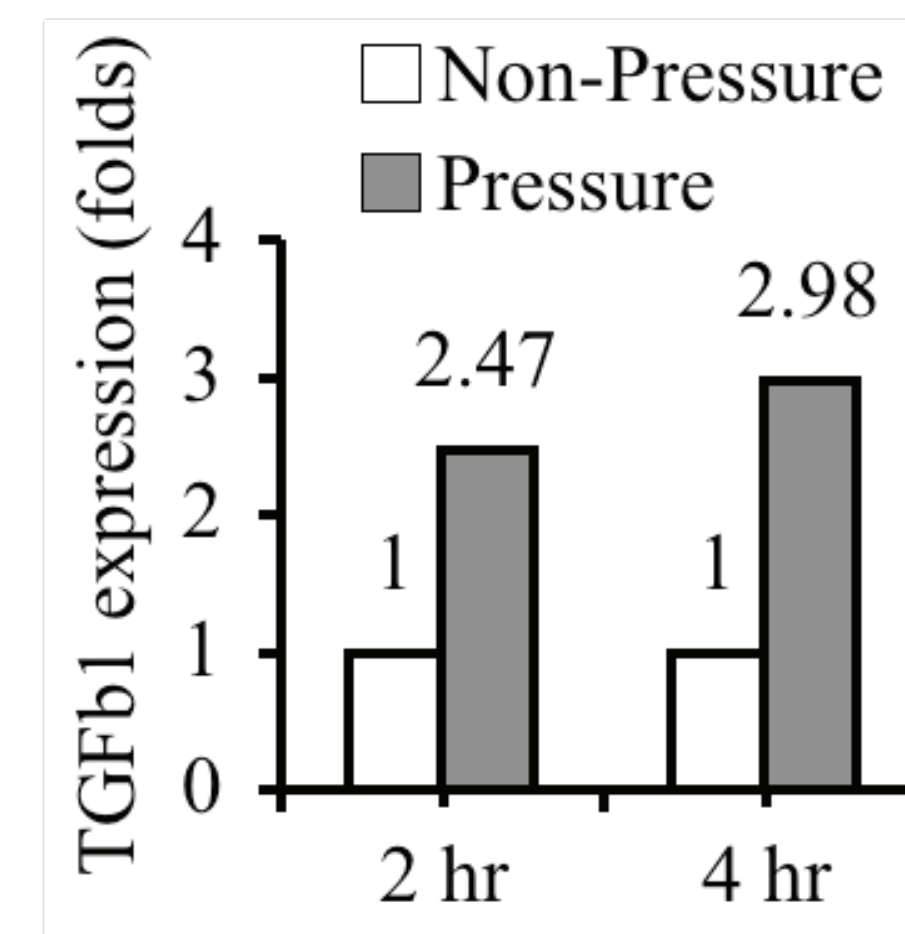


Figure 3. TGF- β 1 mRNA in chondrocytes was increased by ~3-fold after 2 and 4-hrs of hydrostatic pressure treatment.

Expressions of *Ddr2* in bovine explants and TGF- β 1 and p-Smad2 in mouse OA knee joints.

For bovine explant studies, 3mm diameter, 1mm thick cartilage disks from the femoropatellar grooves of 1-2 week-old bovine calves were subjected to one-time mechanical injury (100%/s, 50% final strain) followed by 5 days culture. Non-treated explants were the negative control. Bovine sections were treated with polyclonal antibody against human DDR2. For mouse OA knee joints, heterozygous chondrodysplasia (*chol*+) mice (n=4) at the age of 3 months were collected. Knee sections were incubated with polyclonal antibodies against Tgf- β 1 and p-Smad2.

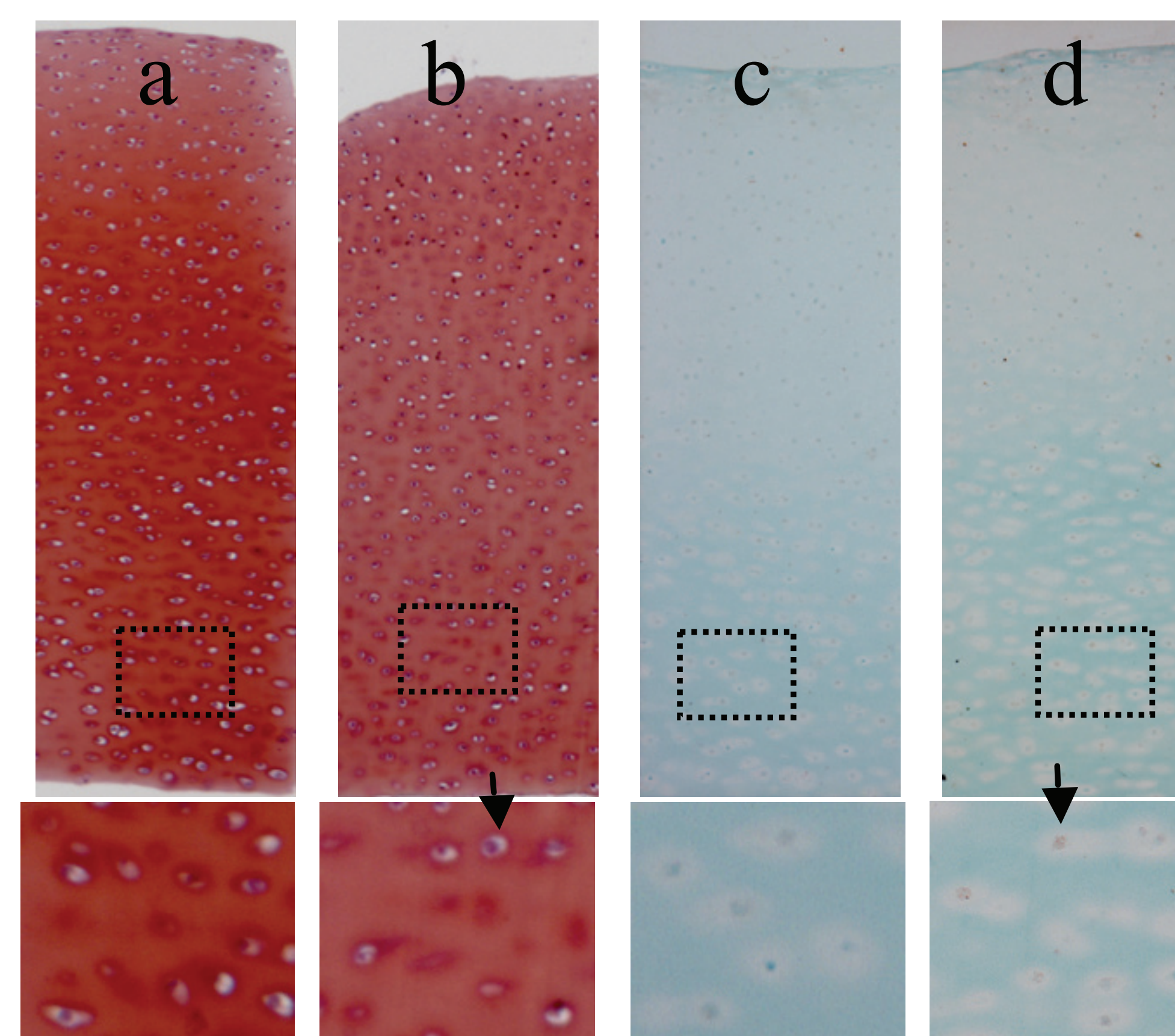


Figure 4. The mechanical injury resulted in reduction of preteoglycan staining, particularly in the pericellular regions (4b), associated with increased expression of *Ddr2* (4d). (4a shows normal histology; 4c shows that *Ddr2* staining was hardly detected in control).

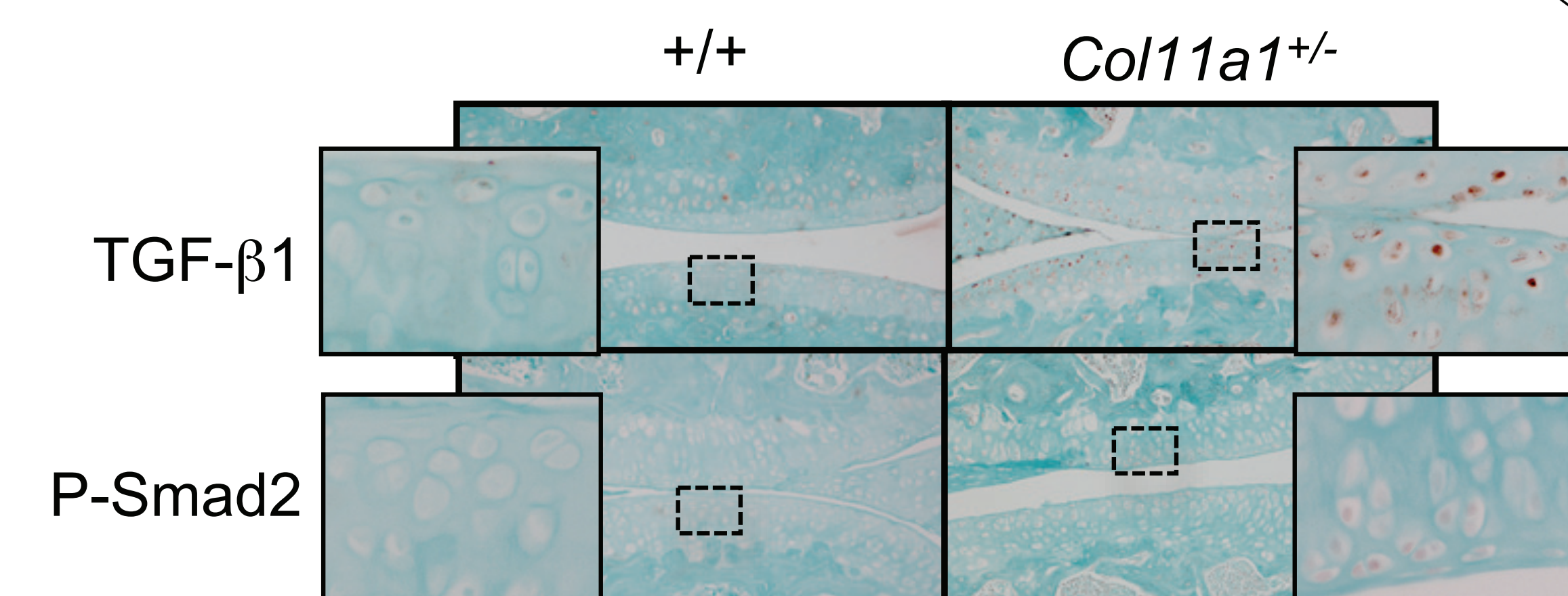


Figure 5: Immunohistostaining for Tgf- β 1 and p-Smad2 was relatively absent in articular cartilages in wild-type mice. However, positive staining for both genes was detected in chondrocytes in the superficial layers of knee joints in *chol*+/+ mice. This increased expression was prior to up-regulated expression of *Htra1* in *chol*+/+ mice.

DISCUSSION: TGF- β 1 is considered an anabolic factor that may act during the early stage of OA by stimulating ECM production. However, data from our present study indicated that

- 1) hydrostatic pressure increased expression of TGF- β 1 in chondrocyte cultures.
- 2) mechanic loading induced and activated TGF- β 1 signaling in articular cartilage of knee joints of a mouse model of OA.
- 3) TGF- β 1 induced expression of HTRA1, which could accelerate cartilage pericellular matrix degeneration.
- 4) mechanic injury of bovine explants caused an increase in expression of DDR2.

We previously reported that mechanical injury of bovine explants caused a significant increase in TGF- β 1 gene expression. These data together suggest a direct link between mechanical stimuli and gene and protein expressions of HTRA1 and DDR2.

CONCLUSION: Mechanical loading-induced TGF- β 1 can mediate cartilage degradation by upregulation of HTRA1/DDR2.

ACKNOWLEDGEMENTS: Supported by NIH/NIAMS Grants R01-AR051989 (to Li and Xu) and R01-AR33236 (to Grodzinsky). [§]Corresponding author. *Authors contributed equally.