



Attenuation of Articular Cartilage Degeneration by the Genetic Deletion of HtrA1 in Mice

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**Attenuation of articular cartilage
degeneration by the genetic deletion of
HtrA1 in mice**

A Thesis Presented by

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to

The Faculty of Medicine
in partial fulfillment of the requirements
for the degree of
Doctor of Medical Sciences

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Table of Contents

Abstract.....	1
Introduction.....	3
Hypothesis and Specific Aims.....	10
Materials and Methods.....	11
Results.....	16
Discussion.....	19
Conclusion.....	21
Figures.....	22
Literature Cited.....	29

Abstract

Objective

To investigate whether the genetic deletion of the serine protease, high temperature requirement protein A1 (HtrA1), will attenuate the progression of articular cartilage degeneration in mouse osteoarthritis (OA) models.

Methods

We used two mouse models for our study. The first is a genetic model of OA and includes two strains of mice. The first strain is a heterozygous genetic deletion of type-XI collagen that results in OA in mice (*Col11a1^{+/-}*). The second strain is a homozygous HtrA1 knockout (*HtrA1^{-/-}*). By crossing the two strains, we can analyze a possible chondral-protective effect of HtrA1-deficiency on the progression of OA.

The second model is a surgical/injury model of OA, in which we performed microsurgery on the knee joints of *HtrA1^{-/-}* mice to destabilize the medial meniscus (DMM). This technique undermines the joint, and rapidly induces OA in the mice. By surgically inducing osteoarthritis in *HtrA1^{-/-}* knockout mice, we can simulate the effect of HtrA1-deficiency on the progression of OA due to surgery or injury.

Conditions of the articular cartilage from the knee joints of the *Col11a1*^{+/-}; *Htra1*^{-/-} mice, *Col11a1*^{+/-} mice, *Htra1*^{-/-} mice, and the surgical mice were then examined by histology, graded on a standard scoring system, and characterized by immunohistochemistry.

Results

We examined the *Htra1*^{-/-} knockout mice and found no overt phenotype abnormalities. We also found that the deletion of *Htra1* attenuates the progressive process of articular cartilage degeneration in *Col11a1*^{+/-} mice. In our surgical model, the degenerative progression towards OA was dramatically attenuated in *Htra1*^{-/-} mice when compared to the controls.

Conclusion

Deletion of *Htra1* attenuates the progression of articular cartilage degeneration in OA models induced either by collagen type-XI haploinsufficiency or by DMM surgery. Therefore, the development of antagonistic drugs that specifically target HTRA1 may be an effective method to treat OA in the future.

Introduction

Arthritis is a leading cause of disability in the United States, and accounts for 19% of all reported disabilities [1]. Of the different types of arthritis, osteoarthritis (OA) is the most common, affecting 27 million people in the United States [2]. This disease is characterized by chronic pain commonly affecting the hands, feet, knees, hips, spine, and temporomandibular joint (TMJ), which greatly restricts a person's ability to carry out daily living activities, and diminishes the overall quality of life [3, 4]. In fact, with osteoarthritis, the TMJ can often be the first joint to show signs of the disease, which means orthodontists and other dental specialists could be the first to recognize and diagnose OA [5].

Currently, there are no well-established mechanisms for the induction and progression of osteoarthritis. Therefore, no effective cure for the disease exists. Additionally, OA of the TMJ is confusing, relatively unrecognized, and due to its multifaceted nature, very difficult to diagnose and manage by dental professionals and even orofacial pain specialists alike. Most current treatments are aimed at alleviating the symptoms of OA, which include physiotherapy, orthopedic aids, pharmacotherapy, and rehabilitation [6]. For OA of the TMJ, non-surgical treatment typically includes reassurance, various occlusal appliances, and anti-inflammatory medications. If symptoms continue to worsen, patients are referred to maxillofacial surgeons for more invasive options such as intra-articular injections, arthrocentesis, or even TMJ replacement [7].

Unfortunately, due to a lack of knowledge of the underlying disease process, these treatments often fall short because they merely address the symptoms of OA rather than modifying the disease process.

The precise etiology of osteoarthritis is unknown, but according to many experts, it is likely to be multifactorial and includes both genetic and environmental components. While the etiology is unclear, several identified factors have been associated with increased risk of developing OA. These factors include age, sex, obesity, lifestyle factors such as alcohol, and activities that cause abnormal or repetitive stress on the joint [8]. For those with a genetic predisposition for the disease, even normal mechanical stress on the joint can result in accelerated articular cartilage degeneration [6].

Regardless of the factors that initiate the disease, the similarities in structural and histological changes suggest that there is a common underlying pathologic process. Based on previous studies conducted in our lab and independent studies conducted by other groups, we propose a mechanistic model of OA involving the following proteins: The transforming growth factor beta 1 (TGF- β 1), the serine protease, high temperature requirement A1 (HTRA1), the cell surface receptor for type-II collagen, discoidin domain receptor 2 (DDR2), and the extracellular matrix degrading enzyme, matrix metalloproteinase 13 (MMP-13) [9-11].

Role of MMP-13 in the pathogenesis of OA: Articular cartilage is composed of proteoglycans and type-II collagen, both of which are substrates of MMP-13 [12]. In addition, MMP-13 expression is hardly detectable in normal mature articular cartilage, but is highly expressed in both human and mouse osteoarthritic cartilages [13, 14]. Experiments conducted on mouse knee joints also shows that the constitutive expression of MMP-13 alone will result in OA-like changes, while removal of the enzyme can prevent articular cartilage erosion [15, 16]. Results from these studies have prompted many pharmaceutical companies to seek drugs that inhibit MMP-13 as a method of treating OA. However, due to the broad biological functions of MMP-13, it would not be a suitable drug target [17]. Therefore, we must look for upstream molecules that regulate the expression of MMP-13.

Role of DDR2 in the induction of MMP-13: DDR2 is a cell surface receptor tyrosine kinase typically found in cartilage, skeletal muscle, skin, and adipose tissue. When examining the expression pattern of DDR2, it was found that DDR2 expression was increased in both human and mouse osteoarthritic tissues, while it was hardly detectable in normal articular cartilage tissues [18, 19]. In addition, several experiments were conducted in our lab to test whether activation of DDR2 can induce MMP-13 expression in chondrocytes. The results indicate that the direct interaction between DDR2 and type-II collagen results in

the expression of both MMP-13 and the DDR2 receptor itself, suggesting a positive feedback loop [19]. Additional studies previously conducted by our lab and by other independent research groups have also shown that the reduction in DDR2 expression can delay articular cartilage degeneration in mouse models of OA [20, 21]. Therefore, if DDR2 is found on chondrocyte cell surfaces, and the interaction between DDR2 and type-II collagen induces MMP-13 expression, then something must be inhibiting that interaction in normal articular cartilage.

Role of HTRA1 and the pericellular matrix in the pathogenesis of OA:

The primary structural and functional units of cartilage are termed chondrons, and they are composed of chondrocytes, their pericellular matrix, and a surrounding capsule [22]. The pericellular matrix is composed of laminin, fibronectin, biglycan, decorin, fibromodulin, matrilin 3, and cartilage oligomeric matrix protein (COMP), while the capsule is composed of mainly type-VI collagen, type-IX collagen, and proteoglycans [23]. Under normal conditions, the pericellular matrix and the capsule separate chondrocytes from the surrounding type-II collagen. When chondrons were physically isolated from human osteoarthritic cartilages, it was demonstrated that the pericellular matrix had become disrupted. This then allows DDR2 to interact with the surrounding type-II collagen, which induces the expression of MMP-13. The serine protease HtrA1 is

believed to be one of the key proteins involved in this process of pericellular matrix disruption [11, 24].

Originally, HtrA1 was identified as an Escherichia Coli heat shock protein, and homologues were subsequently identified in humans yielding 4 isotypes [9]. The first of these isotypes, HtrA1, is found to be highly elevated in human OA cartilages, and it has been shown to be capable to degrading a variety of cartilage matrix proteins including aggrecan, decorin, fibromodulin, matrilin 3, biglycan, COMP, and fibronectin [25]. These are the very proteins that makeup the pericellular matrix of chondrocytes, and degradation of these proteins by HtrA1 will lead to destruction of the pericellular matrix, and eventually of the joint itself [23]. Results from both human and mouse genetic studies show that maintaining the integrity of the pericellular matrix is crucial in preventing the initiation and progression of OA [26, 27]. In fact, even when the expression of other contributing proteins is increased, OA does not develop unless the pericellular matrix is removed first [18, 19, 28]. Thus, further research regarding the relationship between HtrA1 and the pericellular matrix in articular cartilage degeneration can provide invaluable information in the search of a true cure for osteoarthritis.

Role of TGF- β 1 in the induction of HTRA1: In order to complete our mechanistic model, we must look further upstream to see what causes the

induction of HTRA1, and evidence shows that TGF- β 1 could be the missing piece of the puzzle. However, past studies regarding this topic have produced conflicting results, and the exact role that TGF- β 1 plays in cartilage metabolism still remains unclear. For example, some studies indicate that TGF- β 1 may have a protective function and is responsible for cartilage repair and inhibition of cartilage degeneration [29, 30]. On the other hand, more recent studies have shown that prolonged exposure to TGF- β 1 in the joint can induce osteoarthritic changes in mice [31-33].

Regardless of its role, it is clear that the level of TGF- β 1 is also significantly elevated in osteoarthritic tissues compared to that of healthy cartilage tissues in both humans and mouse models [11, 34, 35]. These findings beg the questions: what causes the increase in levels of TGF- β 1 in arthritic chondrocytes, and what are the downstream biochemical effects? A study by Lee et. al., has demonstrated that mechanical injury of bovine cartilage explants results in increased levels of TGF- β 1 gene expression [36]. Additionally, data from Dr. Yefu Li's lab found that hydrostatic pressure on human articular chondrocytes in culture also increased TGF- β 1 expression in these cells [24]. This suggests a direct association between mechanical stimulus and the induction of TGF- β 1 expression. As for the downstream effects, based on results from Dr. Li's lab and other independent researchers, it has been shown that TGF- β 1 may be activated to induce HtrA1 expression in both mouse models of OA and in human primary

chondrocytes. Therefore, it appears that TGF- β 1 is the first step in a biochemical pathway that ultimately leads to irreversible articular cartilage degeneration [37]. Figure 1 summarizes the molecular pathway underlying articular cartilage degeneration as proposed by Dr. Yefu Li's lab.

Hypothesis

Based on our proposed mechanistic model, we hypothesize that the deletion of serine protease high temperature requirement A1 (HtrA1) will attenuate articular cartilage degeneration in mouse osteoarthritis (OA) models.

Specific Aims

Aim 1: To investigate the biological effects of the genetic deletion of HtrA1 on articular cartilage of knee joints in two mouse models of OA

Aim 2: To examine the gene expression profiles of HtrA1 and downstream molecules, Ddr2 and Mmp-13, in articular cartilage of knee joints in two mouse models of OA

Materials and Methods

Experimental animals and genotyping: All mutant mice and their wild-type littermates were maintained in a virus-free animal facility at Harvard Medical School under a 12-hour light/dark schedule. Animals were identified using an ear punch system and genotyping was conducted using DNA isolated from tail biopsies. Polymerase chain reaction (PCR) amplification was performed on the samples, and the PCR products were analyzed to determine genotype. Mice were grouped based on their genotypes and maintained for further study. Euthanization was performed via CO₂ inhalation. All experiments were approved by the Harvard University Institutional Animal Care and Use Committee (IACUC).

Morphological characterization of *HtrA1*^{-/-} and control mice:

Homozygous *HtrA1* global knockout mice were received from collaborators and examined for phenotype abnormalities. PCR products obtained from brain, heart, and skeletal muscle tissues were examined to confirm the complete removal of *HtrA1*. In addition, there have been recent reports indicating that the deletion of the *HtrA1* gene causes an increase in mice bone density. Therefore, a radiographic analysis was performed on 6 month old *HtrA1*^{-/-} mice and their wild-type littermates. The mice were exposed to 35kvp x-rays for 30 seconds, and the results were compared.

Generation of compound mutant mice, *Col11a1*^{+/-};*HtrA1*^{-/-}: *Col11a1*^{+/-} mice are genetically predisposed to developing OA, and these mice typically develop OA-like changes in knee and temporomandibular (TM) joints beginning at 3 months of age, which progresses to severe OA-like changes over the next 9 to 12 months [38]. We crossed these mice with *HtrA1*^{-/-} mice. So by removing *HtrA1* completely from *Col11a1*^{+/-} mice, we were able to determine whether the deletion of *HtrA1* could delay the development of OA in *Col11a1*^{+/-} mice. The knee and TM joints of *Col11a1*^{+/-};*HtrA1*^{-/-} mice were collected at 3, 9, and 15 months of age, and the joints of their *Col11a1*^{+/-}, *HtrA1*^{-/-}, and wild-type littermates were collected at the same ages to serve as controls.

Surgically induced OA mice: *HtrA1*^{-/-} mice and their wild-type littermates were subjected to destabilization of the medial meniscus (DMM) surgery at 10 weeks of age. They were anesthetized with intra-peritoneal injections of Ketamine and Xylazine, at 90µg/g and 25µg/g bodyweight, respectively. The right knees were prepared for aseptic surgery, and the incision was made in the joint capsule just medial to the patellar tendon. Using microsurgical scissors, the medial meniscotibial ligament (MML) was sectioned, and the surgical site was then covered with Vicryl sutures. Sham surgery, in which the medial meniscus ligament was visualized but not sectioned, was also performed on the *HtrA1* knockout mice as controls. Knee joints of the mice were collected at 8 weeks,

and 16 weeks after surgery

Histology and immunohistostaining of knee joints: For *Col11a1*^{+/-}; *HtrA1*^{-/-} mice group, 8 knee joints from 8 animals were collected at the ages of 3, 9, and 15 months of age, and 6 knee joints were collected at the same time points from each of the control groups. In the surgical model, 8 knee joints from sham surgery on *HtrA1*^{-/-} mice, DMM on wild-type littermates, and DMM on *HtrA1*^{-/-} mice were collected at 8 and 16 week time points after surgery. All of the joints were fixed in 4% paraformaldehyde for 24 hours at room temperature. They were then decalcified in EDTA for 6 days, and embedded in paraffin. Each knee joint underwent serial sections each measuring 6 µm from anterior to posterior. Every fifteenth section was processed through Safranin O/fast green staining for histological analysis, and every thirteenth section was processed for immunohistostaining.

For histologic analysis, each section was examined and graded based on the Modified Mankin scoring system (Figure 2) [10]. The features evaluated include chondrocyte periphery staining (range 0-2), spatial arrangement of chondrocytes (range 0-3), and staining intensity of the interterritorial matrix (range 0-4). A healthy articular cartilage would yield a score of 0, and the maximum score for articular cartilage degeneration is 9. After each section was

scored, a mean was calculated to yield a score for the entire joint. For completeness, the overall condition of the knee joint was also evaluated by examining the meniscus, subchondral bone, and possible osteophyte formation. For the surgical mice groups, a different scoring system was used to assess the joints, and this scoring system is the standard recommended by the osteoarthritis research society international (OARSI) histopathology initiative [39]. A minimum score of 0 was given to normal mouse articular cartilage, and a maximum score of 6 was given to joints that had vertical clefts or erosion of the calcified cartilage extending >75% of the articular surface.

The immunohistochemical analysis evaluated for protein expression of HtrA1, Ddr2, and Mmp-13. Each thirteenth section was de-paraffinized and quenched for endogenous peroxidase activity using a 1% hydrogen solution diluted with phosphate buffered saline (PBS). The sections were blocked for one hour with PBS containing 1.5% normal donkey serum, and then treated with chondroitinase ABC (0.25 units/ml). Next, the sections were incubated overnight at 4°C with primary polyclonal antibodies (1:200 dilution) specific to HTRA1, Ddr2, or Mmp-13. After primary antibody treatment, each section was washed three times with PBS and incubated with biotinylated secondary antibodies. Color development was performed using a peroxidase substrate after treatment of the sections using a mixture of avidin and biotinylated horseradish peroxidase.

Negative controls were included in this procedure by staining without primary antibodies.

Statistical analysis: A power analysis was performed prior to this study, which showed that a sample size of at least 6 would be sufficient to detect a 50% reduction of the score in the treatment group with a 95% confidence level. Once the knee joints were scored, the t-test was performed for statistical analysis with $p < 0.05$ being statistically significant, and $p < 0.001$ being statistically highly significant.

Results

To confirm the complete removal of *HtrA1* in the knockout mice, DNA from the brain, heart, and skeletal muscle tissues were collected, and amplified using PCR. Results shown in Figure 3 confirm that the *HtrA1* gene was completely knocked out. We also examined the *HtrA1* knockout mice and found no obvious phenotypic abnormalities compared to wild-type littermates. Additionally, we performed radiographic analysis of our adult *HtrA1*^{-/-} mice at 6 months of age and found no obvious differences in bone density compared to wild-type (Figure 4). Therefore, the *HtrA1* knockout mice should be suitable for this study.

We performed histological examination on the joints and the results are shown in Figure 5. At 3 months of age, chondrocyte clustering was seen in *Col11a1*^{+/-} and *Col11a1*^{+/-};*HtrA1*^{-/-} groups, but no marked difference in the cartilage surface was visible. However, by 9 months of age, fibrillation of the articular cartilage surface was clearly seen in the *Col11a1*^{+/-} group, while none were found in the wild-type and compound mouse group. This fibrillation is a classic morphologic finding at the beginning stages of OA. It involves splitting of the superficial layers of cartilage resulting in pits or clefts in the cartilage surface [40]. This eventually progresses deeper until the cleft extends all the way through the cartilage as seen in the 15 month group. At 15 months, significant cartilage degeneration was seen in the *Col11a1*^{+/-} group with complete destruction of the

superficial layer of cartilage in some areas, while fibrillation was just beginning in the compound mouse group. In other words, articular cartilage degeneration was seen in both groups with the *Col11a1*^{+/-} mutation, however, when *HtrA1* was removed, a marked reduction in the rate of cartilage destruction was seen. The knee joints of *Col11a1*^{+/-};*HtrA1*^{-/-} mice at 15 months were similar to those of *Col11a1*^{+/-} mice at 9 months, suggesting a 6 month delay in the progression of OA. To put things in perspective, the average lifespan of these mice is 30 months.

Results from the modified Mankin scoring system (Figure 6) also corroborate our findings. The knee joints of wild-type littermates at 3 months of age were assigned a score of 0 and used as a reference point for normal articular cartilage. Beginning at 3 months, both the *Col11a1*^{+/-} and the *Col11a1*^{+/-};*HtrA1*^{-/-} groups showed statistically highly significant differences in scores compared to wild-type. However, at 9 months and 15 months, the *Col11a1*^{+/-} group showed statistically highly significant differences in scores compared to both wild-type and the *Col11a1*^{+/-};*HtrA1*^{-/-} groups indicating a much greater degree of articular cartilage degeneration. Consistent with our histological findings, all three groups showed increases in score with age, but the increases were not nearly as steep as in the *Col11a1*^{+/-} group.

Histological analysis of the surgical group also produced corresponding

results (Figure 7). In the wild-type littermates, the articular cartilage appears thinner and there is significant degeneration present. At 16 weeks following surgery, a complete loss of articular cartilage was seen. When HtrA1 was removed, there was a clear delay in the degenerative process following surgery. When the joints were scored, similar results were produced, with the wild-type group showing much higher scores than the *HtrA1* knockout group at both 8 weeks and 16 weeks post DMM surgery (Figure 8).

Our immunohistochemical analysis showed an association of articular cartilage damage with presence of HtrA1, Ddr2, and Mmp-13 expression (Figure 9). Protein expression levels were examined in all of the mouse groups at 9 months of age. We found that HtrA1, Ddr2, and Mmp-13 were highly elevated in *Col11a1*^{+/-} group as indicated by the brown staining, and little or no protein was expressed in the wild-type and *Col11a1*^{+/-};*HtrA1*^{-/-} groups. Immunohistochemical analysis of the surgical group also showed increased expression of Ddr2 and Mmp-13, which was not seen in the sham or *HtrA1*^{-/-} groups (Figure 10). Negative controls were also stained without primary antibody and included in our analysis for reliability.

Discussion

To study the effect that the deletion of *HtrA1* has on articular cartilage degeneration, we generated *Col11a1^{+/-};HtrA1^{-/-}* mice. By using these mice, we were able to investigate a possible chondro-protective role that HtrA1 has on articular cartilage degeneration. Homozygous *HtrA1* knockout mice appear to be normal without any obvious phenotype abnormalities, and therefore, are suitable for our study. Results from our experiments indicated that deletion of *HtrA1* significantly attenuated articular cartilage degeneration in mouse models of OA, induced by either collagen type-XI haploinsufficiency, or DMM surgery.

Interestingly, while the removal of *HtrA1* did attenuate the overall process of articular cartilage degeneration, it did not prevent the early signs of OA, such as chondrocyte clustering. Chondrocyte clustering is characterized by clusters of round chondrocytes surrounded by large lacunae, and is a direct consequence of the elevation in chondrocyte activity. As shown in the 3 month mice group of Figure 6, the *Col11a1^{+/-}* mice had the same results as the *Col11a1^{+/-};HtrA1^{-/-}* mice. This result suggests that, normal chondrocyte metabolism is altered prior to the initiation of cartilage degeneration by *HtrA1*.

Through our immunohistostaining, we found that HtrA1 protein expression was elevated in *Col11a1^{+/-}* mice, as are the protein expression of the downstream

molecules Ddr2, and Mmp-13. We also found that the protein expression of HtrA1, Ddr2, and Mmp-13 were hardly detectable in the *Col11a1^{+/-};HtrA1^{-/-}* mice. This indicates that the absence of HtrA1 was associated with the attenuation of the progressive process of articular cartilage degeneration in *Col11a1^{+/-};HtrA1^{-/-}* mice. All of this, along with our other findings, is consistent with our proposed mechanistic model.

Our explanation for these observations is that HTRA1 is a protease that degrades pericellular components of chondrocytes in articular cartilage. In addition, HTRA1 may also contribute to the development of OA through the degradation of the pericellular network, resulting in dysfunctional activity of chondrocytes. Therefore, maintaining the integrity of the pericellular matrix is one of the key factors preventing the development of OA. However, more in vivo experiments will be needed to fully verify the role of HTRA1 in the pathogenesis of OA.

In summary, OA can develop as a result of a genetic disorder, or as a result of abnormal stress or injury[6, 8]. It can affect any joint in the body, and the TMJ could be the first joint affected by OA [5]. By having both the genetic knockout model and the surgery model, we were able to see the chondral-protective effect of the deletion of *HtrA1* in both the genetic and injury scenarios.

Conclusion

Our results suggest that the inhibition of *HtrA1* can significantly attenuate the process of articular cartilage degeneration caused by either a genetic predisposition for OA, or by surgery/injury. Therefore, the development of antagonistic drugs that specifically target HTRA1 may be an effective method to treat OA in the future. Rather than just managing the symptoms, we could now potentially modify the underlying pathological process of OA and significantly attenuate disease progression.

Figures

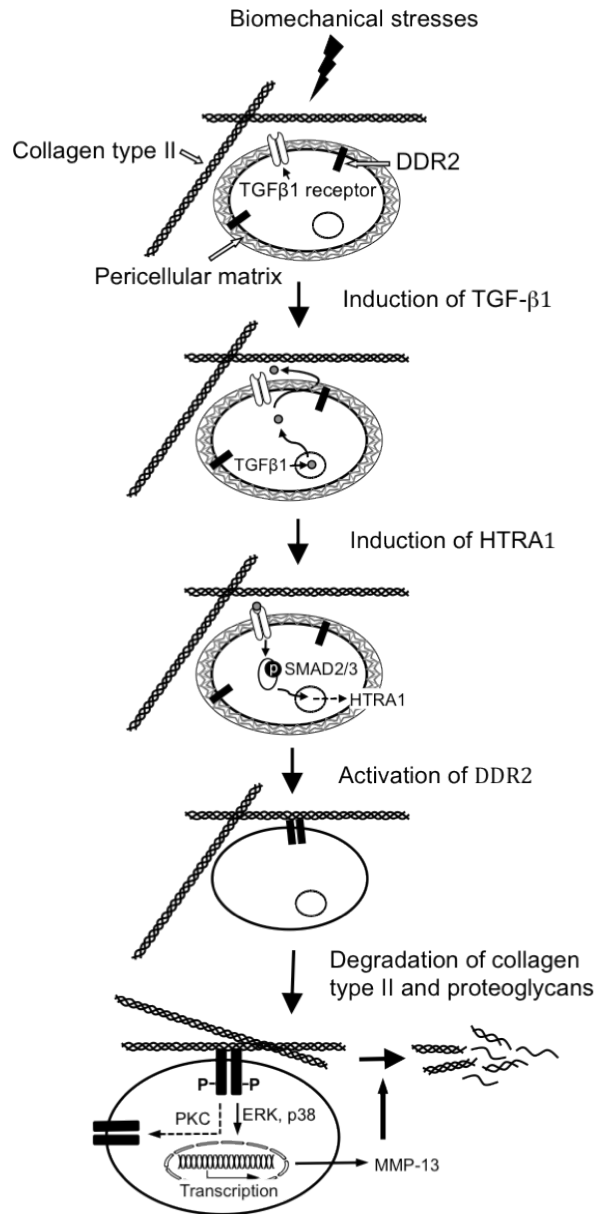


Figure 1: Proposed molecular pathway underlying articular cartilage degeneration

Feature	Grade
Chondrocyte periphery staining	
Normal	0
Slightly enhanced	1
Intensely enhanced	2
Spatial arrangement of chondrocytes	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity	3
Background staining intensity	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3
No dye noted	4

Figure 2: Modified Mankin scoring system used to evaluate articular cartilage degeneration

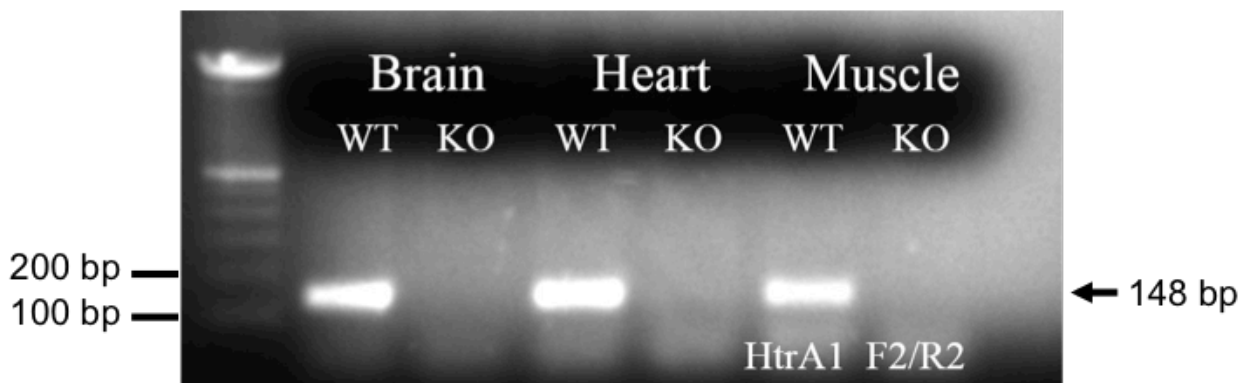


Figure 3: PCR examination of mouse tissues using genomic DNA and primers for HtrA1 exon 1. It shows that HtrA1 gene has been completely removed in HtrA1 Knockout mice.

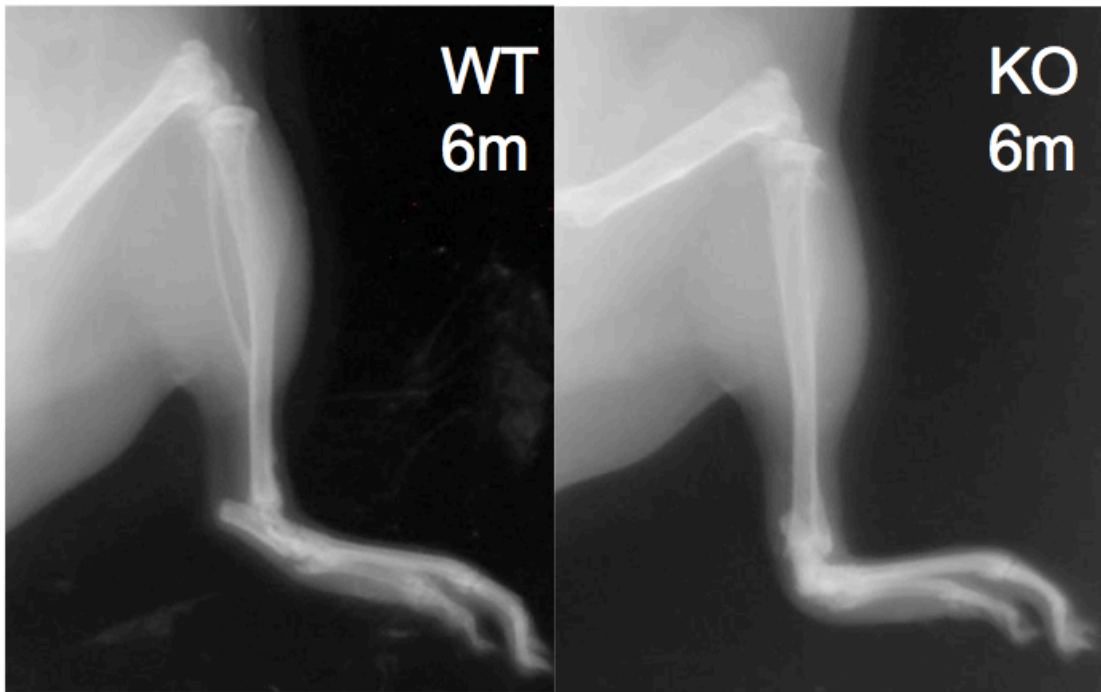


Figure 4: Radiograph of 6 month old wild-type and HtrA1 knockout mice. The mice were exposed to 35kvp x-rays for 30 seconds. No phenotype abnormalities in the HtrA1 knockout mice compared to wild-type. Bone density appears to be the same.

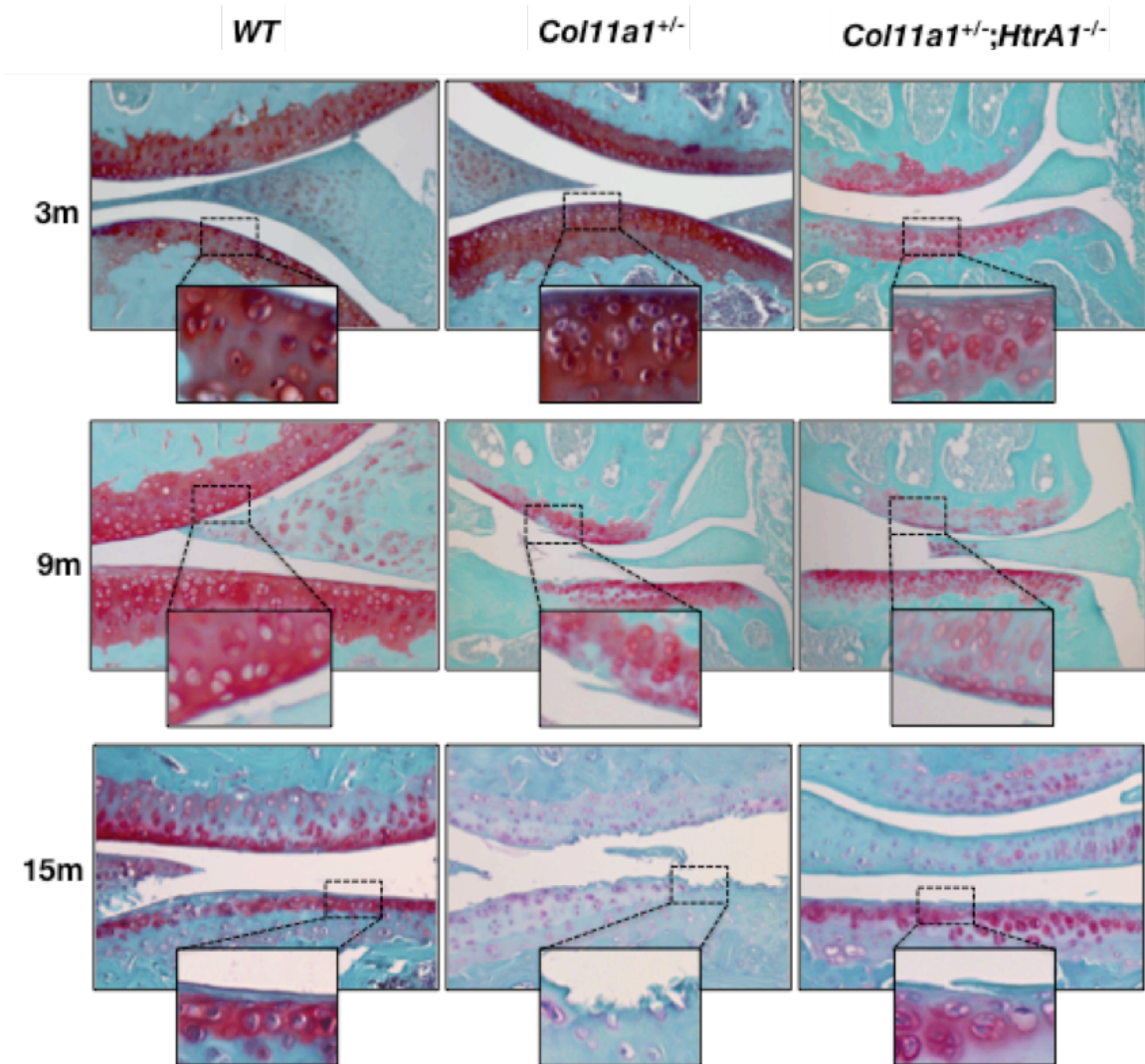


Figure 5: Histology of knee joints from *Col11a1^{+/-};HtrA1^{-/-}*, *Col11a1^{+/-}* and their wild-type littermates. Each image is a section selected to best represent the average score from each experimental group. **3 month group:** Chondrocyte clustering is seen in the *Col11a1^{+/-}* and *Col11a1^{+/-};HtrA1^{-/-}* groups, but no marked difference in cartilage surface. **9 month group:** Fibrillation of the cartilage is clearly seen in the *Col11a1^{+/-}* group but not in the compound or wild-type groups. **15 month group:** Significant cartilage degeneration is seen in the *Col11a1^{+/-}* group while fibrillation is just beginning in the compound mice group.

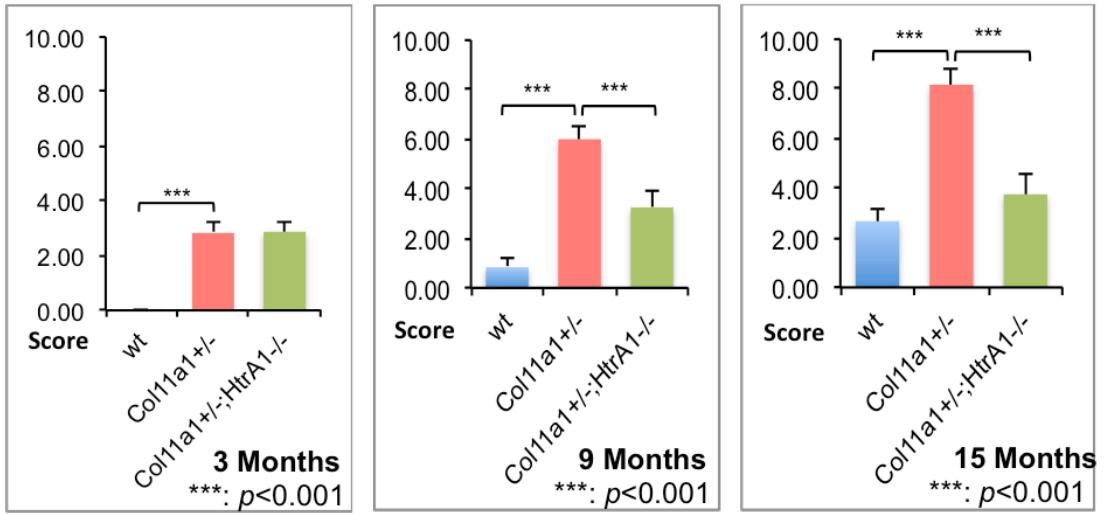


Figure 6: Results from the Modified Mankin Scoring System

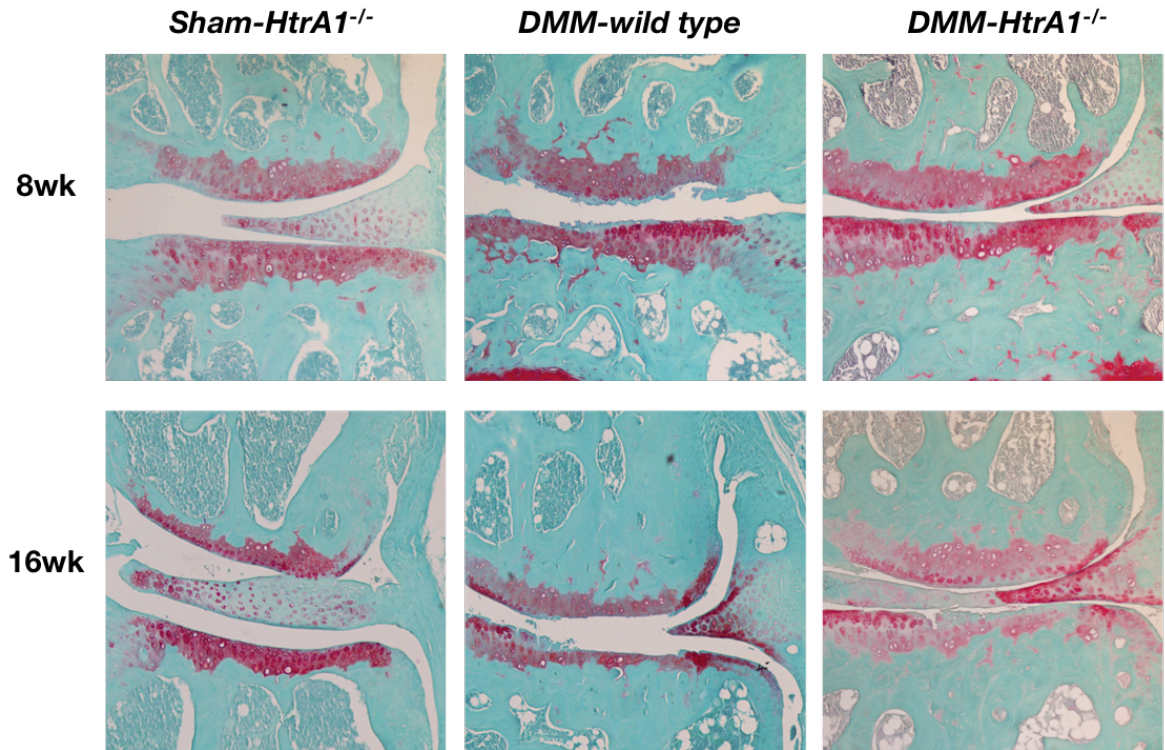


Figure 7: Histology of knee articular cartilage from mice following DMM microsurgery.

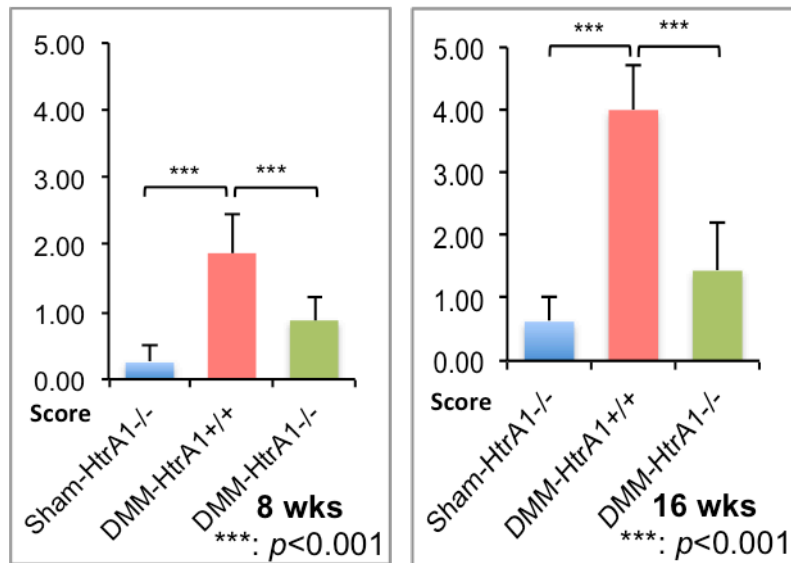


Figure 8: Results from scoring of joints from the surgery groups

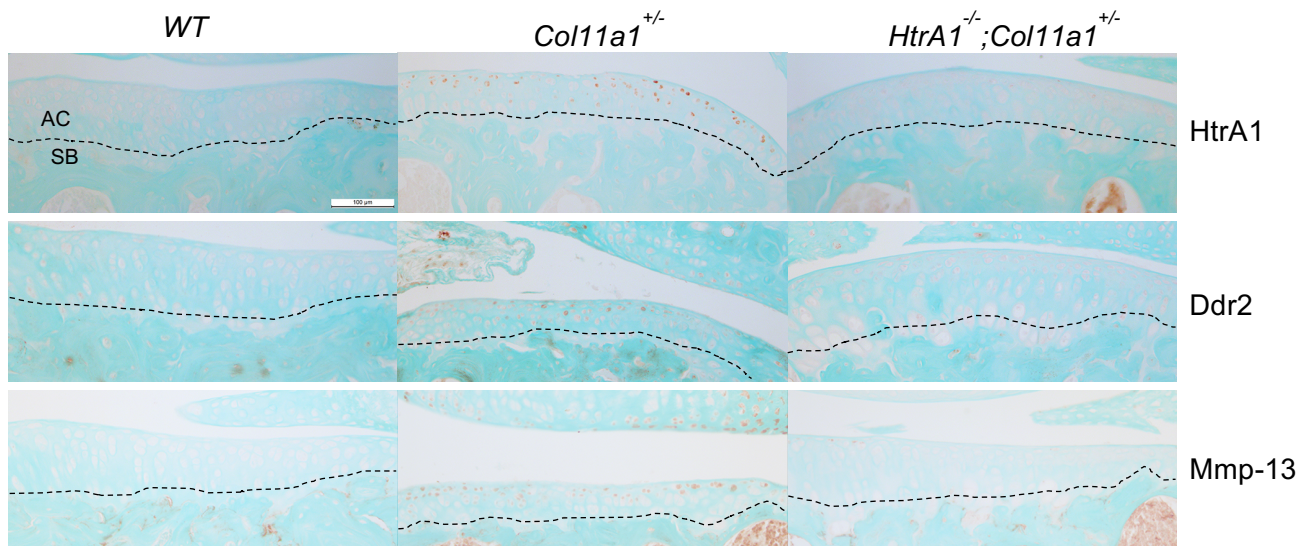


Figure 9: Protein expression in knee joints of mice at 9 months of age. HtrA1, Ddr2, and Mmp-13 expression was seen in *Col11a1*^{+/-} mice, and not in any of the wild-type or knockout groups.

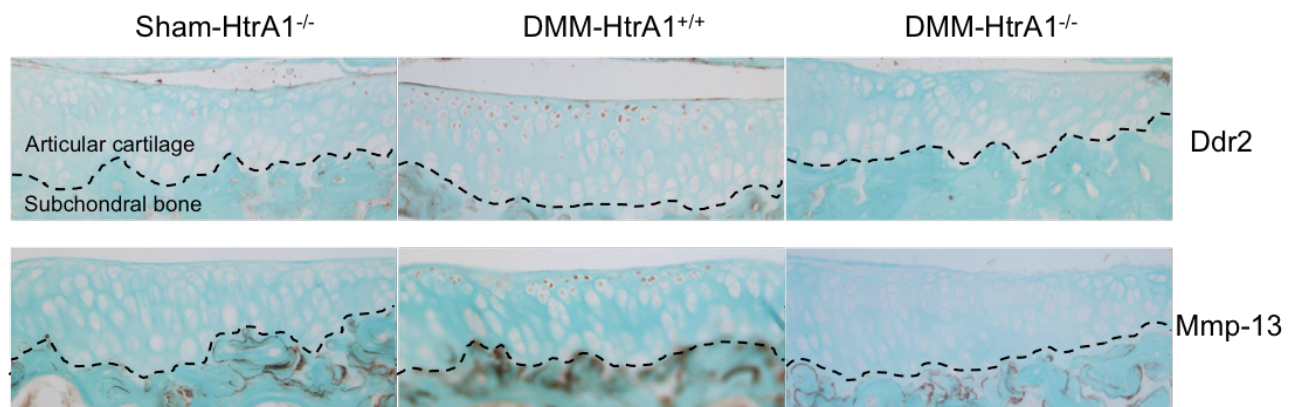


Figure 10: Protein expression in knee joints of surgery group at 8 weeks post-surgery. Ddr2 and Mmp-13 expression was seen in DMM-HtrA1^{+/+} mice, and not in any of the Sham or HtrA1 knockout mice.

Literature Cited

1. Wielage, R.C., et al., *Cost-effectiveness analyses of osteoarthritis oral therapies: a systematic review*. Appl Health Econ Health Policy, 2013. **11**(6): p. 593-618.
2. Lawrence, R.C., et al., *Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II*. Arthritis Rheum, 2008. **58**(1): p. 26-35.
3. Xie, F., et al., *Cost-effectiveness of pharmaceutical management for osteoarthritis pain: a systematic review of the literature and recommendations for future economic evaluation*. Drugs Aging, 2013. **30**(5): p. 277-84.
4. Nelson, A.E., et al., *A systematic review of recommendations and guidelines for the management of osteoarthritis: The Chronic Osteoarthritis Management Initiative of the U.S. Bone and Joint Initiative*. Semin Arthritis Rheum, 2013.
5. PA, T., *Osteoarthritis of the mandibular condyle*. British Dental Journal, 1973. **134**: p. 223-231.
6. Michael, J.W., K.U. Schluter-Brust, and P. Eysel, *The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee*. Dtsch Arztebl Int, 2010. **107**(9): p. 152-62.
7. de Souza RF, L.d.S.C., Nasser M, Fedorowicz Z, Al-Muharraqi MA, *Interventions for the management of temporomandibular joint osteoarthritis*. Cochrane Database of Systematic Reviews, 2012(4).
8. Sakalauskiene, G. and D. Jauniskiene, *Osteoarthritis: etiology, epidemiology, impact on the individual and society and the main principles of management*. Medicina (Kaunas), 2010. **46**(11): p. 790-7.

9. Troeberg, L. and H. Nagase, *Proteases involved in cartilage matrix degradation in osteoarthritis*. *Biochim Biophys Acta*, 2012. **1824**(1): p. 133-45.
10. Xu, L., et al., *Osteoarthritis-like changes and decreased mechanical function of articular cartilage in the joints of mice with the chondrodysplasia gene (cho)*. *Arthritis Rheum*, 2003. **48**(9): p. 2509-18.
11. Xu, L., et al., *Induction of high temperature requirement A1, a serine protease, by TGF-beta1 in articular chondrocytes of mouse models of OA*. *Histol Histopathol*, 2013.
12. Fosang, A.J., et al., *Degradation of cartilage aggrecan by collagenase-3 (MMP-13)*. *FEBS Lett*, 1996. **380**(1-2): p. 17-20.
13. Tetlow, L.C., D.J. Adlam, and D.E. Woolley, *Matrix metalloproteinase and proinflammatory cytokine production by chondrocytes of human osteoarthritic cartilage: associations with degenerative changes*. *Arthritis Rheum*, 2001. **44**(3): p. 585-94.
14. Shlopov, B.V., et al., *Osteoarthritic lesions: involvement of three different collagenases*. *Arthritis Rheum*, 1997. **40**(11): p. 2065-74.
15. Neuhold, L.A., et al., *Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice*. *J Clin Invest*, 2001. **107**(1): p. 35-44.
16. Little, C.B., et al., *Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development*. *Arthritis Rheum*, 2009. **60**(12): p. 3723-33.
17. Hellio Le Graverand-Gastineau, M.P., *OA clinical trials: current targets and trials for OA. Choosing molecular targets: what have we learned and where we are headed?* *Osteoarthritis Cartilage*, 2009. **17**(11): p. 1393-401.

18. Xu, L., et al., *Increased expression of the collagen receptor discoidin domain receptor 2 in articular cartilage as a key event in the pathogenesis of osteoarthritis*. *Arthritis Rheum*, 2007. **56**(8): p. 2663-73.
19. Xu, L., et al., *Activation of the discoidin domain receptor 2 induces expression of matrix metalloproteinase 13 associated with osteoarthritis in mice*. *J Biol Chem*, 2005. **280**(1): p. 548-55.
20. Xu, L., et al., *Attenuation of osteoarthritis progression by reduction of discoidin domain receptor 2 in mice*. *Arthritis Rheum*, 2010. **62**(9): p. 2736-44.
21. Salazar, A., et al., *Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular joints of osteoarthritic mouse models*. *J Oral Pathol Med*, 2014. **43**(4): p. 317-21.
22. Poole, C.A., S. Ayad, and J.R. Schofield, *Chondrons from articular cartilage: I. Immunolocalization of type VI collagen in the pericellular capsule of isolated canine tibial chondrons*. *J Cell Sci*, 1988. **90 (Pt 4)**: p. 635-43.
23. Wilusz, R.E., J. Sanchez-Adams, and F. Guilak, *The structure and function of the pericellular matrix of articular cartilage*. *Matrix Biol*, 2014. **39**: p. 25-32.
24. Li YF, L.Y., Xu L, Servais J, Frank E, Lazarev A, Olsen BR, Grodzinsky AJ, Medici D, *Mechanical loading-induced TGF- β 1 mediates cartilage degradation caused by upregulation of HTRA1/DDR2*. ORS annual meeting, Long Beach, CA, 2011.
25. Wu, J., et al., *Comparative proteomic characterization of articular cartilage tissue from normal donors and patients with osteoarthritis*. *Arthritis Rheum*, 2007. **56**(11): p. 3675-84.

26. Hecht, J.T., et al., *Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia*. Nat Genet, 1995. **10**(3): p. 325-9.
27. Alexopoulos, L.G., et al., *Developmental and osteoarthritic changes in Col6a1-knockout mice: biomechanics of type VI collagen in the cartilage pericellular matrix*. Arthritis Rheum, 2009. **60**(3): p. 771-9.
28. Xu, L., et al., *Intact pericellular matrix of articular cartilage is required for unactivated discoidin domain receptor 2 in the mouse model*. Am J Pathol, 2011. **179**(3): p. 1338-46.
29. Galera, P., et al., *Transforming growth factor-beta 1 (TGF-beta 1) up-regulation of collagen type II in primary cultures of rabbit articular chondrocytes (RAC) involves increased mRNA levels without affecting mRNA stability and procollagen processing*. J Cell Physiol, 1992. **153**(3): p. 596-606.
30. Harvey, A.K., P.S. Hrubey, and S. Chandrasekhar, *Transforming growth factor-beta inhibition of interleukin-1 activity involves down-regulation of interleukin-1 receptors on chondrocytes*. Exp Cell Res, 1991. **195**(2): p. 376-85.
31. Bakker, A.C., et al., *Overexpression of active TGF-beta-1 in the murine knee joint: evidence for synovial-layer-dependent chondro-osteophyte formation*. Osteoarthritis Cartilage, 2001. **9**(2): p. 128-36.
32. van Beuningen, H.M., et al., *Osteoarthritis-like changes in the murine knee joint resulting from intra-articular transforming growth factor-beta injections*. Osteoarthritis Cartilage, 2000. **8**(1): p. 25-33.
33. van Beuningen, H.M., et al., *Transforming growth factor-beta 1 stimulates articular chondrocyte proteoglycan synthesis and induces osteophyte formation in the murine knee joint*. Lab Invest, 1994. **71**(2): p. 279-90.

34. Kawamura, I., et al., *SnoN suppresses maturation of chondrocytes by mediating signal cross-talk between transforming growth factor-beta and bone morphogenetic protein pathways*. J Biol Chem, 2012. **287**(34): p. 29101-13.
35. Schlaak, J.F., et al., *Different cytokine profiles in the synovial fluid of patients with osteoarthritis, rheumatoid arthritis and seronegative spondylarthropathies*. Clin Exp Rheumatol, 1996. **14**(2): p. 155-62.
36. Lee, J.H., et al., *Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression*. Arthritis Rheum, 2005. **52**(8): p. 2386-95.
37. Li YF, X.L., *A molecular understanding of articular cartilage degeneration in development of osteoarthritis*. Harvard School of Dental Medicine, and Harvard Medical School.
38. Polur, I., et al., *Role of HTRA1, a serine protease, in the progression of articular cartilage degeneration*. Histol Histopathol, 2010. **25**(5): p. 599-608.
39. Glasson SS, C.M., Van Den Berg WB, Little CB, *The OARSI Histopathology Initiative - Recommendations for histological assessments of osteoarthritis in the mouse*. Osteoarthritis Cartilage, 2010.
40. DL, G., *General pathology of the peripheral joints*. The Joints and Synovial Fluid, 1980. **2**.