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Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

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This thesis will be organized into three chapters discussing the mechanism underlying the onset and progression of osteoarthritis (OA) in the temporomandibular joint (TMJ). Understanding the mechanism of OA development in the TMJ helps in understanding how OA progresses and how to treat this disease. The goal of this investigation is to examine the process of cartilage degeneration and OA biomarker expression in the TMJ to understand their role in TMJ OA onset and development.

Chapter one covers mechanisms that are altered in TMJ OA during disease progression. Using animal models with different stressors such as mechanical disturbances, direct injury, and changes in the extracellular matrix composition revealed the role of the different mechanisms that are up-regulated and down regulated during cartilage destruction.

Chapter two will cover a paper I wrote that introduces a novel non-invasive technique applied to mice, which induces an early onset of OA in the TMJ. I developed this technique with the aim to provide a new mouse model where the onset and progression of OA more closely mimic the natural TMJ OA progression in humans. The histopathological analysis of the cartilage demonstrates that onset of OA starts at 2 weeks after treatment induction and is aggravated by week eight. This data demonstrated the effectiveness of our technique in inducing OA in the TMJ.

Chapter three will cover a second paper I wrote on the association of RAGE with the progression of OA in the TMJ of mice by using mice with and without RAGE expression. RAGE has been show to contribute to the progression of OA by releasing several pro-inflammatory and catalytic cytokines. Additionally, RAGE has been shown to modulate the expression of specific OA biomarkers, including HtrA-1, Mmp-13, and Tgf-β1 in knee cartilage. The objective of this study was to study the effect of knocking out RAGE on the expression of Mmp-1 3, HtrA-1, and Tgf-β1 in the TMJ. After histophatological and quantitative analysis of biomarkers expression, the results demonstrated for the first time that absence of RAGE expression in the TMJ provides a protective effect against development of TMJ OA in mice.

Keywords: temporomandibular joint, murine, mice, osteoarthritis, RAGE, Mmp-13, HtrA-1, Tgf-β1

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To my parents and siblings, thank you for your encouragement, patience, and love.

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CHAPTER 1: Introduction

Osteoarthritis (OA) of the temporomandibular joint (TMJ) is the most common chronic degenerative disease (1, 2) that results from metabolic imbalances that lead to cartilage degeneration with thinning, fibrillation, fissuring, and loss of the condylar cartilage. The molecular mechanisms underlying this degenerative process indicate that early articular degeneration is characterized by chondrocyte proliferation, cluster formation, and over-production of extracellular matrix (ECM) elements, such as proteoglycans. Contrarily mid and late OA are characterized by progressive degradation of ECM elements, loss of proteoglycan content, and reduction in numbers of chondrocyte (hypoplasia) (3), due to apoptosis, terminal maturation or a combination of both. The molecular differences between OA stages result from the predominance of catabolic processes over anabolic ones as the disease progresses. The onset of these complex processes follows stress produced by mechanical disturbances, direct or indirect injury, and changes in the ECM composition.

Mechanical stress in patients, which is the result of missing teeth or parafunction, is the most frequent trigger of OA in the TMJ. Several studies using animal models such as mice, rats, rabbits, goats, and sheep demonstrated that mechanical stress leads to cartilage degradation (4-7). For example, after 8 and 12 weeks of experimental disruption of the alignment of the teeth on the dental arch of rats , which mimics the absence of the second molar, animals showed signs of cartilage degradation and increased chondrocyte death (4). Also, increasing or decreasing the loading of the TMJ by trimming the dental incisors (out of occlusion), producing a dental cross bite, changing diet, and by forcing the mouth opening resulted in loss of cartilage thickness, altered ECM composition, increased in chondrocyte proliferation, and subchondral bone growth

in the condylar cartilage (4-9). One study examined the effects of inducing a cross bite and changing the diet size for a period of 3 weeks in mice. Results showed that in the group with a large size diet and cross bites, there was an increased expression of a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) mRNA (aggrecanase) in ECM, Receptor Activator of Nuclear Factor κ B (RANK) mRNA in subchondral bone; and a decreased expression of aggrecan mRNA, collagen type I alpha 2 (Col1a2) mRNA, and collagen type II all molecular results that are associated with increase percentage of cartilage degradation (5). Another study showed that after forcing mice mouth open for 1 hr/day for 5 days lead to chondrocyte proliferation and trabecular spacing, which was accompanied with an increase in Col1a2 mRNA, parathyroid hormone-related protein (Pthrp), and transcription factor SOX-9 (Sox9) expression (9).

While these studies reveled the involvement of some genes and proteins in the development of OA in TMJ after mechanical stress, studies made in animals after invasive treatment showed the involvement of more genes and proteins during OA progression (10-15). For example, some studies demonstrated that cartilage post-discectomy showed severe histological features that were coincident with the appearance and increase of discoidin domain receptors 2 (Ddr-2), matrix metalloproteinase-13 (Mmp-13), high temperature requirement protein (HtrA-1) expression, and degraded type II collagen in the cartilage (12, 16). Another study in contrast showed delayed condylar cartilage degeneration in a transgenic Ddr-$2^{0/+}$ mice after surgery (11). All together, these studies demonstrate the key role of HtrA-1, Ddr-2 and Mmp-13 in condylar cartilage degradation.

The injection of chemicals into the joints of rats has also shown to promote cartilage degradation and expression of more proteins and pathways that are involved in TMJ OA

progression (17-21). One study showed that the injection of monosodium iodoacetate (MIA) into the TMJ of rats led to a large increase in the expression of ADAMTS-5 mRNA (aggrecanases), MMP3, MMP13, tumor necrosis factor alpha (TNF-α), Caspase 3/ 8, and proliferating cell nuclear antigen (PCNA), and to a decrease in the expression of mRNA of aggrecan, collagen I, collagen II, and tissue inhibitors of matrix metalloproteinases (TIMP 2). The increase in Capase 3/8 (chondrocyte apoptosis) and patterns of gene expression were consistent with histological and radiological features characteristic of TMJ OA (22). Li at al. (2014) have shown that injecting collagenase into the rat joints produced mild damage to the TMJ cartilage, by significantly increasing the expression of ADAMTs-5 and ADAMTs-5mRNA and significantly decreasing the expression of TIMP-3 mRNA. Therefore, both the imbalance in protein and mRNA expression of aggrecanase and inhibitors of metalloproteinases have been shown to play an important role in the initial stage of condylar cartilage degradation (19). Although the injection of chemicals proved to induce TMJ OA in rats and rabbits, there is no report in the literature of injection- induced TMJ OA in mice.

Cartilage homeostasis is the result of the proper balance and interaction of the ECM elements. Several human chondrodysplasias are the result of mutations that alter the ECM composition, leading to cartilage degeneration and OA. Transgenic mouse models with mutations in collagen type II, IX and XI (Col2a1 (23, 24), Col9a1, Col11a1 (25)), biglycan and fibromodulin ($Bgn^{-/o}$ $Fmo^{-/-}$) (26-28), and $Ddr\text{-}1^{-/o}$(29) genes develop TMJ OA via the activation of several signaling pathways that lead to the up-regulation of catalytic proteases. Thus, alteration of the ECM composition due to genetic mutations disrupts the homeostasis of the cartilage, which activates several mechanisms that promote the cartilage destruction. Jiao et al. (2014) showed that the over-expression of transforming growth factor beta 1 (Tgfβ-1) in

subchondral bone also induces TMJ OA (30). Their findings support the theory that subchondral bone homeostasis plays an important role in OA development in the TMJ. Therefore, OA in TMJ is consequence of direct or indirect alterations of ECM elements.

The study of the TMJ OA in animal models has shown the complexity of this incurable disease that affects many people. Further research will help to elucidate mechanisms and molecules involved in disease progression. This knowledge may lead to the development of therapies for the prevention and cure of OA.

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CHAPTER 2: A Novel Mouse Model of Temporomandibular Joint Osteoarthritis

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Abstract

Objective: Mice present advantages as models to study Osteoarthritis (OA) etiology and pathology. The present study introduces a novel mouse temporomandibular joint (TMJ) model that more closely mimics natural OA progression of the TMJ in humans.

Design: A wire was bound to the right maxillary molars of mice in order to induce a malocclusion, which leads to development of temporomandibular joint dysfunction (TMD) and associated OA. Tissues were histologically analyzed for osteoarthritic characteristics, scored using a standard Modified Mankin system, and compared with an age matched control group.

Results: Experimental mice demonstrated more surface abrasions, hypercellularity and chondrocyte clustering, and decreased proteoglycan staining when compared to control mice. In comparison to controls, summed modified Mankin scores in experimental animals were statistically higher at all time points examined. Confirmatory histological evaluations revealed that experimental mice displayed early onset of OA.

Conclusions: Bonding a wire to induce a malocclusion in mice is an effective inexpensive, novel method to produce an OA model that can be used in future studies to elucidate disease pathogenesis and treatment.

Keywords: temporomandibular joint, murine, osteoarthritis

Introduction

Osteoarthritis (OA) is one of the most common chronic diseases characterized by joint pain, effusion, loss of mobility, and deformity that progresses to functional joint failure. To date there is no treatment to slow or stop its progression. Consequently, OA has become the most common cause of long term disability or physical impairment. It is now considered a major life-altering disorder, and its prevalence in the general population is statistically comparable to major end-stage kidney disease and heart failure. Epidemiological studies shows that over 20 million Americans are affected by OA and that over 500,000 joint replacements are performed annually in United States (1). It has also been reported that more than 80% of people older than 65 years are symptomatic for OA (2-4) and that its incidence is progressively increasing in the middle-aged population (5).

Temporomandibular joint disorder (TMD) is a term encompassing a number of pathological conditions that are primarily caused by the disruption of the dental occlusion. This disorder affects the masticatory musculature, temporomandibular joint, and/or associated structures (6). According to the NIH, approximately 10 million Americans suffer from TMD (7). Symptoms of TMD include severe pain in the soft and/or hard tissues, asymmetrical and/or limited movement of the jaw, joint sounds (clicking), muscular stiffness, and displacement or perforation of the condylar disc. Though symptoms are different for each patient, TMD and the associated malocclusion ultimately result in the development of OA in the TMJ (TMJ OA).

OA is a metabolically active process, where the homeostasis between synthesis and degradation of several extra cellular matrix (ECM) cartilage components is irreversibly disrupted, leading to a loss of cartilage integrity. Despite extensive research, the pathogenesis of the disease is still poorly understood, making effective universal treatment non-existent. These

unknowns necessitate the use of various animal models that induce TMJ OA in order to better understand the biomolecular progression of the disease. Methods currently used to induce TMJ OA include spontaneous, surgical, drug-induced, and mechanical models. Spontaneous models involve the use of genetically altered mice. Researchers have reported TMJ OA in mice with mutations of the *Col2a1* (8, 9), Tgf-β1(10), DDR-1(11), $FgfR^{3P244R}$(13), *Col9a1* and *Col11a1* (14), biglycan and fibromodulin genes (15-17). Drug induced models often involve the injection of solutions like monosodium iodoacetate (MIA) or collagenases (18) into the joint (19, 20). Invasive surgical models include the removal of part or all of the articular disc (12, 21, 22), and perforation of the disc (23).

While all of these models have the general advantage in that they lead to TMJ OA, there are also disadvantages. For instance, a spontaneous model with a genetic mutation may result in blockage of the endoplasmic reticulum or over-activation of pathways that while leading to OA, do not represent common pathways of induction in humans. In addition, inactivating genes in knockout models may interfere with normal early development of healthy cartilage. For surgically invasive models, contamination of the joint is possible, and use of analgesic drugs after surgeries might interfere with the natural progression of OA. Additionally, current surgical models are overly invasive such that although the animal develops early TMJ OA, they do not mimic the natural onset and progression of the disease in humans. Our goal was to create a mouse model that results in development of TMJ OA in a more natural manner to better mimic the progression of the disease in humans. Consequently, our model can be used in future research as an inexpensive, quick option for animal studies that focus on discovering biomolecular components associated with OA, leading to viable treatment options.

Researchers have previously used several techniques to disrupt the dental occlusion in mice, rats, and rabbits (24-27) to study temporomandibular disease (TMD) (26, 28). Inducing such disruptions in mice can prove difficult, due to their small anatomy and the absence of appropriately sized dental tools. However, we developed tools and a quick (7 min), efficient method to alter the dental occlusion in mice. This novel technique is reversible and consists of bonding a wire to the upper maxillary molars of mice at eight weeks of age. Mice were not given an analgesic and their diets were unaltered, allowing for the malocclusion to naturally induce OA. After histological joint analysis and comparison with a control group of age matched mice, we observed the development of TMJ OA as early as two weeks post- misalignment. This novel OA model allows for the study of the expression of OA at the gross and cellular levels during its onset and development in a manner similar to the natural development of TMJ OA in humans. This new technique could aid greatly in elucidating disease pathogenesis and treatment.

Methods and Materials

Facilities and Animals

The experimental procedure was performed on 8-week-old mice, when mice are considered fully mature. Mice were maintained in the animal care facility of Brigham Young University. All experimental procedures were performed following protocol #12-0801, approved by the Brigham Young University IACUC.

Materials

In each of the mice, we disrupted the dental occlusion by bonding a wire to the maxillary molar teeth, similar to the procedure used by Walker et al. (28). We modified the technique by bonding the wire to the molars indefinitely. In addition, we invented a dental platform (Figure 2.1) that allowed the mouse to be restrained during the procedure and tools that helped to provide

a clear operatory field. One tool, referred to as a dental mandibular separator (Figure 2.2), helped by keeping the mouth open during the entire procedure. Another tool, a cheek retractor (Figure 2.2), helped to separate the cheek from the teeth to allow for proper placement of the wire on the molars. Stainless steel wire (0.012" diameter) was cut into 5 mm sections and folded in half.

Disruption of the Dental Occlusion

Mice were anesthetized with intra-peritoneal anesthetic, that contained 1 ml of Ketamine (100 mg/ml), 0.1 ml of Xylazine (100 mg/ml), and 8.9 ml of sterile water. The dose injected was 0.01 ml per 1 gram of mouse body weight. Ophthalmic ointment was applied prior to and post-wire placement to the mouse eyes. Once on the dental platform the mouth was opened with the mandibular separator and cheek was retracted with the cheek retractor. We put drops of 37.5% phosphoric acid gel (Kerr Gel Etchant, Orange, CA, USA) on the maxillary molars and after 20 seconds, we rinsed them and aspirated simultaneously. We dried the teeth by blowing air, after which we added composite adhesive (OptiBond Solo Plus Kerr, Orange, CA, USA), prior to placing a stainless steel wire. The composite was light cured with a micro dental curing light (quartz-tungsten-halogen curing light with radiance of 500Mw/cm2) for 20 seconds. Finally we applied flowable composite (N'Durance Dimer Flow, Septodont, Louisville, CO, USA) and light cured it for 30 seconds (Figure 2.2). Mice were placed in a cage that had a heater pad until they recovered from the anesthetic. After that, they were returned to their proper cages. They did not receive any analgesic prior to and after the procedure. Mice were weighed before the procedure and every week for the duration of the study. In addition, permanence of the wire was checked every week. A pain scoring rubric was used to assess level of discomfort with the wire bonded to the tooth.

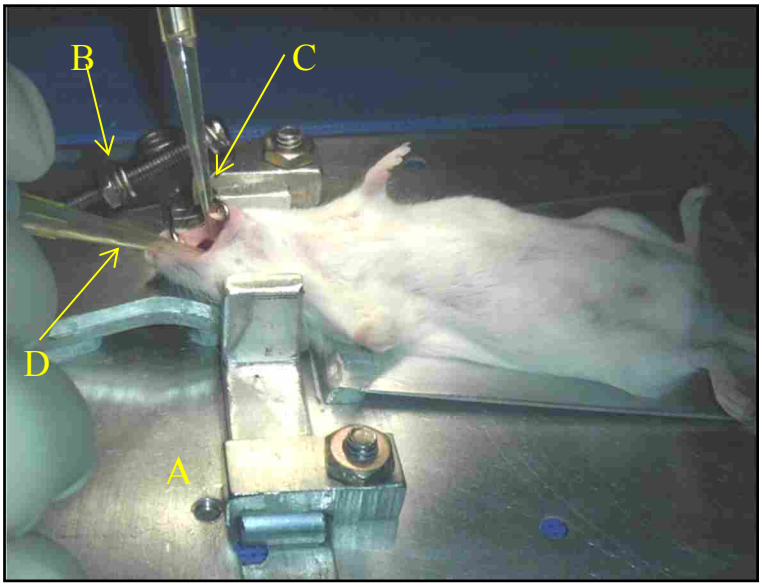

Figure 2.1: Tools Dental Platform.
The dental platform (A) restricts movement and the mandibular separator (B) holds the mouse's mouth open during the entire procedure. Pipet tips connected to the portable dental unit (C, D)

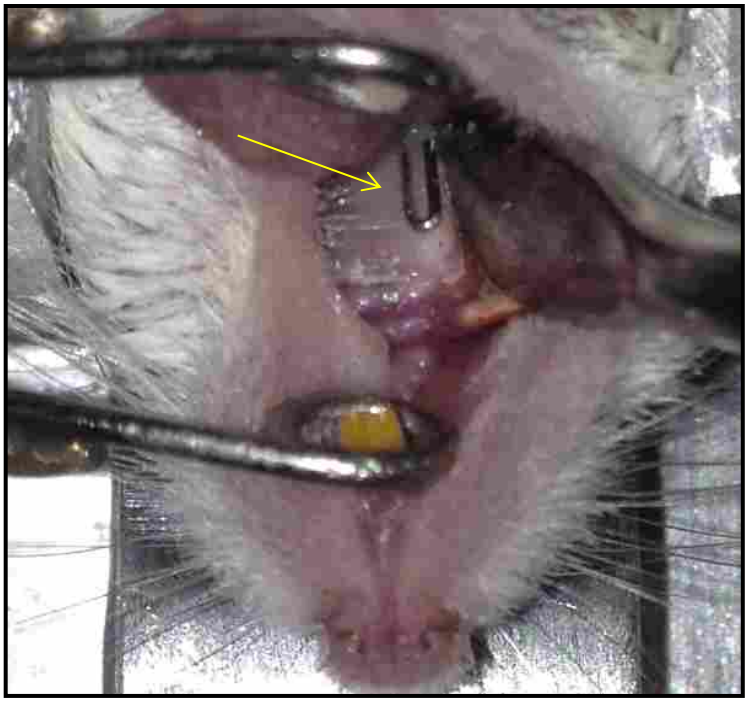

Figure 2.2: Wire Bonded to Teeth.
The wire was bonded to the right upper maxillary molars (A). The murine mandibular separator (B) and cheek retractor (C) were used.

Tissue Harvesting and Processing

Mice 2, 4, 6, and 8 weeks post placement of wire were euthanized using CO_2 and heads were excised and fixed in 4% paraformaldehyde overnight. Each sample was then washed with water every 30 minute over a period of 6 hours. Tissues were decalcified in a decal solution (1:1 ratio of 45% formic acid and 20% sodium citrate), that was changed every 2-3 days for a period of 2-3 weeks. Decalcification of each sample was confirmed through performance of an ammonium oxalate calcium precipitation reaction. They were then dehydrated and embedded in paraffin wax using an automated tissue processor (ThermoFisher Scientific, Waltham, MA, USA). Paraffin blocks were prepared, and TMJs were embedded in paraffin wax with the joint flush with the cutting surface to achieve a frontal cut. TMJ blocks were sectioned at 6 μm through the entire joint from the anterior surface to the posterior part using a Micro HM 325 microtome (Thermo Scientific, Kalamazoo, MI, USA). Four sections were placed per glass microscope slide, yielding approximately 15-20 slides, or approximately 60-80 sections per joint, depending on the animal.

Histological Analysis

Slides from control and experimental mice at each time point were stained with Safranin-O/Fast Green to evaluate the histopathological state of the TMJ. Using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA, USA), photographs of each joint were taken at 10X, 20X, and 40X. The joint cartilage sections were analyzed using a modified Mankin score system (9, 29-32) to quantify the cartilage's pathological state. The modified Mankin scoring system is based on a subset of scores, including cartilage erosion scoring (0-6), chondrocyte periphery staining (0-2), spatial arrangement of chondrocytes (0-3), and background staining intensity (0-3) (33-35). Scores were calculated by

summing the four sub-criteria scores, where zero represented unaltered articular cartilage and 14 represented severe OA.

Statistical Analysis

Statistical significance of the combined Mankin scores at each time point for experimental and control groups was performed using a two-way analysis of variance test (ANOVA) conducted by the department of statistic at BYU.

Results

Modified Mankin scoring used for the semiquantitative assessment of histological OA showed an overall higher score in experimental mice than of control mice at all times points (Figure 2.3 and 2.4). The experimental mice summed scores increased over time, reaching a peak at 8 weeks (W) post disruption of normal occlusion. There was a statistically significant difference between Mankin scores of the two groups of mice at 2 ($p=0.032$), 4 ($p<.0001$), 6 ($p<.0001$), and 8W ($p<.0001$).

The cartilage erosion scores of experimental group compared with that of the control group were higher at all times points (Figure 2.5). However, there was a statistically significant increase at 4W ($p=0.026$) in experimental when compared with control. Interestingly, scores in experimental and control group showed an increase at 4W, decrease at 6W and increase again at 8W. There was also a statistically significant difference ($p=0.017$) when cartilage erosion in 8W experimental mice were compared to 2W experimental mice.

The chondrocyte periphery staining of the experimental group showed an increase at 2W, its highest value at 4W, and a dramatic drop during the following weeks (Figure 2.6). These scores, with the exception of 6W scores, were always higher in mice after placement of wire when compared to control mice. There was a statistically significant increase in chondrocyte

periphery staining at 4W ($p=0.0007$) in the experimental group compared with that of the control group.

Spatial arrangement: Scores for spatial arrangement of chondrocytes in the experimental group increased over time and were always higher than that of the control group (Figure 2.7). These values were significantly higher at 4 ($p=0.0005$), 6 ($p<.0001$), and 8 ($p<.0001$) weeks in mice after placement of wire when compared with the control group. The overall scores for the control groups were lower at all time points.

Background staining scores in mice after the wire was bonded were similar in experimental mice at 2 and 4W. However, they increased in the following weeks, reaching a peak value at 8W. Control group scores reached their peaks at week 4, decreased at week 6, and increased again at week 8. Experimental scores, with the exception of scores at 4W, were higher when compared with that of control scores. However, these differences were not statistically significant. There was a statistically significant difference between scores at 2W in the control and experimental groups when compared with 8 week scores of the experimental group ($p=0.0017$, $p=0.0022$) (Figure 2.8).

Mice in our study showed a decrease in total body weight by the first week after misalignment of the TMJ. However, they recovered and increased in weight to normal levels (36) during the following weeks (Figure 2.9). This showed that mice are capable of adapting to the induced malocclusion. Even though mice were developing OA in the TMJ they did not appear to suffer any pain or change dietary habits.

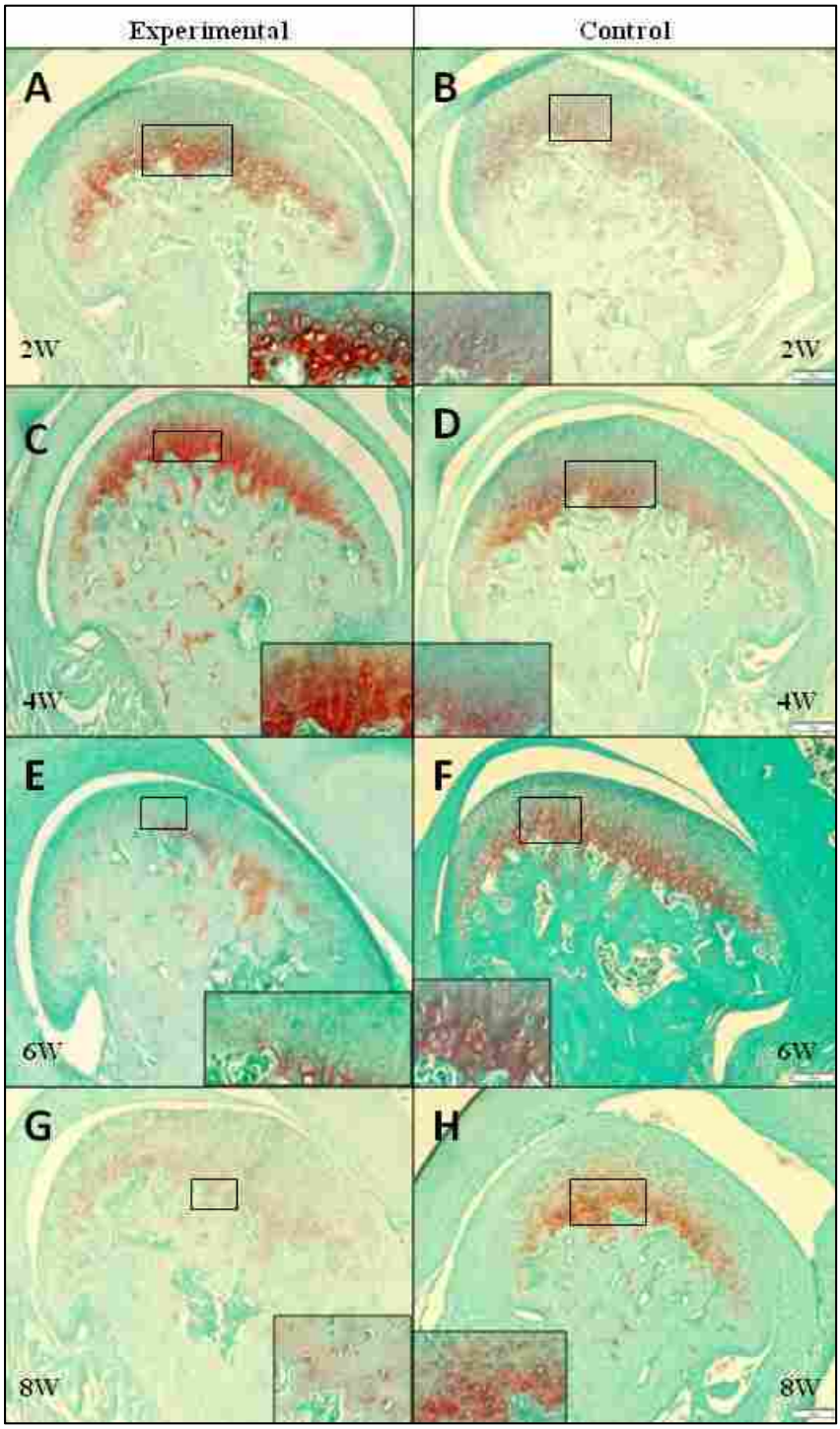

Figure 2.3: Safranin O/ Fast Green Staining.
Proteoglycan expression observed by SO staining in experimental and control mice. An increase in proteoglycan expression was observed in experimental mice at 4W (week) (C) as compared with control mice (D). However, proteoglycan expression decreased at 6W (E) and 8W (G) in experimental mice, as compared with control mice at 6W (F) and 8W (H). At 4W hypercellularity is accompanied with the appearance of clusters in experimental mice (C), features that are missing in control mice of the same age group (D). However, hypocellularity was observed in 8W experimental mice (G) when compared with 8W control mice (H).

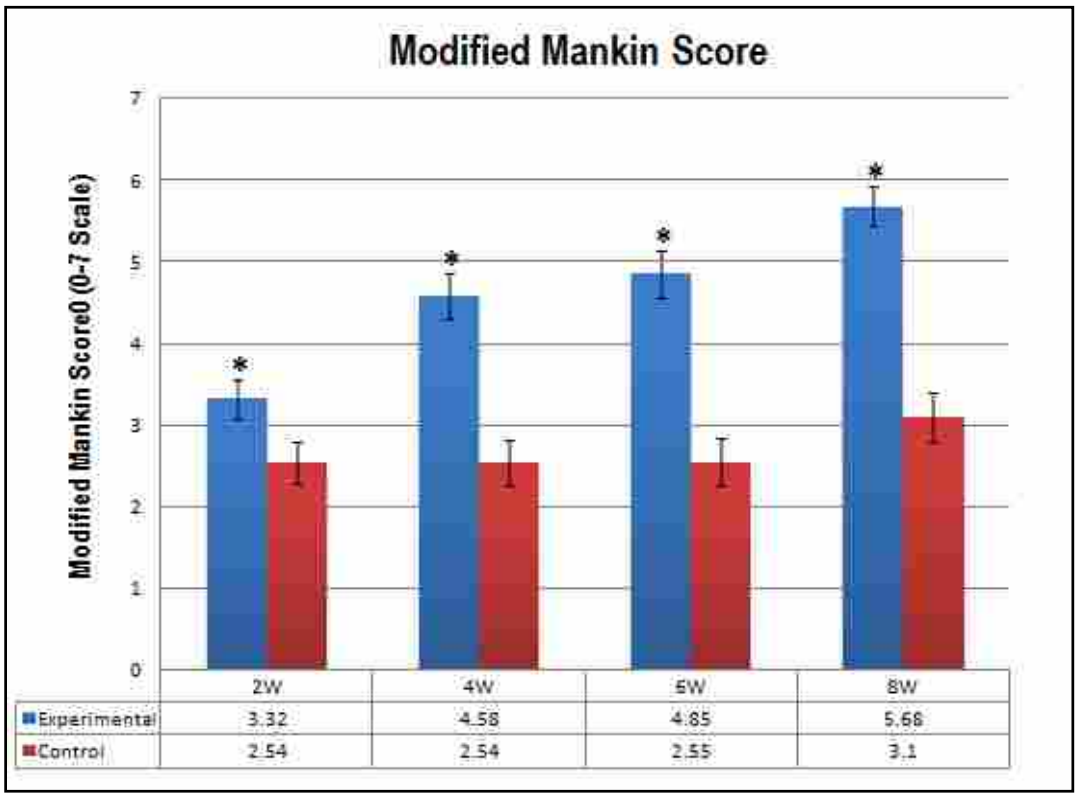

Figure 2.4: Modified Mankin Score.
Modified Mankin score shows an overall higher score in experimental group when compared with that of control mice at all time points. These differences are statistically significant at 2W ($p=0.032$), 4W ($p<0.0001$), 6W ($p<0.0001$), and 8W ($p<0.0001$).

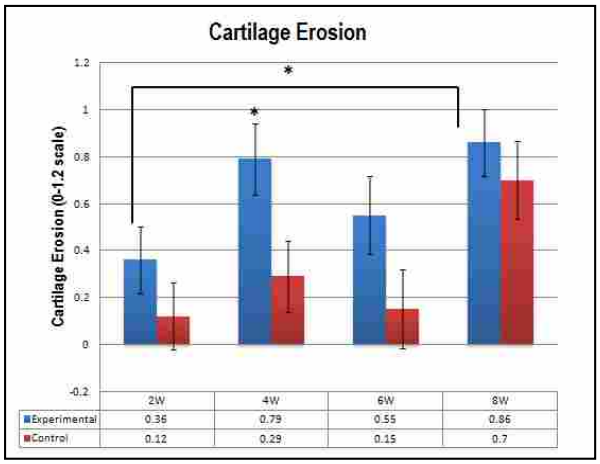

Figure 2.5: Cartilage Erosion.
Cartilage erosion scores demonstrated a significant increase in the experimental group when compared with that of the control group at four weeks (p=0.026), and between 2W and 8W experimental mice (p=0.017). Increased erosion scores in experimental mice are indicative of cartilage fibrillations, OA.

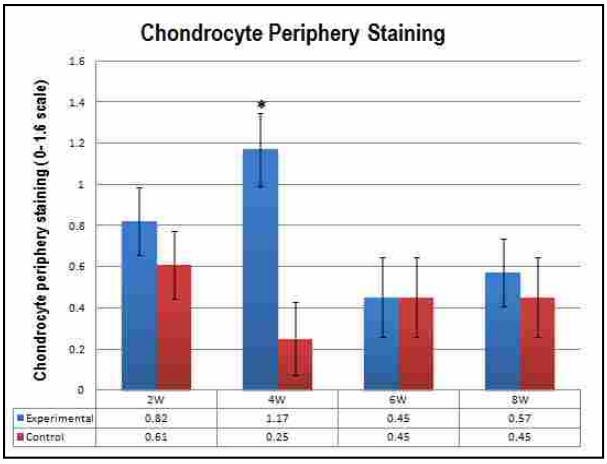

Figure 2.6: Chondrocyte Periphery Staining.
There is a significant difference in the chondrocyte periphery staining scores of 4W experimental group (p=0.0007) when compared with control. This indicates that stressed chondrocytes of experimental mice by altered occlusion increased proteoglycan expression.

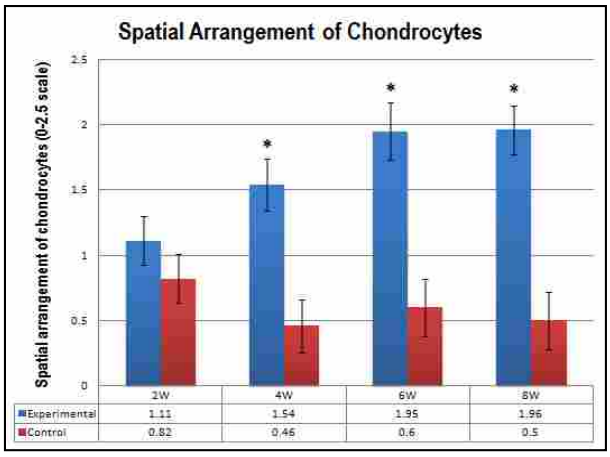

Figure 2.7: Spatial Arrangement of Chondrocytes.
Spatial arrangement scores are statistically different between 4 (p=0.0005), 6 (p<0.0001) and 8 (p<0.0001) groups of mice. This difference is caused by increased hyperplasia and clustering of chondrocytes in experimental mice. These changes worsen over time and are characteristic of osteoarthritic cartilage.

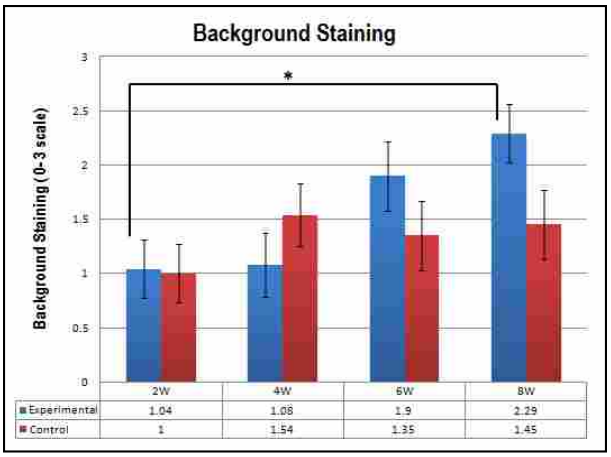

Figure 2.8: Background Staining.
There is a significant increase in the background staining scores of 2W and 8W experimental groups of mice (p=0.0017). This increase is caused by the development of OA and associated decrease in proteoglycan expression and chondrocyte quantity.

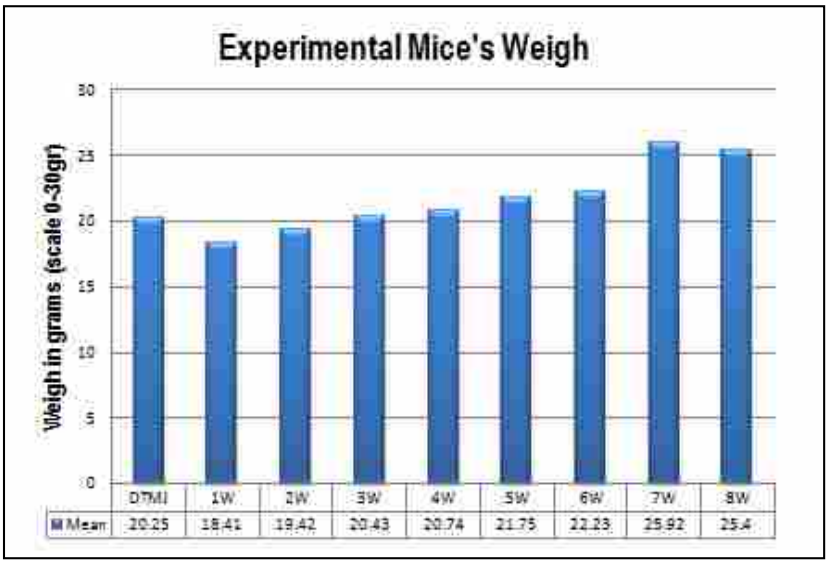

Figure 2.9: Mice Weight.
Experimental mice showed a decrease in weight after alteration of the dental occlusion and then a gradual increase in the following weeks.

Table 2.1: Modified Mankin Score Table.

Cartilage Erosion Scoring	
Smooth non-eroded Cartilage	0
Rough non-eroded Cartilage	1
Superficial fibrillation	2
Separation of uncalcified from calcified cartilage	3
Erosion of uncalcified cartilage only	4
Erosion extending into calcified cartilage	5
Erosion down to subchondral bone	6
Chondrocyte periphery staining	
Normal	0
Slightly enhanced	1
Intensely enhanced	2
Spatial arrangement of chondrocytes	
Normal	0
Diffuse hypercellularity	1
Clustering	2
Hypocellularity	3
Background staining intensity	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3
No dye noted	4

Discussion

In this study, we analyzed the tissue to determine whether our model induced the development of TMJ OA. The histology of the condylar cartilage consists of the articular proliferative, chondroblastic, and hypertrophic layers. Disruptions of cartilage homeostasis are reflected in changes in the cartilage surface, cellular, and extracellular arrangement.

In our study the superficial cartilage in the control group after 2W was smooth compared with that of the experimental group at 8W, where the surface was rough and superficial fibrillations were present (Figure 2.3G). The cartilage erosion score showed an interesting pattern of behavior such that erosion increased at 4W, decreased at 6W, and increased again at 8W in the experimental and control groups (Figure 2.5). We hypothesize that cartilage repair mechanisms were elevated after initial damage to the cartilage surface at 4W. This repair process is reflected in the decrease of the surface erosion score at 6 weeks. However, the homeostatic imbalance caused by the malocclusion prevents long term repair, resulting in increased surface erosion that is typical of OA at 8W in experimental mice. Knee cartilage is articular hyaline cartilage consisting solely of Type II collagen whereas the cartilage of the TMJ is condylar fibrocartilage, composed of Type I and Type II collagen (37, 38). It has been suggested that condylar cartilage is unique in that it has the ability to repair itself, unlike cartilage of the knee (39). This may explain the sudden drop in severity of surface erosion in the group of 6 week experimental mice, but this phenomenon needs to be explored further in future studies.

Even though the cartilage of experimental mice showed surface fibrillation (Figure 2.3G), we did not see any erosion or separation of uncalcified cartilage and erosion extending to the calcified region or subchondral bone. Our study only monitored mice until 8W post malocclusion, at which point experimental mice demonstrated surface abrasions, markedly

decreased proteoglycan levels, and hypocellularity with clustering of chondrocytes (Figure 2.3E, and G). These factors are typical of osteoarthritic tissues and imply that given more time, more dramatic destruction of cartilage surface would occur if monitored to a further time point (40).

Mature chondrocytes reside in the chondroblastic layer (41, 42) and they actively produce collagen type I, II, proteoglycans, and various other ECM components (38, 43). In our study, Safranin –O staining showed a strong staining for proteoglycans in the chondroblastic and hypertrophic layer at 2W (Figure 2.3A and C), which reached its maximum value at 4W in the experimental group of mice (Figure 2.3C and Figure 2.6). This increase in proteoglycan concentration shows hyperactivity in stressed chondrocytes after inducing the malocclusion in our model and is compatible with early onset of OA (9, 44, 45). In the following weeks, the proteoglycan expression decreased dramatically in the experimental group (Figure 2. 3E and G, and Figure 2.6) showing a failed attempt of self-repair and chondrocyte death. The decrease of proteoglycans means that catabolic processes are taking place, causing the cartilage to be unable to repair itself. These features are characteristic of osteoarthritic cartilage shown in our mouse model.

Experimental mice scores in comparison with that of the control mice showed a statistically significant increase for spatial arrangements of chondrocytes at 4, 6, and 8W (Figure 2.7). In response to stress, chondrocytes proliferate and increase in number as an attempt to self-repair and to fix the homeostatic imbalance associated with OA (46). This may explain why tissues at 2W in the experimental group show chondrocyte hyperplasia in comparison to 2W control mouse tissues (Figure 2.3A and B). At 4 weeks, there is a considerable increase in clusters in the proliferative and hypertrophic layers of the experimental group when compared with that of the control group (Figure 2.3C and D). However, at 6 and 8W there is a dramatic

decrease of chondrocytes in the hypertrophic, chondroblastic, and proliferative layers of the experimental mice (Figure 2.3E and G). These cellular changes are compatible with the progression of osteoarthritis that leads to cartilage atrophy (9, 17).

The background staining decreases at 8W in experimental group due to the lack of chondrocytes (Figure 2.3G) and likely increase in catabolic factors, demonstrating dramatically reduced proteoglycan expression in the cartilage. These changes are compatible with the development of TMJ OA in other mice models (8, 17). There is a significant statistical difference in the background staining score between 2 and 8W experimental mice (Figure 2.8). This shows that mouse cartilage, after 8W of malocclusion, develops OA-like changes.

Our hypothesis that the disruption of the occlusion would lead to OA in the TMJ was successfully demonstrated, as shown by histological and statistical analysis. This study introduces a novel TMJ OA mouse model that involves an inexpensive, quick, and replicable procedure. The mechanical nature of the malocclusion resembles the natural development of OA in humans, making this an ideal model in future studies that aim to elucidate the pathogenesis of TMJ OA and discover a treatment.

Authors' Contributions

DLK, EMCM designed the experiment. EMCM designed the mouse model, tools and perform all experimental procedures. PRR provided the mice. DLK, EMCM, PRR and DKM contributed to writing the manuscript. All the authors assisted in carrying out experimental procedures and interpreting the results.

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Competing Interests

No author had competing or conflicting interests during the duration of this study.

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CHAPTER 3: Expression of Osteoarthritis Biomarkers in Temporomandibular Joints of Mice with and Without Receptor for Advanced Glycation End Products (RAGE)

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Abstract

Objective: Osteoarthritis (OA) in the temporomandibular joint (TMJ) is a chronic degenerative disease that may be the result of sustained chronic inflammation. Recent experiments have shown that the receptor for advanced glycation end products (RAGE) is involved in OA progression. The objective of this research was to study the expression of Mmp-13, HtrA-1, and Tgf-β1, OA biomarkers in TMJ of mice with and without RAGE expression after induced TMJ misalignment.

Design: We induced a non invasive TMJ misalignment on 8-week-old RAGE knockout (KO) and wild type (WT) mice. We assayed for Mmp-13, HtrA-1, and Tgf-β1 biomarkers in condylar cartilage at 2 and 8 weeks post-misalignment by immunohistochemistry of the right TMJs.

Results: Immunohistochemistry and histopathological tissue analysis in RAGE KO mice after misalignment showed lower Mmp-13 and HtrA-1 expression, as well as lower summed modified Mankin scores in condylar joints when compared to those of WT.

Conclusions: We conclude that knocking out RAGE protects the TMJ from the typical development of OA by decreasing Mmp-13 and HtrA-1 expression.

Keywords: temporomandibular joint, murine, mice, osteoarthritis, RAGE, Mmp-13, HtrA-1, Tgf-β1.

Introduction

Osteoarthritis (OA) is a chronic degenerative disease that affects the joints, including the temporomandibular joint (TMJ). Even though OA is associated with several inherited chondrodysplasia syndromes, in most adults it seems to be associated with risk factors such as aging, obesity, repetitive stress, joint misalignment, acute injury, and genetic predisposition. OA results in a painful and progressive degeneration of the articular surface with thinning, fissuring, and ultimate loss of the protective articular surface of the joint.

OA is increasingly viewed as a metabolically, active dynamic process, where destruction and repair occur simultaneously, after being triggered by a variety of biochemical and mechanical insults (1, 2). This dynamic process is carried out by the expression of pro-inflammatory cytokines during OA onset, and its progression explains why OA is currently viewed as an inflammatory disease (3). Unfortunately, the exact inflammatory mechanism that is activated or up-regulated and the exact cytokine/s responsible for OA onset and development are not well understood. Loeser et al. showed that chondrocytes express the receptor for advanced glycation end-products (RAGE), which is up-regulated in OA in humans (4). RAGE, a member of the superfamily of pattern recognition receptors (PRR), is a transmembrane receptor that is composed of three immunoglobulins like domains. V and C domains provide a large cationic surface area that mediates the binding of most RAGE ligands. An additional transmembrane domain that is attached to a highly charged 43 amino acid intra-cytoplasmic domain mediates intracellular signal transduction [17]. RAGE binds to several ligands, such as advanced glycation end-products (AGEs)(5), high-mobility group box-1(HMGB1/amphoterin) (6, 7), several pro-inflammatory cytokine-like mediators of the S100/calgranulin family (8), β amyloid peptides, Mac-1 (9, 10), and specific DNA and RNA structures (7). After binding its ligands, RAGE

mediates gene expression and pro-inflammatory response through the activation of a variety of signal transcriptional pathways, including PI3K,/Akt (11), RhoGTPases (12), Jak/STAT (13), Src family kinases (14), MAPKs/Erk1/2, JNK, p38 and IKK/NF-κB (15). The downstream gene product results of its signal transcription include NF- κB, Cox-2, pro-inflammatory cytokines TNF-α (16, 17), IL-1β(8), IL-6(18), IL-8(19), MCP-1(19), INF-α(20), and matrix metalloproteinases, such as Mmp-13 (21). The release of these products is sustained by a positive feedback loop, a feature characteristic of RAGE signaling (22, 23). This is the reason why RAGE expression is up-regulated in chronic inflammatory states (24, 25) such as OA (4), diabetes (26), atherosclerosis, sepsis, rheumatoid arthritis, Alzheimer's Disease (7), lung fibrosis (27), acute respiratory distress syndrome (ARDS), polycystic kidney disease (28), and chronic obstructive pulmonary disease (COPD). In OA, the cartilage deterioration is due to sustained degradation of type II collagen that has been shown to be carried out by expression of Mmp-13 (collagenase 3), a downstream RAGE product that is increased as a result of RAGE activation (29).

We and others have recently demonstrated that knees of osteoarthritic mice show high levels of Mmp-13 and high temperature requirement A serine protease (HtrA-1), indicating their key role in the initiation and progression of OA (30-33). HtrA-1 is a serine protease that is involved in various aspects of protein quality and cell fate. During development, its expression is significantly increased; however, in adult articular cartilage, its expression is not significantly detectable (34). Pathological levels of HtrA-1 have been implicated with the development of several diseases, such as rheumatoid arthritis, OA, cancer, macular degeneration, muscular dystrophy, and aging (35-40). HtrA-1 has three mechanisms of action. First, it hydrolyzes multiple substrates present in the pericellular (PCM) (36) and extracellular matrix (ECM),

including aggrecan, biglycan, fibromodulin, decorin, cartilage oligomeric matrix protein (COMP), (41) and fibronectin (42, 43). Second, it induces expression of metalloproteases (MMPs) (34). Third, it inhibits growth factor family activity (34). Because of its mechanism of action, HtrA-1 expression has been implicated in the onset and progression of OA; patients with OA have been shown to express high levels of HtrA-1 in their synovial fluid and cartilage (35, 44). HtrA-1 has also been shown to be associated with centrosomal modulation of microtubule stability (45). This suggests that its aberrant expression plays a role in cytoskeletal disruption associated with cell death, or at least in pathological processes like OA. Upon destruction of the PCM by HtrA-1, the triple helical type 2 collagen makes contact with exposed discoidin domain receptor 2 (Ddr2) located in the chondrocyte cell membrane, thereby causing further up-regulation of this receptor (36) and subsequent expression of Mmp-13 (46). Thus, in OA, the up-regulation of Mmp-13 as a result of RAGE and HtrA-1 signaling accelerates cartilage degeneration. We and others have show that OA is additionally linked to low levels of an anabolic cytokine, Tgf-β1(30), which is inhibited by high levels of HtrA-1.

Transforming growth factor (Tgf-β1) is a multifunctional growth factor that modulates chondrocyte cell differentiation, proliferation, and synthesis of proteoglycans (47-50) and type II collagen (51, 52). Chondrocytes secrete inactive Tgf-β1 (53), which once activated, triggers a series of reactions that result (54) in sustained cartilage integrity. Normal cartilage expresses moderate levels of Tgf-β1, as opposed to osteoarthritic cartilage, where Tgf-β1 expression is almost absent (55). Additionally, high levels of Tgf-β1 have been show to promote osteophyte formation and synovial fibrotic features of OA (56). The overall effects of Tgf-β1 counteract the catabolic effects of IL-1 (55), TNF-α, and Mmp-13 by stimulating synthesis of ECM (57), down

regulating the expression of cytokine (IL-1) receptors (51) and Mmp-13 (58, 59), and inhibiting chondrocyte terminal differentiation into osteophytes (60).

We have shown that Tgf-β1 expression disappears as HtrA-1 expression appears following surgical destabilization of the knee and that these events are associated with an increase in Mankin scores of knee joints (30). Furthermore, we have observed that the expression of HtrA-1 and other catabolic factors are attenuated in a RAGE knockout (KO) mouse following surgical destabilization. These results support the theory that inflammation may provide the trigger for RAGE, HtrA-1 up-regulation, and Tgf-β1 down regulation, which results in the up-regulation of Mmp-13 and onset of OA. Our lab and others have shown that Tgf-β1, HtrA-1, and Mmp-13 play a key role in the development of TMJ OA in transgenic and invasive OA mouse models (44, 61).

In our present study, we investigate the interaction of RAGE, HtrA-1, Tgf-β1, and Mmp-13 activity signaling in TMJ OA. Because these cytokines have been shown to play key roles in the onset and development of OA, we use mice with and without RAGE expression in order to elucidate the receptor's role in this process. Contrary to other experiments, we induce TMJ OA by producing a non-invasive misalignment of the TMJ (manuscript in review). To our knowledge, this is the first study to elucidate the role of RAGE expression in TMJ OA development after induction by a non-invasive trigger. We therefore hypothesize that the absence of RAGE expression will attenuate or prevent the TMJ from developing OA.

Methods and Materials

Facilities and Animals

Balb/C wild type mice (n=56) and RAGE knockout mice (n=56) were kindly provided by Dr. Paul Reynolds at Brigham Young University. The experimental procedure was performed

on 8-week-old mice, when mice are considered fully mature. Mice were maintained in the animal care facility of Brigham Young University. All experimental procedures were performed following protocol #12-0801, approved by the Brigham Young University IACUC.

Materials

In each of the mice, we produced a TMJ misalignment by disrupting the dental occlusion through bonding a wire to the right maxillary molar teeth, similar to the procedure used by Walker et al. (62). We modified the technique by bonding the wire to the molars indefinitely (manuscript in revision).

Tissue Harvesting and Processing

Mice 2, 4, 6, and 8 weeks post placement of wire were euthanized using CO_2 and heads were excised and fixed in 4% paraformaldehyde overnight. Each sample was then washed with water every 30 minute over a period of 6 hours. Tissues were decalcified in a solution (1:1 ratio of 45% formic acid and 20% sodium citrate) that was changed every 2-3 days for a period of 2-3 weeks. Decalcification of each sample was confirmed through performance of an ammonium oxalate calcium precipitation reaction. Tissues were then dehydrated and embedded in paraffin wax using an automated tissue processor (ThermoFisher Scientific, MA). Paraffin blocks were prepared, and TMJs were embedded in paraffin wax with the joint flush with the cutting surface to achieve a frontal cut. TMJ blocks were sectioned at 6 μm through the entire joint from the anterior surface to the posterior part using a Microm HM 325 microtome (Thermo Scientific, Kalamazoo, MI). Four sections were placed per glass microscope slide, yielding approximately 15-20 slides, or approximately 60-80 sections per joint, depending on the animal.

Histological Analysis

Slides from control and experimental mice at each time point were stained with Safranin-O/Fast Green to evaluate the histopathological state of the TMJ. Using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA, USA), photographs of each joint were taken at 10X, 20X, and 40X. The joint cartilage sections were analyzed using a modified Mankin score system (2, 63-66) to quantify the cartilage's pathological state. The modified Mankin scoring system is based on a subset of scores, including cartilage erosion scoring (0-6), chondrocyte periphery staining (0-2), spatial arrangement of chondrocytes (0-3), and background staining intensity (0-3) (67-69). Scores were calculated by summing the four sub-criteria scores, where zero represented unaltered articular cartilage and 14 represented severe OA.

Immunohistochemistry Analysis

Immunohistochemistry (HIC) was performed on sections of joints from WT and RAGE-KO mice at 2, 4, 6, and 8 weeks post-TMJ misalignment. Separate slides were stained with antibodies against HtrA-1, Tgf-β1, and Mmp-13. Antibodies against HtrA-1 (ab38611) and Tgf-β1 (ab92486) were purchased from Abcam (Cambridge, MA), and those against Mmp-13 (SC-8989) from Santa Cruz Biotechnology (Santa Cruz, Ca). Slides were deparafinized and then blocked for 1 hour. All antibodies were diluted, applied to specimens, and incubated overnight at 4ºC. On the second day, samples were rinsed with PBS and then incubated with an avidin/biotin ABC mix (Vectastain elite ABC Kit, Vector Laboratories, Inc. Burlingame, CA). Slides were rinsed a second time with PBS and incubated with biotinylated secondary antibody. After a third PBS rinse, a color reaction was initiated to achieve a red/brown stain using a peroxidase

substrate VECTOR NovaRED substrate kit, (Vector Laboratories, Burlingame, CA). A cover slip was placed prior to photographing. Negative controls were prepared by staining without the addition of primary antibody. Positively stained cells were counted in an area of the lateral side of the joint, the side to which the wire was bonded. Following quantitative analysis, percentages were compared to wild type controls. Photographs of each joint were taken at 40X magnifications using a light microscope equipped with an Olympus digital camera (Olympus America Inc. Center Valley, PA).

Statistical Analysis

Statistical significance of the combined Mankin scores at each time point for experimental and control groups was performed using a two-way analysis of variance test (ANOVA). Staining was analyzed quantitatively by calculating the percentage of cells staining positive for the respective biomarkers in a defined area of the lateral side of the condylar cartilage. All quantitative analysis were performed using ImageJ (National Institutes of Health, Bethesda, MD). The quantitative results were subsequently analyzed statistically using ANOVA to detect differences in the mean percentages of positive staining for OA biomarkers between the RAGE KO and WT samples.

Results

Histopathological results at 8 weeks post-TMJ misalignment show that RAGE KO mice have statistically low modified Mankin scores when compared with WT mice of the same age ($p<.0036$) (Figure 3.3). We observed increased Safranin O staining for proteoglycans of the condylar cartilage 2 weeks after TMJ misalignment, which was greatly reduced in WT cartilage and slightly reduced in RAGE KO cartilage at 8 weeks after TMJ misalignment (Figure 3.1). In addition, controls group show an overall low Mankin score in comparison to experimental

groups, with the exception of RAGE KO at 8 weeks (16 weeks old), unexpectedly showed an elevated Mankin score that is not typical of OA OA (Figure 3.3).

Immunohistochemical analysis of Mmp-13 shows low percentages of positive cells (42.9%) at eight weeks post TMJ-misalignment in RAGE KO mice when compared with WT (70%) (Figure 3.7). However, results did not show a statistically significant difference ($p=.363$). Wild type experimental mouse Mmp-13 expression at 8 weeks shows a statistically higher value (70%) when compared with age matched WT control group (26%)($p<.0091$). All control groups showed lower Mmp-13 expression than age matched experimental groups.

Percentages of positive HtrA-1 staining in WT mice at 2 and 8 weeks after TMJ misalignment are higher (59-62%) when compared with RAGE KO at 2 and 8 weeks post-TMJ misalignment (42-41%) (Figure 3.7). Interestingly, we found that control groups have elevated HtrA-1 expression at 2 week (10 weeks old) (62-67%), which subsequently decrease at 8 week (16 weeks old) (42-54%) (Figure 3.7). Tgf-β1 positive chondrocyte staining shows comparable percentages in all mouse groups at 2 weeks after induced OA, which were slightly reduced by week 8, with the exception of RAGE KO controls, where values increased from 49% to 61% at week eight (Figure 3.7).

Discussion

Early onset of osteoarthritis is characterized by over-production of proteoglycans, hypercellularity, and chondrocyte clustering. Contrary to other invasive TMJ OA mouse models where these features appear at 4 weeks post-OA induction, we found that the same OA features appeared at 2 weeks after a non-invasive OA induction in both RAGE KO and WT mice (Figure 3.2)(31, 70). In subsequent weeks, we observed gradual loss of proteoglycan expression, a reduction in chondrocyte number, and the appearance of superficial fibrillations. At 8 weeks,

RAGE KO mice expressed more proteoglycans and clustering than WT mice, which had significantly reduced proteoglycan expression and chondrocyte number (Figure 3.2). In order to evaluate if there was a statistical difference between the morphological conditions of the condylar cartilage in both mice, we analyzed them using a modified Mankin score system (30, 31, 67). The results revealed that there is a statistical difference between RAGE KO and WT scores at 8 weeks post experimental OA induction (Figure 3.3). This finding demonstrates and agrees with previous work in knee OA that postulates that absence of RAGE does provide a protective effect against OA progression (30). We found an unexpectedly high Mankin score in RAGE KO control mice at 16 weeks. We attribute this to reduced background staining due to low proteoglycan expression, which is accompanied by chondrocyte hypocellularity. This finding could be explained by the absence of RAGE expression during development, which appears to result in reduced chondrocyte number. A reduction in the number of chondrocytes appears to subsequently alter proteoglycan expression in a process that is reversed by the stress induced by TMJ misalignment.

In order to elucidate the underlying molecular events through the expression of catabolic and anabolic cytokines in the onset and development of OA in TMJ and link them with the expression of RAGE, we examined the expression patterns of Mmp-13, HtrA-1, and Tgf-β1. Mmp-13 is a downstream product of RAGE activation and sustained up-regulation (4, 29). Our results confirm our hypothesis that knocking out RAGE decreases Mmp-13 expression from 70% to 40% (Figure 3.4 and 3.7). We are the first to effectively demonstrate that after a non-invasive TMJ OA induction, RAGE KO mice, when compared with WT, showed low Mmp-13 expression in TMJ. This result coincides with observations made by Larkin et al, who studied similar molecular processes in articular cartilage of knee joints (30). These findings show the role of

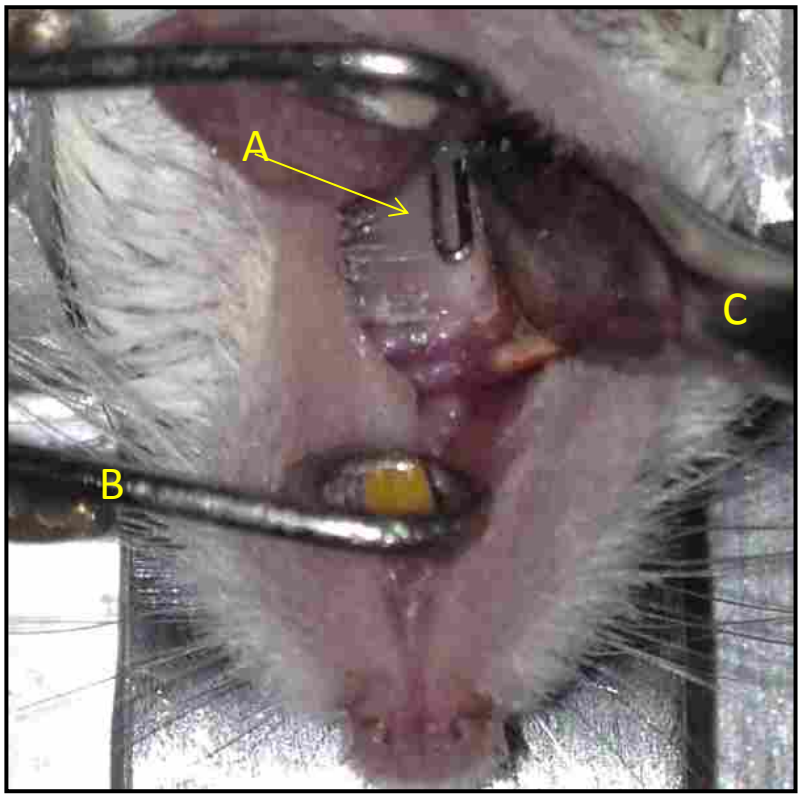

Figure 3.1: Wire Bonded to Teeth.
Misalignment of the temporomandibular joint was produced by bonding a stainless steel wire to the right upper maxillary molars (A). The murine mandibular separator (B) and cheek retractor (C) were used (manuscript in revision).

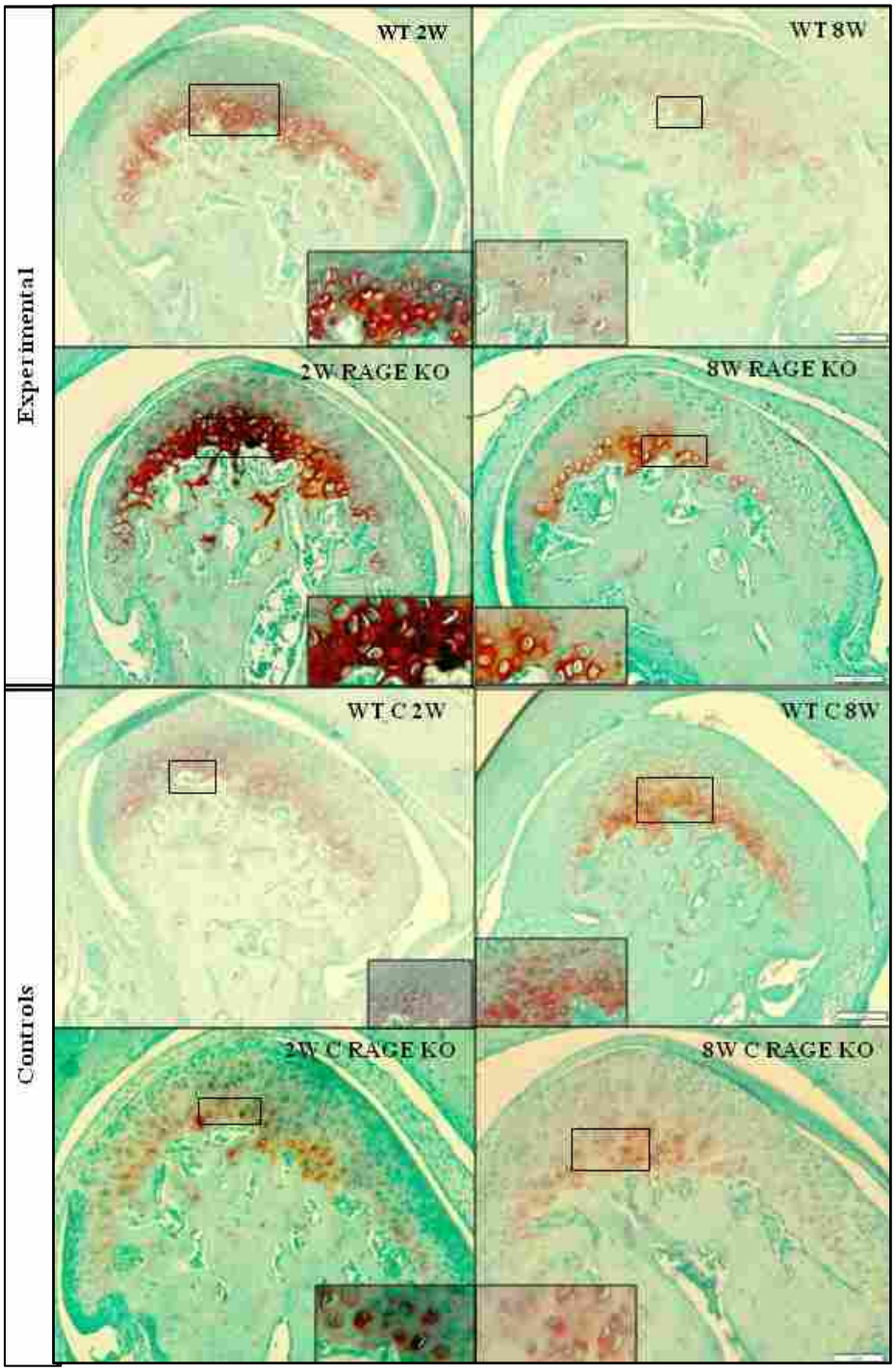

Figure 3.2: Safranin-O/Fast Green Staining.
Safranin-O/Fast Green staining of proteoglycan expression of experimental and control WT and RAGE KO mice. 2 week samples after TMJ misalignment demonstrated intense proteoglycan staining clustering formation, and hypercellularity features typical of early OA. 8 week WT experimental mice showed less proteoglycan staining, and hypocellularity than RAGE KO experimental mice. Control group show steady proteoglycans expression.

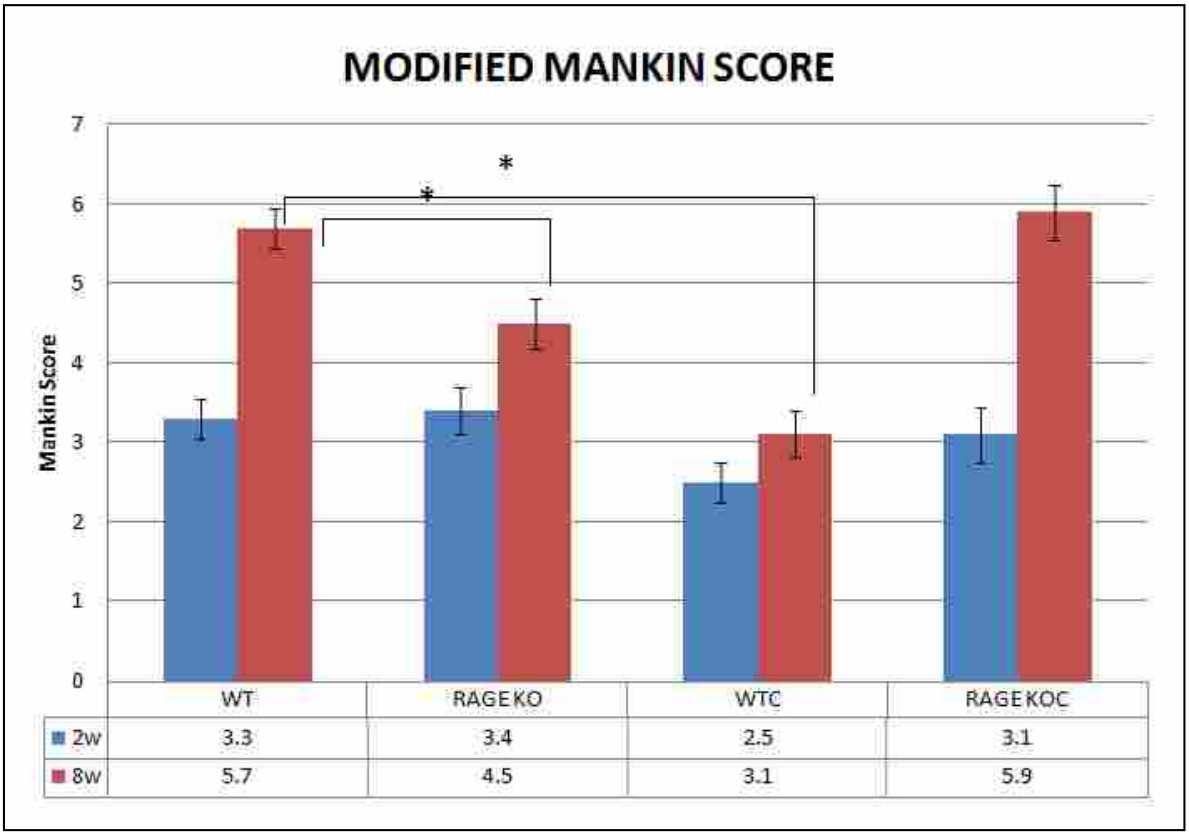

Figure 3.3: Modified Mankin Score WT-RAGE KO.
Modified Mankin score totals of all groups. There is a statistically significant difference between Mankin scores of the experimental WT with control WT mice at 8 weeks ($p<.0001$), demonstrating the effectiveness of the misalignment model in inducing early OA in the TMJ. 8 week experimental RAGE KO mice had statistically lower scores than 8 week WT ($p < .0036$), demonstrating a slight protective effect by knocking out RAGE receptors. 8 week RAGE KO control mice scored surprisingly high, we attributed this to a lack of receptor expression from birth, resulting in fewer chondrocytes and subsequent reduced proteoglycan expression.

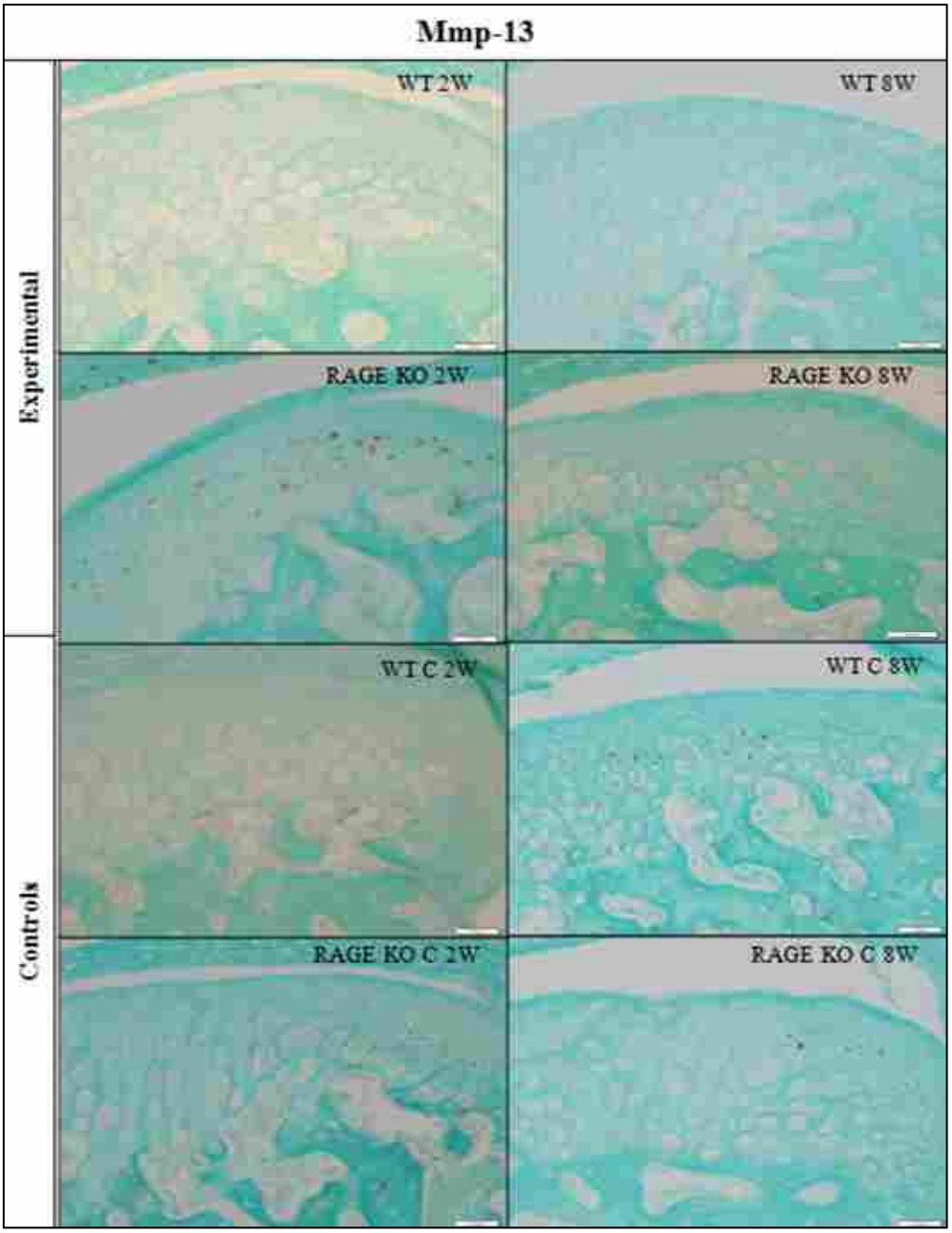

Figure 3.4: IHC Staining - Mmp-13.
At 2 week and 8 week WT experimental mice, show the highest levels of positive Mmp-13 staining. RAGE KO experimental mice show reduced staining, indicating less Mmp-13 activation, and reduced extracellular matrix (ECM) degradation in comparison to WT mice.

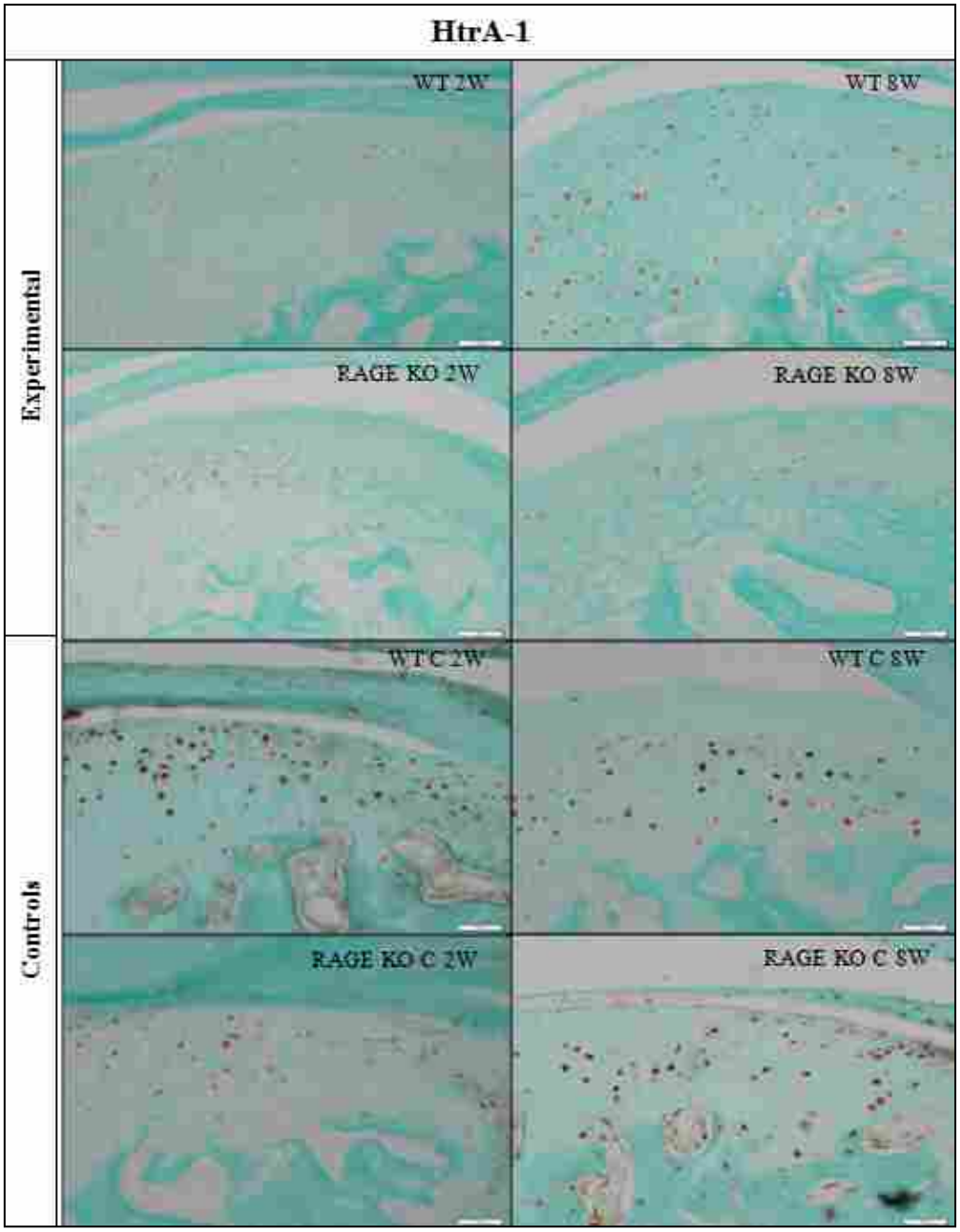

Figure 3.5: IHC Staining - HtrA-1.
At 2 week and 8 week WT experimental mice show higher levels of positive HtraA-1 staining than RAGE KO mice, demonstrating the misalignment's direct effects on pericellular matrix (PCM) and ECM degradation.

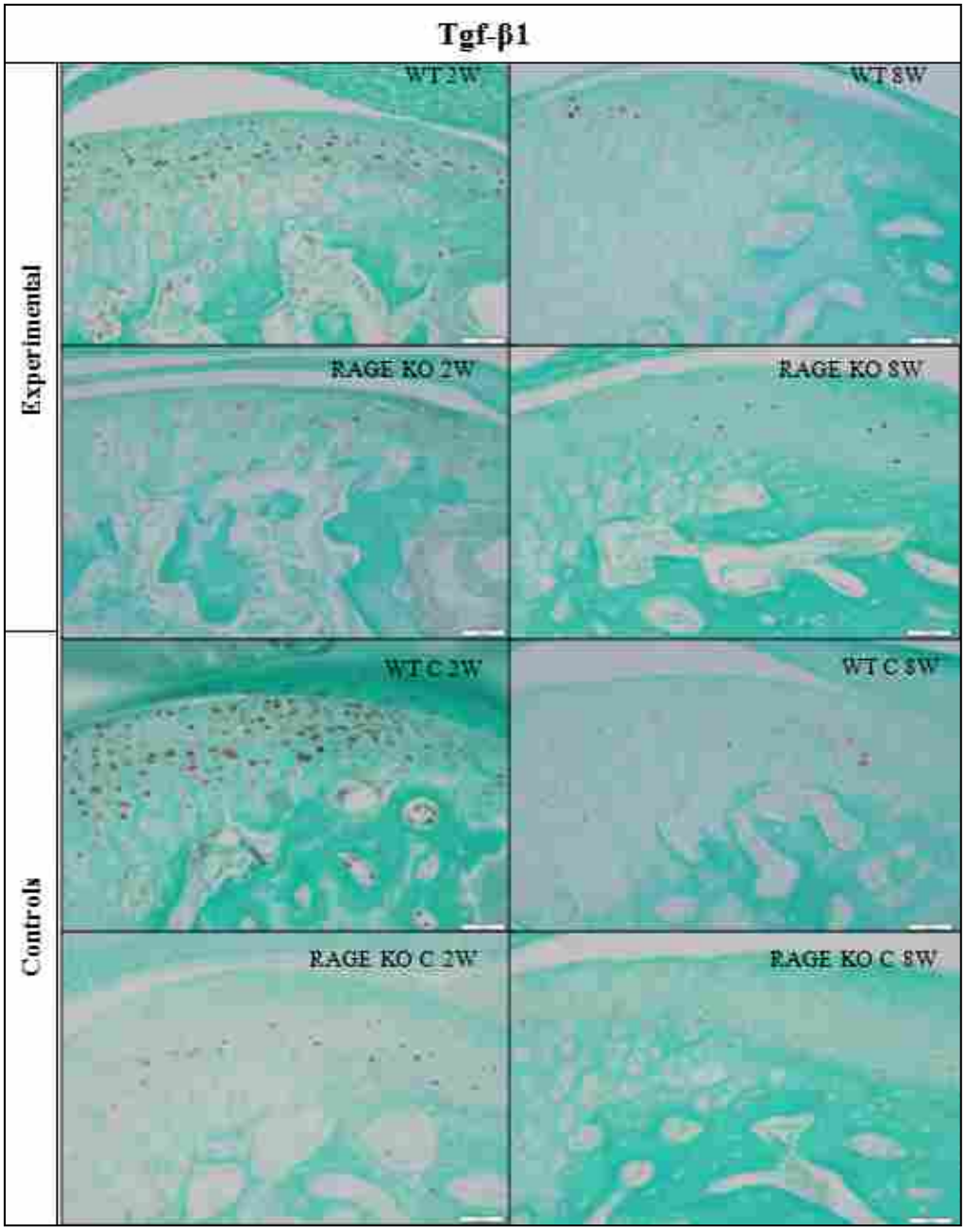

Figure 3.6: IHC Staining - Tgf-β1.
At 2 week and 8 weeks, WT and RAGE KO experimental mice show higher levels of positive Tgf-β1 staining. Misalignment of the TMJ induces high and constant Tgf-β1 expression over time.

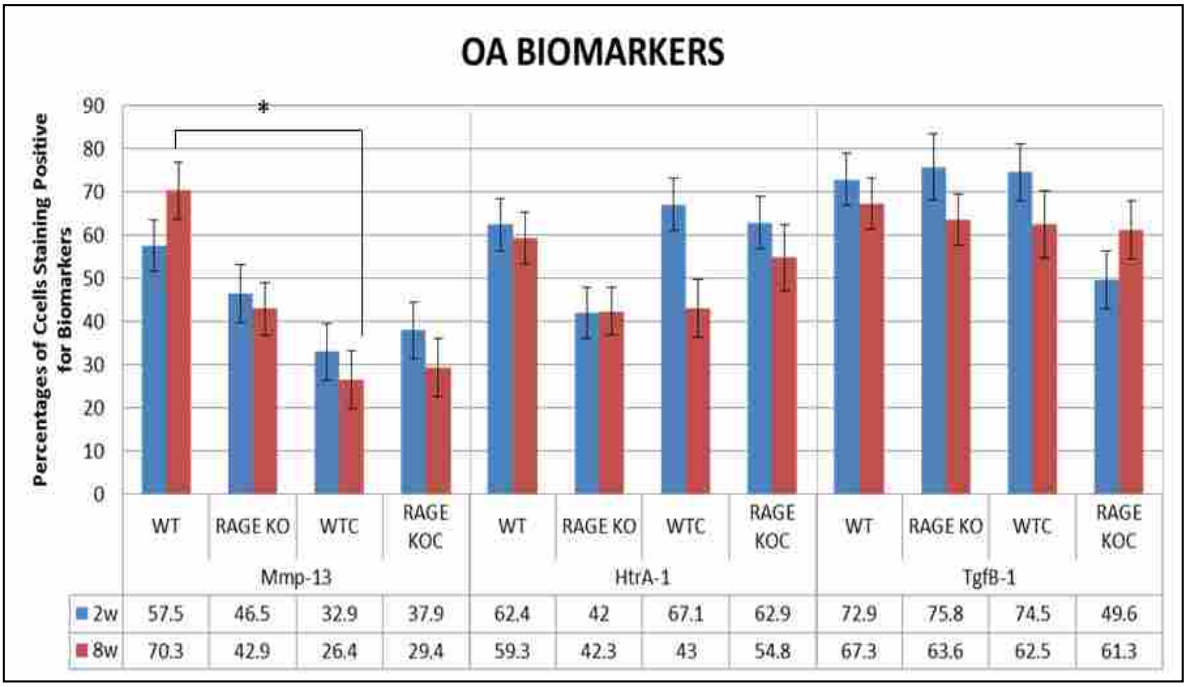

Figure 3.7: Osteoarthritis (OA) Biomarkers: Mmp-13, HtrA-1, and Tgf-β1.
This figure shows percentages of positively stained chondrocytes expressing Mmp-13, HtrA-1, and Tgfβ-1. WT experimental mice had overall higher percentages of Mmp-13 and HtrA-1 staining than RAGE KO experimental mice, demonstrating the protective effect of knocking out RAGE receptors. Additionally, there was a statistical difference between 8 week WT experimental and control mice for Mmp-13 ($p < .0091$), showing the effectiveness of the misalignment in inducing OA in the TMJ. There were no significant differences in percentages of Tgf-β1 in all groups.

RAGE in the expression of Mmp-13 and subsequent OA development. Knocking out RAGE did not attenuate all Mmp-13 expression, and we attribute this to the fact that TMJ misalignment activates other pathways that induce expression of Mmp-13. Some of these pathways are HtrA-1, Ddr-2, Mmp-13, and Toll–like Receptor 4 pathways (31, 44, 61, 71).

HtrA-1 has been show to increase as OA progresses in the knee joints and TMJs of humans and mice (37, 72, 73). Its gradual increase in levels contributes to cartilage degradation, final chondrocyte differentiation, and inhibition of growth factor activity (34). In our study, we did not observe a gradual increase of HtrA-1 over time; however, we found that HtrA-1 expression in WT mice was 20% higher than in RAGE KO mice (Figure 3.5 and 3.7). We cannot explain why there was not an observable up-regulation of this protease over time, but we hypothesize that this event may be related to the steady and high Tgf-β1 expression that may be triggered by the cartilage's self-repair mechanism as a result of induced stress (Figure 3.6 and 3.7). Thus, we hypothesize that high Tgf-β1 expression may be counteracting HtrA-1 activity by inducing chondrogenesis, ECM synthesis, inhibition of the terminal chondrocyte differentiation, and blocking self-inhibition (74).

Another study on the knee joint shows that there is an inverse expression pattern of Tgf-β1 and HtrA-1 in early and late OA (30). Although HtrA-1 expression in experimental group was higher in WT (62%-59%) than in RAGE KO (42%-42.3%) (Figure 3.7), there was not a statistically significant difference. However, this result suggests that there might be a link between RAGE and HtrA-1 pathways that is blocked by the absence of RAGE signaling. Further studies need to be conducted in order to understand this possible link. Genetically altered and surgical TMJ OA mouse models have shown HtrA-1 expression in 3 months old mice and 4 weeks post surgical OA induction. In contrast, in our model, HtrA-1 expression appears at 2

weeks (2.5 month of age) after OA induction (44). This shows that our model is effective in inducing early OA biomarker expression.

Our results show that WT mice at 8 weeks have cartilage degradation that is reflected by high summed Modified Mankin scores and elevated levels of catabolic biomarker expression. We expect that even higher scores due to severe fibrillations would appear if mice were monitored after 8 weeks post misalignment. We hypothesize that these scores would be the result of prolonged HtrA-1 and Mmp-13 expression. This is the first study to demonstrate that absence of RAGE in the TMJ provides a protective effect against the development of progression of TMJ OA in a non-invasive mouse model. Therefore, our findings, in conjunction with our previous study focusing on knee joints, concludes that RAGE plays a key role in OA progression [30]. Thus, RAGE deactivation or aggressive reduction of inflammation could be potential targets to prevent the progression of TMJ OA.

Authors' Contributions

DLK, EMCM designed the experiment. EMCM performed all experimental procedures. PRR provided the mice. DLK, EMCM, and DKM contributed to writing the manuscript. All authors assisted in carrying out experimental procedures and interpreting the results.

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Ethical Approval

All animal work described in this paper was approved by the University Institutional Animal Care and Use Committee (IACUC Protocol 12-0801; Kooyman and Chavez PI).

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