

Brief report

Early-onset osteoarthritis of mouse temporomandibular joint induced by partial discectomy

L. Xu^{††*}, I. Polur[‡], C. Lim[‡], J. M. Servais[‡], J. Dobeck[‡], Y. Li[‡] and B. R. Olsen^{‡§}

[†] The Forsyth Institute, 140 The Fenway, Boston, MA 02115, USA

[‡] Department of Developmental Biology, Harvard School of Dental Medicine, 188 Longwood Ave., Boston, MA 02115, USA

[§] Department of Cell Biology, Harvard Medical School, 240 Longwood, Boston, MA 02115, USA

Summary

Objective: The objective of this study is to characterize mouse temporomandibular joint (TMJ) following partial discectomy, since there is no documentation of whether or not partial discectomy can induce early-onset osteoarthritis (OA) in mouse TMJ.

Methods: Partial discs of TMJ in mice were removed by microsurgery. Histology was performed to characterize articular cartilages from the TMJ of mice. The morphology of the articular cartilages was evaluated using a modified Mankin scoring system. Immunohistostaining was carried out to examine the expression of discoidin domain receptor 2 (Ddr2), a type II collagen receptor, matrix metalloproteinase-13 (Mmp-13), and Mmp-derived type II collagen fragments in the articular cartilage of condyles from the mouse TMJ.

Results: Articular cartilage degeneration was seen in the mouse TMJ post-discectomy, including increased proteoglycan staining in the extracellular matrix at 4 weeks, the appearance of chondrocyte clusters at 8 weeks, reduced proteoglycan staining and fibrillation at 12 weeks and the loss of articular cartilage at 16 weeks. Increased immunostaining for Ddr2, Mmp-13, and Mmp-derived type II collagen fragments was detected.

Conclusion: Results indicate that partial discectomy induces early-onset OA in mouse TMJ and that increased expression of Mmp-13, likely due to the elevated expression of Ddr2, may be one of the factors responsible for the early-onset OA in mouse TMJ.

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Key words: Temporomandibular joint, Articular cartilage, Discectomy, Osteoarthritis.

Introduction

The fibrous disc in temporomandibular joint (TMJ) serves as a cushion during joint movements, evenly distributing pressure-loads on the articular surfaces of the condyle and the fossa of temporal bone^{1,2}. Data from studies with rats and rabbits indicate that a partial or total removal of the disc in TMJ results in early-onset osteoarthritis (OA)^{3–5}. However, to our knowledge, there are no reports on discectomy of mouse TMJ. Clinical studies suggest that injury to the disc might be the most frequent cause of TMJ OA^{6–8}. Therefore, an understanding of the molecular basis of articular cartilage degeneration in TMJ may provide novel insights into the pathogenesis of TMJ OA.

Investigation of the mechanisms of OA with the complicated etiology is a formidable challenge. However, the task is made simpler by the fact that there is a typical pattern of OA progression regardless of the nature of the initiating events⁹. The earliest indication of articular cartilage degeneration is the over-production of proteoglycans and other extracellular matrix molecules, and the appearance

of chondrocyte clusters. Then, gradual loss of proteoglycans occurs in the surface of articular cartilage, along with cleavage of type II collagen fibrils. Cracks gradually develop along the surface, producing a histological image of fibrillation^{10–12}. This pattern of the degeneration indicates that there may be a common chain of molecular events underlying the degeneration. Many studies suggest that articular cartilage degeneration is mediated by numerous biochemical factors^{13–16}. Matrix metalloproteinase (Mmp)-13 is one of these factors and is of particular importance because of its ability to cleave triple-helical type II collagen^{17,18}. Mmp-13 is expressed at a very low level in normal articular cartilage and at a high-level in degenerative ones^{19–21}. This is consistent with the observation that constitutive expression of Mmp-13 results in OA-like changes in mouse knee joints²². Results from our recent studies indicated that the activity and expression of Mmp-13 were increased in mouse OA knee joints. We also found that the increased expression of Mmp-13 might result from the elevated expression of a cell membrane type II collagen receptor, discoidin domain receptor 2 (Ddr2)²³. Furthermore, we found that increased expression of Mmp-13 was associated with a high-level of Ddr2 expression in human OA cartilages. Thus we hypothesize that increased expression of Ddr2 may be a common event in articular cartilage degeneration.

To investigate if the expression of Ddr2 is increased in degenerative TMJ induced by a non-genetic factor, we

*Address correspondence and reprint requests to: L. Xu, The Forsyth Institute, 140 The Fenway, Boston, MA 02115, USA. Tel: 1-617-432-1764; Fax: 1-617-432-5867; E-mail: lin_xu@hms.harvard.edu

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performed microsurgery to destabilize mouse TMJ by removing a portion of the intra-articular disc. We then examined the articular cartilage of surgically destabilized joints for evidence of articular cartilage degeneration, changes in Ddr2 and Mmp-13, and alterations in the amount of Mmp-derived type II collagen fragments.

Materials and methods

PARTIAL DISCECTOMY OF TMJ IN MICE

The experimental procedure was performed following approval from the Forsyth Institutional Animal Care. Eighty C57BL/6j mice at the age of 3 months (Jackson Laboratory, Maine) were used for the surgery. Each mouse was anesthetized with intra-peritoneal 70 µg Ketamine–15 µg Xylazine/g bodyweight. An incision was made over the left TMJ and then through the subcutaneous and muscle layers. The lateral part of the disc was removed. All of the removed discs were saved to evaluate association, if any, of the cartilage damage with the size of the removed discs. For control (sham-surgery), TMJ in mice underwent a similar preparation and surgical procedure but their discs were not cut. Buprenex (analgesic) was also provided subcutaneously at 15 ng/g bodyweight twice per day for 3 days post-surgically. Mice were fed with powder food for a week immediately after the surgery and then with regular food for the remainder of the experiment. Body weights of all the mice were recorded prior to the surgery and three times per week after the surgery, in order to monitor whether the mice were losing weight as a result of the procedure.

HISTOLOGY

At 2, 4, 8, 12 and 16 weeks post-surgery, eight heads from the discectomy and eight heads from the sham-surgery were fixed in 4% paraformaldehyde for 6 h at room temperature. The experimental procedure for histology has been described in our previous study²⁴. Serial sections were cut at thickness of 6 µm. It took about 100 sections to cover a mouse TMJ from anterior to posterior. Every fifteenth section (total six sections) was collected for evaluation of each TMJ. Mouse articular cartilages were evaluated based upon pericellular and background Safranin-O staining, chondrocyte arrangement, and structural condition of the articular cartilage. The score for normal mouse articular cartilage is 0 and the maximum score for degenerative articular cartilage is 10, see Table I.

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Polyclonal antibodies against Ddr2 (Santa Cruz Biotechnology, CA, USA), Mmp-13 (Chemicon, Temecula, CA, USA), and type II collagen fragments (C1–2C, IBEX Tech. Inc., Montreal, QC, Canada) were used for immunostaining. Isotype-matched normal immunoglobulins G (IgGs) (Vector Laboratory, CA) were used for negative control staining. Every fifteenth section from four randomly selected TMJ in each group (2, 4 and 8 weeks post-surgery) was collected for the staining. Sections were incubated with polyclonal antibodies (1:200), and then incubated with biotinylated secondary antibody. Color detection was performed with peroxidase substrate after incubation with a mixture of avidin and biotinylated horseradish peroxidase (HRP).

STATISTICAL ANALYSIS

Data from the modified Mankin scoring system were non-parametric (ordinal). Thus, the Mann–Whitney test was used to examine differences between discectomy and sham-surgery.

Table I
Modified Mankin scoring system used to evaluate articular cartilage in mice

(1) Pericellular Safranin-O staining	
a. Normal	0
b. Slightly enhanced	1
c. Intensely enhanced	2
(2) Background Safranin-O staining	
a. Normal	0
b. Slight increase or decrease	1
c. Severe increase or decrease	2
d. No staining	3
(3) Arrangement of chondrocytes	
a. Normal	0
b. Appearance of clustering	1
c. Hypocellularity	2
(4) Cartilage structure	
a. Normal	0
b. Fibrillation in superficial layer	1
c. Fibrillation beyond superficial layer	2
d. Missing articular cartilages	3

Results

DISCECTOMY

There were no significant losses or gains in the body weights of the experimental and control mice. The sizes of the removed discs from mouse TMJ varied from a half to two thirds of the disc. Results from the histological analysis indicated that variations in the size of the removed disc were not correlated with the severity of the articular cartilage degeneration. Instead, the severity of the cartilage damage was associated with the time course following the surgery.

HISTOLOGY

Results (Fig. 1) showed that the morphology of TMJ in sham-surgery mice at different time points were similar to the appearance of TMJ of wild-type mice¹⁰. There were no differences observed between the discectomy and sham mice 2 weeks post-surgery. However, increased Safranin-O staining for proteoglycans was seen throughout the entire articular cartilage of the condyle and in the articular cartilage of the fossa from mice 4 weeks post-discectomy. At 8 weeks post-discectomy, chondrocyte clusters were observed. At 12 weeks, fibrillation was seen in the discectomy TMJ. At 16 weeks post-discectomy, the loss of articular cartilage was evident. No osteophytes and subchondral sclerosis were observed in discectomy mice.

To evaluate the morphological condition of the articular cartilage of TMJ, eight scores representing eight animals from each group at each time point were obtained. Then each set of scores was used to calculate an average score

Table II
Modified Mankin scores of mouse TM joints

Time point of post-surgery	Sham										Discectomy									
	No. of individual animals										No. of individual animals									
	1	2	3	4	5	6	7	8	Aver.	1	2	3	4	5	6	7	8	Aver.		
4 weeks	0	0	1	1	0	1	0	0	0.38	1	1	1	2	1	1	1	1	1.13*		
8 weeks	0	0	0	0	1	1	1	0	0.38	3	4	3	3	4	3	3	3	3.24*		
12 weeks	1	0	1	1	1	1	1	0	0.75	7	6	6	5	6	6	5	6	5.88*		
16 weeks	0	0	1	1	1	1	0	1	0.63	7	6	8	6	6	7	6	6	6.50*		

* *p* value < 0.001.

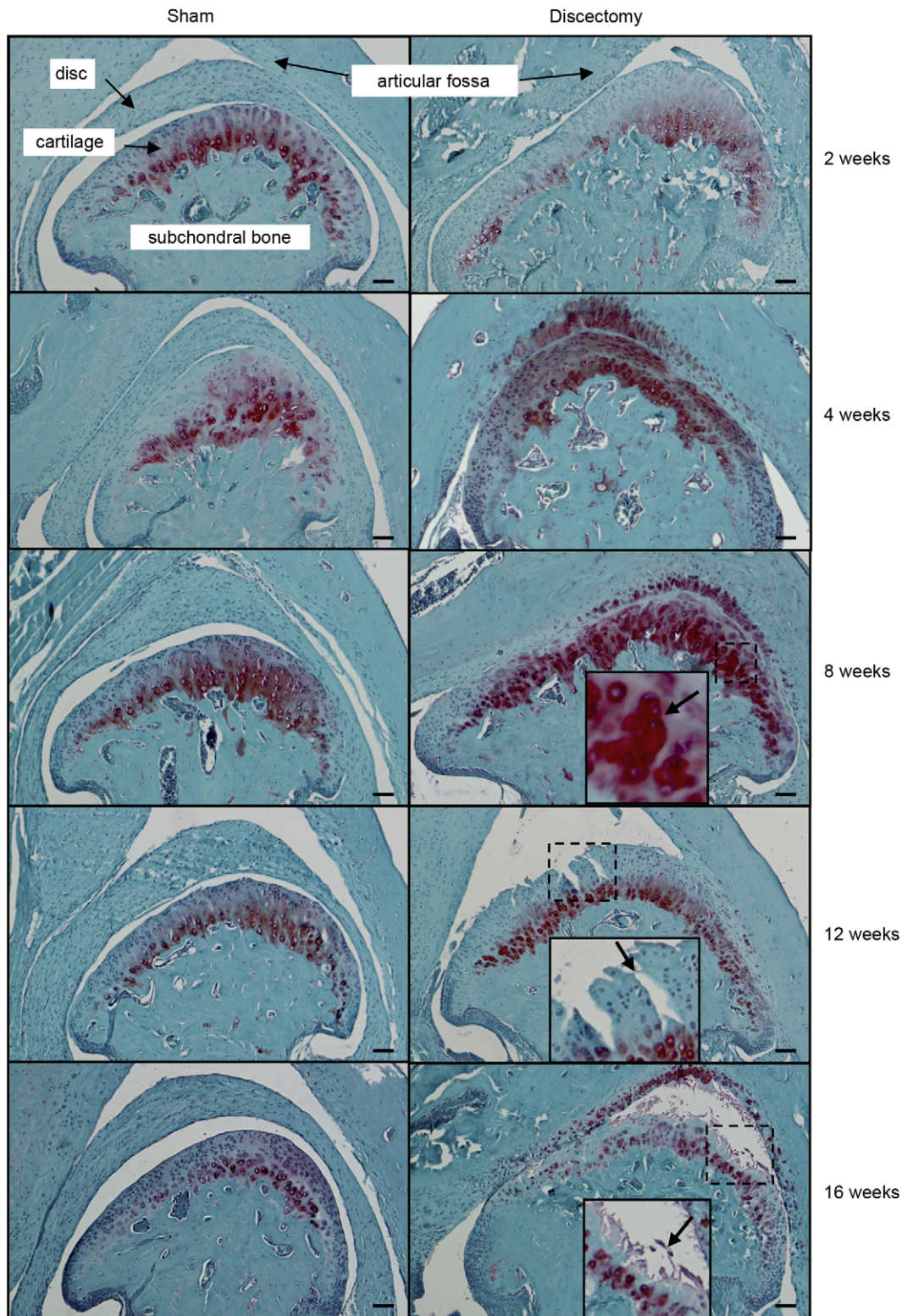


Fig. 1. Histology of TMJ in mice post-sham-surgery and partial discectomy. There were no significant differences between the discectomy and sham mice at 2 weeks post-surgery. However, at 4 weeks post-discectomy, increased Safranin-O staining for proteoglycans was seen throughout the entire articular cartilage of the condyle and in the articular cartilage of the fossa. At 8 weeks post-discectomy, chondrocyte clusters appeared (see insert and arrow) and slight reduction of Safranin-O staining in the fossa of the temporal bone and the superficial layer of the condyles was observed. At 12 weeks, fibrillation was observed (see insert and arrow) in the discectomy mice. At 16 weeks, a dramatic overall reduction of Safranin-O staining and the loss of articular cartilage (see insert and arrow) were also observed in the discectomy mice. No significant morphologic changes were observed in the subchondral plate and joint margins in the sham-surgery or discectomy mice (Bar = 50 μ m).

for each group at each time point. A statistical comparison of the scores (Table II) obtained from the sham and discectomy mice, using the Mann–Whitney test, indicated a significant difference between the two groups at each time point; the *P* value was less than 0.001. Two-week post-sham mice were used as a normal control (score = 0). There were no significant differences between the morphologies of TMJ of discectomy and sham mice at 2 weeks post-surgery. However, at 4 weeks, the morphologic changes observed in the discectomy mice were significantly different from those observed in the sham mice. As the length of time following the surgery increased, further degeneration of the

articular cartilage was observed in the discectomy mice; indicating a close association between the post-surgery time course and the progression of articular cartilage degeneration. There were no significant changes observed among the sham-surgery mice at the different time points.

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Results (Fig. 2) showed that the expression of Ddr2 and Mmp-13 was increased in the articular cartilage of discectomy TMJ. Mmp-13 was seen in the majority of the sections selected at random from mice at 8 weeks post-discectomy.

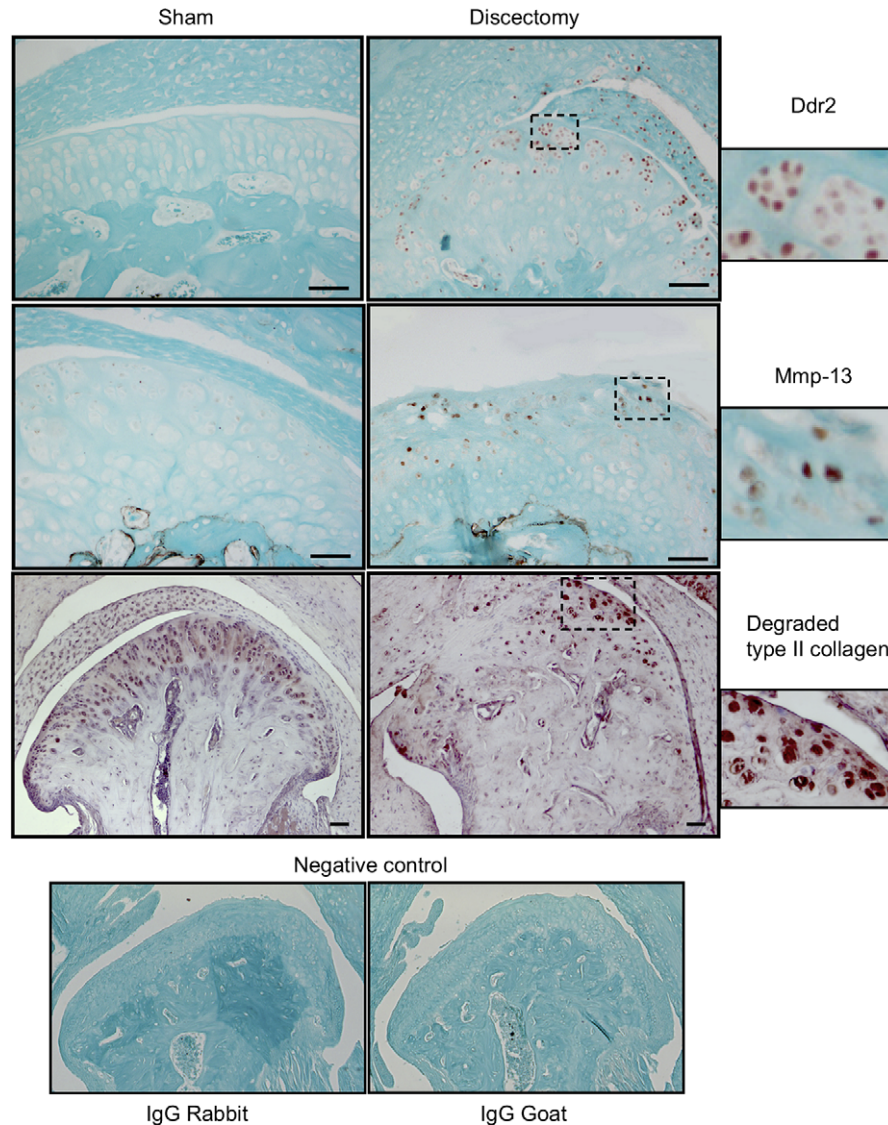


Fig. 2. Immunostaining of Ddr2, Mmp-13 and Mmp-derived type II collagen fragments in TMJ of sham and discectomy mice. Ddr2 and Mmp-13 positive cells (see brown color staining cells) were observed in the TMJ of the discectomy mice at 8 weeks following the surgery. However, Ddr2 and Mmp-13 positive cells were hardly detected in the TMJ of the sham-surgery mice at 8 weeks. No positively stained cells were detected in the sham and discectomy mice at 2 and 4 weeks (data not shown). More intense staining for Mmp-derived type II collagen fragments (see insert) was observed in the superficial layer of articular cartilage in the TMJ of mice 8 weeks post-discectomy than was observed in the sham-surgery mice. Diffuse non-significant staining for Mmp-derived type II collagen fragments in the extracellular matrix appeared in both sham and discectomy mice at 8 weeks. Immunostaining for Mmp-derived type II collagen fragments was undetectable in the articular cartilage of TMJ from mice 4 weeks post-sham and partial discectomy (data not shown). Stainings with isotype-matching normal IgG (rabbit IgG for Ddr2 and Mmp-derived type II collagen; goat IgG for Mmp-13) and without primary antibody (data not shown) were also performed as negative controls. No positive stainings were observed in the controls. Counter staining for Ddr2 and Mmp-13 is Fast Green and for degraded type II collagen is hematoxylin (Bar = 50 μ m).

The number of positively stained cells for these two genes was consistent throughout the samples. Cells positively stained for Ddr2 and Mmp-13 were hardly detectable in the TMJ obtained from the sham mice at 8 weeks post-operation. At 2 and 4 weeks after the surgery, cells positively stained for Ddr2 and Mmp-13 were not detected in the TMJ of mice from either the sham or discectomy mice (data not shown). Results also indicated that there were increased amount of type II collagen fragments in the articular cartilage of TMJ in mice 8 weeks post-discectomy. There were hardly any detectable type II collagen fragments in the articular cartilages of TMJ from mice at 2 and 4 weeks after the sham-surgery or the discectomy (data not shown).

Discussion

To our knowledge, this is the first time to demonstrate that partial discectomy can induce early-onset OA in mouse TMJ. The data revealed a typical pattern of articular cartilage degeneration following partial discectomy. Although precise mechanisms that initiate early-onset OA in TMJ of discectomy mice are unknown, we believe that a partial removal of the disc can dramatically affects the distribution of the pressure and the capacity for load absorption on surfaces of the TMJ, resulting in the presence of excessive mechanical force on a small area of articular surfaces. This results in early-onset OA. In this study, we used a modified Mankin scoring system to characterize morphologic changes of TMJ. Others and we have used this system in previous studies^{10,25}. We found that this modified score system reliably represented the morphological conditions of mouse joints at different developmental stages. We notice that there are other scoring systems for mouse or small animal joints that have been used in numerous studies since there is no a consensus in the grading system for mouse articular cartilage or small animals.

To understand mechanisms underlying articular cartilage degeneration in TMJ of discectomy mice, we examined expression patterns of Ddr2 and Mmp-13. One question that remained was which molecules, if any, can stimulate chondrocytes to synthesize and release Mmp-13 in articular cartilage, prior to the significant degradation of the cartilage at the early stage of the degeneration. We found that the activation of Ddr2 resulted in increased expression of Mmp-13 in chondrocytes *in vitro*²³. We also observed that the levels of *Ddr2* and *Mmp-13* mRNA and proteins were elevated in the articular cartilage of human OA hip and knee joints. In addition, we found increased expression of Ddr2 and Mmp-13 in knee and TMJ of two genetic mouse OA models. These results suggest that Ddr2 may play an important role in the pathogenesis of OA. *Ddr2* is a cell membrane tyrosine kinase receptor and preferentially bound to native type II collagen^{26–28}. There is little or no type II collagen molecules around chondrocytes in the pericellular region²⁹. This suggests that there is little or no contact between chondrocytes and type II collagen molecules in mature articular cartilage under normal conditions. Consequently, we speculate that the exposure of the collagen network to chondrocytes, under any circumstance, such as after depletion of proteoglycans, will permit interaction of type II collagen with chondrocytes, resulting in the activation of Ddr2. The activated Ddr2 induces the expression of Mmp-13 as well as expression of Ddr2 itself. In this study, we found that expression of Ddr2 and Mmp-13 was increased in the articular cartilage of the TMJ at 8 weeks post-partial discectomy, when the degradation of proteoglycans was already

evident. This is consistent with our hypothesis that regardless of the nature of OA initiation events (genetic or non-genetic factors), degradation of proteoglycans increases exposure of chondrocytes to type II collagen. This, in turn, elicits interaction of Ddr2 with type II collagen, resulting in elevated expression of the receptor itself and induction of Mmp-13 expression in chondrocytes. Therefore, inhibitors of Ddr2 or its downstream effectors may turn out to be useful for the treatment of OA.

Conflicts of interest

Any of the authors in this study does not have any financial and personal relationships with any organization that could influence (bias) this work.

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