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Delayed progression of condylar cartilage degeneration, by reduction of the discoidin domain receptor 2, in the temporomandibular joints of osteoarthritic mouse models

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Summary

Objective—To determine whether reduction of the discoidin domain receptor 2 (Ddr2) delays the progression of condylar cartilage degeneration in the temporomandibular joint (TMJ) of mouse models with osteoarthritis (OA).

Methods—Double-heterozygous (*Col11a1*- and *Ddr2*-haploinsufficiency, *Col11a1*^{+/-};*Ddr2*^{+/-}) mice were generated. TMJs of *Ddr2*^{+/-} mice were subjected to partial discectomy. Condylar cartilage from the TMJ of *Col11a1*^{+/-};*Ddr2*^{+/-} mice, surgically treated (discectomy) *Ddr2*^{+/-} mice and their corresponding controls was characterized by means of histology and evaluated using a scoring system specific to mouse joints.

Results—The progression of condylar cartilage degeneration was significantly delayed in the TMJ of *Col11a1*^{+/-};*Ddr2*^{+/-} mice compared with those of the *Col11a1*^{+/-} mice. The progression of condylar cartilage degeneration in the TMJ of *Ddr2*^{+/-} mice following discectomy was also significantly delayed when compared with their wild-type littermates.

Conclusion—Reduced expression of *Ddr2* delays the progression of condylar cartilage degeneration, induced either by type XI collagen-haploinsufficiency or by a partial discectomy, in TMJ.

Keywords

osteoarthritis; temporomandibular; discectomy; *Ddr2*; mouse

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Contributions

AS, IP and LX participated in study conception and design, acquisition, analysis and interpretation of data, drafting and revision of the manuscript and final approval of the version to be submitted. JS and YL performed experiments and participated in acquisition and analysis of data, drafting and final revision of the manuscript.

Competing interests

The authors have no conflicts of interest to declare.

Introduction

Condylar cartilage degeneration of the temporomandibular joint (TMJ) is one of the leading causes of TMJ disorders (TMDs) (1,2). The incidence of TMD caused by degenerative joint disorder increases in populations over the age of 60 (3). A molecular understanding of the process of condylar cartilage degeneration would provide valuable information towards the search for novel therapeutic targets in the treatment of TMDs. The goal of this study is to determine whether reduced expression of a cell surface tyrosine kinase receptor for native type II collagen, discoidin domain receptor 2 (Ddr2), can attenuate the progression of condylar cartilage degeneration in the TMJ of mouse models with osteoarthritis (OA).

DDR2 was originally cloned as a cell membrane receptor tyrosine kinase (RTK) (4). A study demonstrates that type II collagen molecules contain a specific binding site for DDR2, located within a segment of 234 amino acid residues (5). Moreover, type II collagen is recognized preferentially by DDR2 (6). Results from our studies indicate that the expression of *Ddr2* is increased in the TMJ of genetic and non-genetic forms of mouse models of OA (7). The increased expression of *Ddr2* is associated with elevated expression of matrix metalloproteinase 13 (Mmp-13)--a major extracellular matrix-degrading enzyme. Reduced expression of *Ddr2* delays the progression of articular cartilage degeneration in the knee joints of mouse models with OA (8). However, the question of whether reduced expression of *Ddr2* can also slow down the progression of condylar cartilage degeneration in TMJ remains to be answered.

In our previous study, we observed that *Col11a1*-haploinsufficient mice exhibited early onset condylar cartilage degeneration in TMJ (9). We also discovered that partial removal of the disc (discectomy) initiated early onset condylar cartilage degeneration in the TMJ of mouse models (10). In the present study, we created double-heterozygous mice (*Col11a1*^{+/-};*Ddr2*^{+/-}) and evaluated the condition of their TMJs and those of their wild-type littermates for evidence of changes in condylar morphology. We also examined morphologic changes in the condylar cartilage of TMJ from *Ddr2*^{+/-} mice and their WT littermates following discectomy.

Methods

Generation of double-heterozygous (*Col11a1*^{+/-};*Ddr2*^{+/-}) mice

The spontaneous mouse strain mutation, heterozygous chondrodysplasia (or *Col11a1*^{+/-} mice), is an autosomal-recessive disorder. *Col11a1*^{+/-} mice develop normally, without obvious skeletal abnormalities at birth. Histologic studies show that *Col11a1*^{+/-} mice begin to develop OA-like changes in their TMJs at the age of 3 months and that severe OA-like pathology develops over the course of 9 months. A *Ddr2*-null mutant mouse strain (*Ddr2*^{-/-}) was provided by Regeneron at New York. A detailed description of the generation of the *Ddr2*-null mutant mouse strain can be found in our previous publication (8). In order to generate *Col11a1*^{+/-};*Ddr2*^{+/-} mice, *Col11a1*^{+/-} mice were bred with *Ddr2*^{+/-} mice. *Col11a1*^{+/-};*Ddr2*^{+/-}, *Col11a1*^{+/-}, *Ddr2*^{+/-} mice and their WT littermates were identified by PCR and maintained under a daily schedule of 12 hours with light and 12 hours without

light for further experimentation. The experimental procedure for PCR has been described in our previous publications (8).

Partial discectomy of the TMJ in mice

The surgical procedure was performed following approval from the Institutional Animal Care and Use Committee. The discectomy procedure has also been described in our previous publications (10). Briefly, *Ddr2*^{+/-} mice and their wild-type littermates were prepared for surgery at the age of 3 months. An incision was made over the left TMJ and then through the subcutaneous and muscle layers. The lateral part of the disc was removed. The TMJ of the control (sham surgery) mice underwent a similar surgical procedure, except their discs were not cut.

Histology

For the histologic evaluation, eight mice from each of the genetic backgrounds--*Col11a1*^{+/-}, *Col11a1*^{+/-};*Ddr2*^{+/-} and their wild-type littermates--were sacrificed at the ages of 3, 6, and 9 months; their TMJs were collected. The surgically-treated mice--eight from the *Ddr2*^{+/-} discectomy group, eight from the wild-type discectomy group, and eight from the sham surgery group--were sacrificed at 4, 8, 12 and 16 weeks post-surgery; their heads were collected for evaluation. All of the heads were fixed in 4% paraformaldehyde for 6 hours at room temperature and processed for paraffin embedding. The heads were cut along the midsagittal plane and the left half of the head was embedded in paraffin. The condyles were sectioned into approximately 100 serial sections at a 6 µm-thickness in an anterior-posterior direction. Every tenth section was collected for Safranin O/Fast green staining. The pathologic condition of the joints was evaluated by a scoring system designed to assess the histology of OA in mouse joints; the system is recommended by the OARSI histopathology initiative (11). The score for normal mouse condylar cartilage is 0 and the maximum score for degenerated condylar cartilage is 6. For the purposes of this study, we added an additional score of 0.5 to indicate increased glycosaminoglycan staining.

Statistic analysis

In this study, each TMJ was represented by 9 to 10 paraffin sections. Each section was scored. An average score for each TMJ was then calculated from the 9 to 10 individual scores. Consequently, eight average scores were obtained for each experimental group since there were eight TMJs in each group. A final mean for each experimental group was then calculated from the eight averages. A two-sample *t* test with a significance level of 0.05 was used to determine whether a significant difference between any two mean scores was present.

Results

Delayed progression of condylar cartilage degeneration in the TMJ of *Col11a1*^{+/-};*Ddr2*^{+/-} mice

At 3 and 6 months of age, the proteoglycans were predominantly distributed in the deep zone of the condylar cartilage in wild-type mice. However, the *Col11a1*^{+/-} mice were found to have a diffuse distribution of proteoglycans throughout the entire layer of condylar

cartilage. The *Col11a1*^{+/-};*Ddr2*^{+/-} mice showed less diffuse distribution of proteoglycans. At 9 months of age, the damage to condylar cartilage was significantly more severe in the *Col11a1*^{+/-} mice--degradation of proteoglycans and fibrillations (see arrow in Figure 1a) were observed. The *Col11a1*^{+/-};*Ddr2*^{+/-} mice only displayed an over-production of proteoglycans at this time point. The degenerative damage to the TMJ of *Col11a1*^{+/-};*Ddr2*^{+/-} mice at 9 months of age was similar to that observed in the *Col11a1*^{+/-} mice at 6 months of age, suggesting a 3 months delay in the progression of condylar cartilage degeneration in the *Col11a1*^{+/-};*Ddr2*^{+/-} mice (Figure 1a).

At 3 months of age, the TMJ of the wild-type littermates scored a zero, indicating normal condylar cartilage. No significant score increases were observed in the wild-type mice as they aged. However, significant score increases were observed in aging *Col11a1*^{+/-} mice. Similar, but less significant, score increases were observed in aging *Col11a1*^{+/-};*Ddr2*^{+/-} mice. Significant score difference was observed between the *Col11a1*^{+/-} mice and *Col11a1*^{+/-};*Ddr2*^{+/-} mice at 9 months of age (Table 1a).

Delayed progression of condylar cartilage degeneration in the TMJ of *Ddr2*^{+/-} mice, induced by discectomy

We found that at 4 weeks following surgery, there were no significant differences in condylar morphology between sham wild-type mice and *Ddr2*^{+/-} mice that had undergone discectomy. However, over-production of proteoglycans was observed in the condylar cartilage from discectomy wild-type mice (Figure 1b). At 8 weeks following discectomy, the condylar cartilage from the wild-type mice exhibited chondrocyte clustering with increased glycosaminoglycan staining in the pericellular matrix. However, the *Ddr2*^{+/-} mice displayed no significant condylar cartilage changes at that time point. At 12 weeks post-surgery, *Ddr2*^{+/-} mice revealed chondrocyte clustering (see arrow in Figure 1b) with increased glycosaminoglycan staining in the pericellular matrix. This recapitulated what was observed in the condylar cartilages of TMJ from wild-type mice at 8 weeks post-discectomy. Furthermore, reduced glycosaminoglycan staining and fibrillation (see arrow head in Figure 1b) were evident in the condylar cartilages of TMJ of wild-type mice at 16 weeks post-discectomy, whereas, no fibrillation was seen in the *Ddr2*^{+/-} mice following surgery. Compared with the post-surgical wild-type mice, the post-surgical *Ddr2*^{+/-} mice exhibited relatively minor damage to the condylar cartilages. There was a consistent 4 weeks delay in condylar cartilage degeneration in the *Ddr2*^{+/-} mice.

At 4 weeks post-sham surgery, the TMJ of the wild-type littermates scored a zero, indicating the presence of normal condylar cartilage. While the scores for the sham surgery mice remained the same at each time point, the scores for the post-discectomy wild-type mice steadily increased with each successive time point. The *Ddr2*^{+/-} mice also exhibited increasing scores with each successive time point following discectomy. However, the scores remained significantly lower than those observed in the wild-type littermates (Table 1b).

Discussion

Data from this present study indicates that reduced expression of Ddr2 significantly attenuates the progression of condylar cartilage degeneration, induced by either type XI collagen-haploinsufficiency or partial discectomy, in the TMJ of the mouse models. This result is consistent with our previous finding that elevated expression of Ddr2 may play a role in the acceleration of condylar cartilage degeneration in the TMJ of mice, suggesting that inhibition of Ddr2 activity and its down-stream signaling molecules may slow down the progression of condylar cartilage degeneration in TMJ. The question remains as to how DDR2 is kept inactivated in normal articular cartilage or, conversely, how it is activated to induce MMP-13 in degenerative articular cartilage.

The articular cartilage is a specialized avascular connective tissue covering the joint surfaces. The chondron, consisting of the chondrocyte and its pericellular microenvironment, has been considered the primary structural and functional unit of articular cartilage (12). In normal conditions, chondrocytes are well-protected by their pericellular matrix network. The pericellular matrix separates chondrocytes from native type II collagen located in the territorial and interterritorial extracellular matrix. It is conceivable that disruption of the pericellular matrix exposes chondrocytes to the type II collagen. This, in turn, elicits an interaction between the type II collagen and DDR2. The activation of DDR2 induces MMP-13. The end result is OA. In fact, numerous independent research groups, in addition to ours, have reported that the disruption of the pericellular matrix of chondrocytes by a serine protease, HTRA1 (high temperature requirement A1) is associated with early onset articular cartilage degeneration of joints (13,14). We propose that HTRA1-DDR2-MMP13 may be a rate-limiting sequence of molecular events mediating the progression of articular cartilage degeneration. It is worth mentioning that results from a very recent study, conducted by an independent research group, support our hypothesis (15).

In summary, the results from our present study suggest that preventing activation of DDR2--either by protecting the pericellular matrix or directly inhibiting DDR2 activation--and subsequently the down-stream signaling molecules involved in induction of MMP-13, may be a novel therapeutic approach to treating TMDs associated with condylar cartilage degenerative disorders and OA in general.

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a

b

Figure 1.

a. Histology of the condylar cartilage of TMJ from *Coll1a1*^{+/-}, *Coll1a1*^{+/-};*Ddr2*^{+/-} mice and their wild-type littermates.

Each image shown is one representative section selected from 72–80 sections of an experimental group (9–10 sections from each TMJ and 8 joints in each experimental group). The representative section was selected based on the average score from the each experimental group. A progression in condylar cartilage degeneration in TMJ was seen in both *Coll1a1*^{+/-} and *Coll1a1*^{+/-};*Ddr2*^{+/-} mice, but with a reduced progressive rate in the *Coll1a1*^{+/-};*Ddr2*^{+/-} mice. In particular, degenerative damage to the TMJ condyles of

Col11a1^{+/-};*Ddr2*^{+/-} mice at the age of 9 months was similar to that of *Col11a1*^{+/-} mice at the age of 6 months, suggesting a 3 months degenerative delay in the *Col11a1*^{+/-};*Ddr2*^{+/-} mice. (Bar=50 μ m)

b. Histology of the condylar cartilage of TMJ from mice following discectomy microsurgery.

Each image shown is one representative section selected from 72–80 sections of an experimental group (9–10 sections from each TMJ and 8 joints in each experimental group). The representative section was selected based on the average score from the each experimental group. A delay of the degenerative process was seen in the *Ddr2*^{+/-} mice following discectomy. In particular, the damage to the TMJ condyles of *Ddr2*^{+/-} mice at 12 weeks following the surgery was similar to that of wild-type littermates at 8 weeks following the surgery, suggesting, at least, a 4 weeks degenerative delay in the *Ddr2*^{+/-} mice. (Bar=50 μ m)

Table 1

- a. Average scores for the *Col11a1*^{+/-} and *Col11a1*^{+/-};*Ddr2*^{+/-} TMJs.
 b. Average scores for the discectomy TMJs.

a. Histologic scores for TMJ in <i>Col11a1</i>^{+/-} & <i>Col11a1</i>^{+/-};<i>Ddr2</i>^{+/-} mice			
Age (months)	Mean ± SD		<i>p</i>-value
	<i>Col11a1</i> ^{+/-}	<i>Col11a1</i> ^{+/-} ; <i>Ddr2</i> ^{+/-}	
3	0.09 ± 0.07	0.09 ± 0.07	0.9151
6	0.18 ± 0.10	0.09 ± 0.03	0.0719
9	1.65 ± 0.57	0.23 ± 0.09	0.0004

b. Histologic scores for TMJ in discectomy mice			
weeks	Mean ± SD		<i>p</i>-value
	+/+ Discectomy	<i>Ddr2</i>^{+/-} Discectomy	
4	0.34 ± 0.14	0.07 ± 0.05	0.0011
8	0.47 ± 0.12	0.14 ± 0.08	0.0001
12	2.41 ± 0.63	0.46 ± 0.15	0.0001
16	3.80 ± 0.61	1.04 ± 0.19	< 0.0001

The *p*-value is less than 0.05 is considered significance.