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Discoidin Domain Receptor 2 as a Potential Therapeutic Target for Development of Disease-Modifying Osteoarthritis Drugs

Q25 Lauren B. Manning,* Yefu Li,[†‡] Nithya S. Chickmagalur,[†] Xiaolong Li,[†§] and Lin Xu[†‡]

From the Departments of Prosthodontics* and Developmental Biology,[†] Harvard School of Dental Medicine, Boston, Massachusetts; the Faculty of Medicine,[‡] Harvard Medical School, Boston, Massachusetts; and the State Key Laboratory of Oral Diseases,[§] Department of Orthodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, China

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Address correspondence to Yefu Li, M.D., Ph.D., REB Room 504, or Lin Xu, M.D., Ph.D., REB Room 514A, 188 Longwood Ave, Boston, MA 02115. E-mail: yefu_li@hms.harvard.edu or lin_xu@hms.harvard.edu.

Osteoarthritis (OA) is the most common form of arthritis disorders, but the identification of therapeutic targets to effectively prevent OA has been increasingly difficult. The goal of this investigation is to provide experimental evidence that discoidin domain receptor 2 (DDR2) may be an ideal target for the development of disease-modifying OA drugs. *Ddr2* was conditionally deleted from articular cartilage of adult mouse knee joints. *Aggrecan-CreERT2;floxed Ddr2* mice, which were generated by crossing *Aggrecan-CreERT2* mice with floxed *Ddr2* mice, then received tamoxifen injections at the age of 8 weeks. The mice were then subjected to destabilization of the medial meniscus (DMM) surgery. At 8 and 16 weeks after DMM, mice were euthanized for the collection of knee joints. In a separate experiment, *Aggrecan-CreERT2;floxed Ddr2* mice were subjected to DMM at the age of 10 weeks. The mice then received tamoxifen injections at 8 weeks after DMM. The mice were euthanized for the collection of knee joints at 16 weeks after DMM. The progressive process of articular cartilage degeneration was significantly delayed in the knee joints of *Ddr2*-deficient mice in comparison to their control littermates. Articular cartilage damage in the knee joints of the mice was associated with increased expression profiles of both Ddr2 and matrix metalloproteinase 13. These findings suggest that DDR2 may be an ideal target for the development of disease-modifying OA drugs. *(Am J Pathol 2016, ■: 1–11; http://dx.doi.org/10.1016/j.ajpath.2016.06.023)*

Q3 Osteoarthritis (OA) is the most common form of arthritis disorders.[1] Because the molecular mechanism underlying the development of OA is largely unknown, it is difficult to identify therapeutic targets that effectively prevent and treat OA. Currently, existing drugs for the treatment of OA provide, at best, symptomatic relief from pain and inflammation.[2] Current pharmacological interventions that address chronic pain and inflammation do not prevent articular cartilage degeneration, which eventually leads to OA. Therefore, the identification of targets for the development of disease-modifying OA drugs (DMOADs) has become more pressing than ever. The objective of this present investigation is to determine whether a cell surface receptor tyrosine kinase, discoidin domain receptor 2 (DDR2), for native collagen type II is an ideal target for the development of DMOADs.

What led us to investigate the role of DDR2 in the development of OA? The answer is the classic biochemistry event in living systems, known as enzyme induction. An example of such a system is the inducible lactose-metabolizing enzyme, by which the enzyme is induced by its own substrates. Bacterial *Escherichia coli* is unable to directly use disaccharide lactose for consumption. However, under the condition in which lactose is solely present, the enzyme β-galactosidase is induced in *E. coli*. The enzyme then breaks down lactose into monosaccharides, galactose and glucose, which the bacteria is able to metabolize. We believe that the induction of matrix metalloproteinase 13 (MMP-13) in chondrocytes may

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represent a similar situation. For cartilage tissue turnover, extracellular matrix molecules, such as type II collagen, need to be degraded. MMP-13 can degrade type II collagen. However, type II collagen cannot enter chondrocytes to physically induce MMP-13. Thus, the collagen has to interact with a cell surface molecule(s) and send signals into chondrocytes to induce the expression of MMP-13 and the subsequent release of MMP-13 into the extracellular space. If this is the case, the question becomes which cell surface molecule(s) transduce the signal into chondrocytes? Initially, we examined whether integrin *α2β1* was the cell surface receptor responsible for this transduction.[3] However, results from our previous study indicated that the blockage of the integrin *α2β1* did not prevent the induction of MMP-13 in chondrocytes by native type II collagen.[4] Moreover, the activation of the integrin *α2β1* and the stimulation of type II collagen to chondrocytes showed a synergistic effect on the induction of MMP-13 in the cell. This suggests that there is another cell surface receptor responsible for transducing the signal to induce MMP-13 expression. We speculated that DDR2 might be such a receptor,[5,6] and results from our previous investigations supported our speculation.[2,7,8]

In one of our previous investigations, we removed one copy of *Ddr2* in two mouse models of OA.[9] We found that the reduction in the expression of Ddr2 attenuated articular cartilage degeneration in knee joints of the mouse models. However, several critical questions remain to be addressed before we deem DDR2 as an ideal target to develop DMOADs. First, in that study, we were unable to evaluate the chondroprotective effect of the complete removal of *Ddr2* from mature articular cartilage of mouse knee joints, as a means of investigating how critical Ddr2 is to the progression of OA. This is because of the fact that homozygous conventional *Ddr2* knockout mice that we used in the study exhibit short stature (dwarfism) and such cannot be used to study OA. Second, we were unable to determine whether inhibition of Ddr2, after the onset of articular cartilage degeneration, could still prevent the joint from being destroyed. This was because of the lack of a floxed *Ddr2* mouse strain; therefore, a complete deletion of *Ddr2* was not possible at a desirable time in articular cartilage. Third, numerous investigations report there is a biological effect secondary to the absence of DDR2 on articular cartilage development in human.[10−12] Thus, the full potential chondroprotective effect of the *Ddr2* deletion by a conventional knockout technique could be compromised or misleading. To address these questions, in this present investigation, we used a conditional knockout technique to delete *Ddr2* from mature articular cartilage of mouse knee joints to eliminate any potential developmental growth abnormalities that might arise. We then investigated the chondroprotective effect of the deletion of *Ddr2* on knee articular cartilage before and after the onset of cartilage degeneration induced by destabilization of the medial meniscus (DMM).

In this present study, we have investigated what the potential chondroprotective effect on mouse knee joints is. To address this question, we generated a floxed *Ddr2* mouse strain. We then specifically removed *Ddr2* from articular chondrocytes before, or after the onset of, articular cartilage degeneration induced by DMM. We then examined the morphology of articular cartilage in knee joints of *Ddr2*-deficient mice and their control littermates, for evidence of changes in histology and in protein expressions of Ddr2 and Mmp-13. We also examined the expression profile of *Ddr2* in normal adult mice.

Materials and Methods

Generation of the Floxed *Ddr2* Mice

All animal experimental procedures were performed after approval from the Harvard Medical School Institutional Animal Care Committee. Embryonic stem cells containing the floxed *Ddr2* allele were purchased from EUCOMM (Wellcome Trust Genome Campus, Hinxton, Cambridge, UK). The embryonic stem cells were injected into blastocysts with C57BL/6 genomic background to generate chimeric mice. The chimeric mice were then bred with wild-type, C57BL/6, mice to generate heterozygous floxed *Ddr2* mice. The floxed *Ddr2* allele contains several elements, including FRT-flanked En2SA-IRES-LacZ-βact-Neo cassette, Q4 which may have potential effects on the development of mice. Thus, by crossing floxed *Ddr2* mice with FLPeR mice, the DNA fragment containing these elements was removed, eliminating the potential for undesired developmental abnormalities.

Mouse Genotyping

Mouse genotyping for *Aggrecan-CreERT2* and the floxed *Ddr2* gene was performed. Genomic DNA was isolated from the mice tails. To determine whether mice were *Aggrecan-CreERT2* positive (*AgcCreERT2*$^{+/-}$), the forward primer 5′-TAACTACCTGTTTTGCCGGG-3′ and the reverse primer 5′-GTCTGCCAGGTTGGTCAGTAA-3′ were used. The PCR for *AgcCreERT2* was set for primary denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and elongation at 72°C for 1 minute, with final elongation at 72°C for 10 minutes. To determine whether mice were homozygous for the floxed *Ddr2* gene (*Ddr2*$^{flox/flox}$), the forward primer 5′-AGTAGGTGCTAGCTACCTCCCACC-3′ and the reverse primer 5′-CTGCTTCCTCCCAGGTACCTTCCC-3′ were used. The PCR for floxed *Ddr2* was set for primary denaturation at 94°C for 3 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and elongation at 72°C for 30 seconds, with final elongation at 72°C for 10 minutes.

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