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Genetic Inhibition of Fibroblast Growth Factor Receptor 1 in Knee Cartilage Attenuates the Degeneration of Articular Cartilage in Adult Mice

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Abstract

Objective—Fibroblast growth factor (FGF) family members are involved in the regulation of articular cartilage homeostasis. The aim of this study was to investigate the function of FGF receptor 1 (FGFR-1) in the development of osteoarthritis (OA) and its underlying mechanisms.

Methods—FGFR-1 was deleted from the articular chondrocytes of adult mice in a cartilage-specific and tamoxifen-inducible manner. Two OA models (aging-associated spontaneous OA, and destabilization-induced OA), as well as an antigen-induced arthritis (AIA) model, were established and tested in *Fgfr1*-deficient and wild-type (WT) mice. Alterations in cartilage structure and the loss of proteoglycan were assessed in the knee joints of mice of either genotype, using these 3 arthritis models. Primary chondrocytes were isolated and the expression of key regulatory molecules was assessed quantitatively. In addition, the effect of an FGFR-1 inhibitor on human articular chondrocytes was examined.

Results—The gross morphologic features of *Fgfr1*-deficient mice were comparable with those of WT mice at both the postnatal and adult stages. The articular cartilage of 12-month-old *Fgfr1*-deficient mice displayed greater aggrecan staining compared to 12-month-old WT mice. *Fgfr1* deficiency conferred resistance to the proteoglycan loss induced by AIA and attenuated the development of cartilage destruction after surgically induced destabilization of the knee joint. The chondroprotective effect of FGFR-1 inhibition was largely associated with decreased expression of matrix metalloproteinase 13 (MMP-13) and up-regulation of FGFR-3 in mouse and human articular chondrocytes.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. L. Chen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Conclusion—Disruption of FGFR-1 in adult mouse articular chondrocytes inhibits the progression of cartilage degeneration. Down-regulation of MMP-13 expression and up-regulation of FGFR-3 levels may contribute to the phenotypic changes observed in *Fgfr1*-deficient mice.

Osteoarthritis (OA) is one of the most prevalent chronic joint diseases and is characterized by progressive cartilage destruction and insufficient extracellular matrix synthesis. Thus far, none of the strategies employed to prevent and treat OA have been effective. The final option for advanced OA is the surgical approach of total joint replacement (1). Studies have shown that changes in growth factor signalings and their downstream target genes may be involved in the development of OA (2–5). However, the pathogenic mechanisms of OA are still largely unknown. Better understanding of the molecular events in OA development would provide important information that could facilitate the identification of novel therapeutic targets for the prevention and treatment of OA.

Chondrocytes are the only cell type in adult human articular cartilage in which the main responsibility is the synthesis and degradation of extracellular matrix (6). Numerous molecules and pathways, such as hypoxia-inducible factor 2 α , discoidin domain receptor 2, and hedgehog signaling, in the articular chondrocytes have been shown to be involved in cartilage metabolism (7–11). Recently, the role of fibroblast growth factor (FGF) family members in the regulation of cartilage homeostasis has received specific attention (12). FGF-18 is a well-established anabolic growth factor that contributes to the metabolism of articular cartilage (13,14). Although several lines of evidence support the tight association between FGF-2 and articular cartilage metabolism, the role of FGF-2 in cartilage homeostasis is controversial (12,15).

Some studies of human articular chondrocytes have demonstrated that FGF-2 stimulates the production of matrix metalloproteinase 13 (MMP-13), which is the main collagenase responsible for collagen degradation (16). Results of other studies have suggested that FGF-2 functions as a chondroprotective factor in cartilage homeostasis (17–19). It has been reported that FGF-2 can suppress interleukin-1 (IL-1)–induced catabolic effects on human cartilage (18). In mice with *Fgf2* deficiency, spontaneous OA, as well as instability-induced OA, is accelerated (19). In response to tissue injury or mechanical compression, FGF-2 can be released from the extracellular matrix to activate intracellular ERK signaling and regulate expression of chondrocyte-specific genes, suggesting that FGF-2 plays a homeostatic role in articular chondrocytes (20).

The involvement of FGF ligands in the maintenance of articular cartilage suggests that FGF receptors (FGFRs) may also play an important role in articular cartilage homeostasis. Notably, FGFR-1 and FGFR-3 are highly expressed in human articular chondrocytes and have been implicated in cartilage metabolism (21). Valverde-Franco et al showed that *Fgfr3*-knockout (KO) mice exhibited abnormal cartilage metabolism and early signs of OA, suggesting that FGFR-3 signaling may have a chondroprotective role during the development of OA (22). It is hypothesized that activation of FGFR-1 may exert antianabolic and procatabolic effects on adult human articular cartilage (12). However, since conditional deletion of *Fgfr1* is known to have a lethal effect on mouse embryos (23), it is impossible to investigate the in vivo function of FGFR-1 in articular cartilage homeostasis using conventional *Fgfr1*-KO mice.

In this study, we investigated the role of FGFR-1 in articular cartilage function postnatally, using mice with tamoxifen-inducible and cartilage-specific conditional knockout (cKO) of the *Fgfr1* gene (hereinafter referred to as *Fgfr1* cKO mice). We evaluated the effects of FGFR-1 on the function of articular chondrocytes and the degeneration of articular cartilage in mice using 2 models of OA, as well as an antigen-induced arthritis (AIA) model. Our

study demonstrated that *Fgfr1* deficiency in mice attenuates articular cartilage degeneration in all 3 arthritis models. In addition, we found that blockade of FGFR-1 signaling could antagonize the IL-1 β -induced up-regulation of MMP-13 and enhance the expression of FGFR-3 in human articular chondrocytes.

MATERIALS AND METHODS

Animals

Fgfr1^{fl/fl} mice and *Col2a1-CreERT²* mice were generated in the laboratories of Dr. Deng and Dr. Di Chen, respectively (23,24). Both *Col2a1-CreERT²* and *Fgfr1^{fl/fl}* mice were backcrossed and maintained on a C57BL/6J background. *Col2a1-CreERT²;Fgfr1^{fl/fl}* mice with *Fgfr1* deficiency were obtained from offspring of the breeding of *Fgfr1^{fl/fl}* mice with *Col2a1-CreERT²*-transgenic mice. The efficiency of Cre recombination in the cartilage of these adult mice was evaluated in a manner as previously reported (25,26). *Fgfr1* cKO mice and their Cre-negative wild-type (WT) control littermates were intraperitoneally injected, at age 8 weeks, with tamoxifen at a dose of 1 mg/10 gm body weight, administered daily for 5 days. Genotyping for *Fgfr1* and the Cre transgene was carried out using polymerase chain reaction (PCR) (primer sequences available from the corresponding author upon request).

Surgical model of OA in mice

Before surgical induction of OA, mice were intraperitoneally injected, at age 8 weeks, with tamoxifen, administered daily for 5 days. Destabilization of the medial meniscus (DMM) surgery was performed on the right knee joints of 10-week-old male mice, according to previously described procedures (27). DMM surgery will lead to the development of progressive cartilage degeneration. As a control, sham surgery was performed with medial capsulotomy only. Pathologic changes in the medial tibial plateau and medial femoral condyle of the knee joint were scored at 4 weeks or 8 weeks postoperatively. All procedures were approved by the Institutional Animal Care and Use Committee of Daping Hospital (Chongqing, China).

Mouse model of AIA

Induction of AIA in mice was performed according to previously described methods (28). Briefly, to induce inflammatory arthritis, 10-week-old male mice were immunized by intradermal injection at the base of the tail with a 100- μ l emulsion containing 100 μ g of methylated bovine serum albumin (mBSA; in 0.9% saline) and an equal volume of Freund's complete adjuvant (catalog no. F5881; Sigma). Ten days after immunization, mBSA (10 μ l of 20 μ g/ml mBSA in 0.9% sterile saline) or an equal volume of vehicle was injected intraarticularly into the right or left knee joints. All knee joints were harvested on day 17 after immunization and processed for histologic assessment.

Histologic assessment

Microscopic scoring of mouse cartilage degeneration was performed in accordance with the recommendations of the Osteoarthritis Research Society International (OARSI) (29). Briefly, after the mice were killed, the knee joints were dissected free of skin and muscle. The joints were then fixed in 4% paraformaldehyde, decalcified in 20% formic acid, and embedded in paraffin. Either sagittal or frontal serial sections were obtained across the entire knee joint. Specifically, three 5- μ m sections were placed on each slide, with each joint fully harvested in ~10-slide intervals. Some intervening sections were used for immunostaining. Sections of the joints at every 50 μ m (~10 slides per joint) were stained with Safranin O-fast green to assess cartilage destruction.

Histologic grading of cartilage degeneration was performed using the OARSI-recommended subjective scoring system (on a scale of 0–6) (29). In addition, cartilage aggrecan depletion was scored (on a scale of 0–3) as a complement measure of cartilage degeneration, as previously described (30). All sections from WT and cKO mouse joints in each of the 3 arthritis models were stained on the same day, and all slides in each shelf were stained simultaneously to avoid staining variations between slides. The intensity of Safranin O staining in the growth plate of the femora and tibiae was used as an internal control between batches.

The severity of cartilage destruction was expressed as a summed score (sum of the 4 highest scores in all slides) and as a maximal score for the medial femora and medial tibiae separately within each section. We summed the 4 highest scores from 4 sections that showed the most significant histologic changes in all slides from each joint, to demonstrate both the severity and the extent of cartilage damage.

Immunohistochemistry

Tissue sections were deparaffinized using xylene and deprived of endogenous peroxidase activity with 3% H₂O₂, and then blocked with normal goat serum for 30 minutes. Sections were incubated overnight with rabbit anti-FGFR-1 polyclonal antibody (1:100 dilution; Abcam), rabbit anti-FGFR-3 polyclonal antibody (1:100 dilution; Santa Cruz Biotechnology), rabbit anti-MMP-13 polyclonal antibody (1:50 dilution; ProteinTech), and rabbit anti-type X collagen polyclonal antibody (1:200 dilution; Calbiochem-Merck). After rinsing the sections with phosphate buffered saline, a horseradish peroxidase-conjugated secondary antibody was applied, followed by staining with diaminobenzidine.

Isolation and culture of chondrocytes

To obtain mouse primary chondrocytes, we harvested the knee joints from 5-day-old *Col2a1-CreER^{T2};Fgfr1^{fl/fl}* mice and digested the joints with 0.1% collagenase (Gibco). Chondrocytes were maintained in 6-well plates at 1 million cells per well. On culture day 3, the cells were treated with 4-hydroxytamoxifen (4OH-TM) (1 μ M) or vehicle for 48 hours. Alternatively, chondrocytes were stimulated with recombinant human IL-1 β (1 ng/ml; R&D Systems) for 24 hours after incubation with 4OH-TM (i.e., 4OH-TM was still present during the culture period after the addition of IL-1 β).

To obtain human articular chondrocytes, surgically removed human cartilage tissue was harvested from patients with OA who were undergoing total knee joint replacement or from patients who were hospitalized at Daping Hospital (Chongqing, China) because of a traffic accident. Collection of these samples was approved by our Institutional Review Board and Ethics Committee, and samples were obtained following the patients' provision of written consent. Articular chondrocytes were digested from the cartilage according to previously described methods (18). Isolated chondrocytes were seeded onto 12-well plates at 1 million cells per well and cultured in Dulbecco's modified Eagle's medium/F-12 containing 10% fetal calf serum for 3 days. Prior to treatment with IL-1 β , the chondrocytes were preincubated with the FGFR-1 inhibitor PD166866 (100 nM; Sigma) for 2 hours and then incubated with IL-1 β (1 ng/ml) and PD166866 for 24 hours.

Overexpression of *Fgfr1* in ATDC5 cells

ATDC5 prechondrocytes were maintained in 6-well plates at 1 million cells per well, and pcDNA3.1 or pcDNA3.1-*Fgfr1* plasmids were transfected using Lipofectamine 2000 (Invitrogen), in accordance with the manufacturer's instructions. Cultures were harvested for extraction of RNA from the cells after 48 hours of incubation.

Real-time PCR

RNA was extracted from the chondrocytes using TRIzol reagent (Invitrogen). Total RNA was reverse-transcribed (RT) to complementary DNA (cDNA) using a PrimerScript RT reagent kit (Takara). For real-time PCR, cDNA was amplified with a Two-Step QuantiTect SYBR Green RT-PCR kit (Takara) in an Mx3000P PCR machine (Stratagene). Relative gene expression was calculated with MxPro software, which was provided by the manufacturer (Stratagene). All samples were measured in triplicate and normalized to an internal control, cyclophilin A. The annealing temperature was 57°C, and primer sequences were designed using the Primer Premier program, version 5.0.

Western blotting

Proteins were lysed from the articular chondrocytes using a radioimmunoprecipitation assay buffer containing protease inhibitors (Roche). Proteins of the same quality were separated on sodium dodecyl sulfate–polyacrylamide electrophoresis gels and transferred to PVDF membranes (Millipore). The membranes were blocked with 5% milk resolved in Tris buffered saline–Tween buffer, and probed by Western blotting with diluted antibodies. The primary antibodies used were anti–MMP-13 (Protein Group), with β -actin as a loading control (Sigma).

Statistical analysis

Results are expressed as the mean $\pm$ SD. All statistical comparisons between groups were conducted using Student's unpaired *t*-test, utilizing the SPSS program (version 13.0). *P* values less than 0.05 were considered statistically significant.

RESULTS

Histologic characterization of the joints of *Fgfr1* cKO mice

We first confirmed the efficiency of Cre-mediated recombination in adult mice by lacZ staining of the articular cartilage. Consistent with previous results (26), we observed positive staining for lacZ in the articular chondrocytes of *Col2a1-CreERT²;Rosa26R* mice at 5 months of age (results not shown), which indicated that generation of *Col2a1-CreERT²* mice could lead to efficient Cre recombination in mouse articular chondrocytes at the adult stage. In addition, no lacZ-positive chondrocytes were observed in *Col2a1-CreERT²; Rosa26R* mice that had not received treatment with tamoxifen (results available from the corresponding author upon request).

To examine the role of FGFR-1 in articular cartilage homeostasis, *Fgfr1* cKO mice and their WT littermates received, at age 8 weeks, treatment with tamoxifen, administered intraperitoneally for 5 days. Thereafter, spontaneous changes in the histologic features of the articular cartilage were analyzed in *Fgfr1* cKO and WT male mice at ages 3, 6, and 12 months. No gross abnormalities in the articular cartilage and no changes in the morphologic features of the joints were observed in either the *Fgfr1* cKO or WT mice. Staining of whole knee joint sections with Safranin O revealed no obvious differences in cartilage thickness or alterations in histologic features between *Fgfr1* cKO and WT mice at ages 3 and 6 months (Figure 1A).

In WT mice at age 12 months, the intensity of Safranin O staining of the knee joints appeared to be greatly decreased in most cartilage regions (Figure 1A, arrow) compared to that in WT mice at age 6 months, but the integrity of tidemark was still well preserved and the intensity of Safranin O staining in the growth plate was comparable (Figure 1A, open arrowhead) (additional results available from the corresponding author upon request). In contrast, in the knee joints of *Fgfr1* cKO mice, there were no apparent changes in

proteoglycan intensity at age 12 months compared to that at age 6 months (Figure 1A and results available from the corresponding author). Compared to WT littermate control mice, the intensity of Safranin O staining of cartilage was greater in *Fgfr1* cKO mice at age 12 months (Figure 1A, arrow, and results available from the corresponding author).

We also scored alterations in the proteoglycan content in the medial femoral condyle and medial tibial plateau across the whole joint, and the 5 highest scores from an individual joint were summed. We observed that the summed scores of proteoglycan loss in both the tibia and the femur were significantly decreased in *Fgfr1* cKO mice compared to WT mice at age 12 months (Figure 1B). These results indicate that progression of spontaneous, aging-related proteoglycan loss is attenuated by *Fgfr1* deficiency.

Resistance to AIA after cartilage-specific knockout of *Fgfr1*

To determine whether FGFR-1 contributes to the development and progression of chemically induced arthritis, we created an AIA model in *Fgfr1* cKO and WT mice and assessed the knee joints for histologic alterations. As expected, we observed that administration of mBSA led to the loss of sulfated proteoglycan in the articular cartilage of WT and *Fgfr1* cKO mice. However, the extent of proteoglycan loss in the *Fgfr1* cKO mice was less than that in WT control mice (Figures 2A and B). In the saline-injected knee joints, there were no apparent gross changes in either histologic features or proteoglycan content in the articular cartilage of either *Fgfr1* cKO or WT mice (Figures 2A and B). These results indicate that mice with cartilage-specific knockout of *Fgfr1* are resistant to the proteoglycan loss induced by AIA.

To quantify the extent of cartilage degeneration, Safranin O-stained sections of mBSA-treated knee joints were evaluated. Results of Safranin O staining of the medial femoral condyle and medial tibial plateau revealed that proteoglycan loss (as assessed using both the summed and maximal scores) was reduced in *Fgfr1* cKO mice compared to WT mice (Figure 2C). These findings support the hypothesis that suppression of FGFR-1 protects against inflammation-induced proteoglycan depletion.

Delayed progression of articular cartilage destruction following DMM surgery in *Fgfr1* cKO mice

To determine the effect of *Fgfr1* deficiency on the development of OA induced by injury, we performed DMM surgery in the right knee joints of *Fgfr1* cKO and WT mice. It has been reported that female mice in this model are resistant to surgery-induced cartilage degeneration (31), and therefore we used only male mice in these experiments. DMM surgery in the right knee joints resulted in a progressive degeneration of cartilage, with little or no synovitis. Moreover, we observed that the expression of FGFR-1 was progressively up-regulated in WT mice following DMM surgery (results available from the corresponding author upon request). In contrast, the knee joints of sham-operated mice of either genotype (*Fgfr1* deficient or WT) were normal or showed only mild changes at 4 or 8 weeks after the procedure (Figures 3A and 4A).

Histologic analysis of the knee joints showed that cartilage degeneration developed both at 4 and at 8 weeks after DMM surgery (Figures 3A and 4A). However, following DMM surgery, the responses related to instability-induced OA development were significantly different between the *Fgfr1* cKO mice and their WT littermates. At 4 weeks after DMM surgery, the joints of WT mice exhibited a large depletion of proteoglycan in the superficial layer and some erosion that extended to the calcified cartilage (Figure 3A). In contrast, the knee joints of *Fgfr1* cKO mice displayed only mild aggrecan loss within 4 weeks after DMM surgery (Figure 3A).

To quantify the extent and severity of the cartilage damage, we compared the summed and maximal structural damage scores in the knee joints of *Fgfr1* cKO and WT mice at 4 weeks following surgery. We found that the summed and maximal scores for cartilage damage in the femur and tibia were decreased significantly in *Fgfr1* cKO mice compared to WT mice at 4 weeks following DMM surgery (Figure 3B).

Within 8 weeks after DMM surgery, the knee joints of WT mice showed loss of uncalcified cartilage in some areas and exposed subchondral bone, whereas the knee joints of *Fgfr1* cKO mice exhibited significantly lower levels of damage (Figure 4A). In the *Fgfr1* cKO mice, progression of cartilage erosion was evident, especially in the medial tibial plateau, but the summed and maximal cartilage damage scores were still significantly lower in the *Fgfr1* cKO mice compared to WT mice (Figure 4B). These results demonstrate that the development of OA induced by destabilization is significantly attenuated in mice with *Fgfr1* deficiency.

Down-regulated expression of MMP-13 and increased expression of FGFR-3 in mice with *Fgfr1* deficiency

To explore the mechanisms underlying the delayed progression of joint degeneration in *Fgfr1* cKO mice, we analyzed chondrocyte gene expression in *Fgfr1*-deficient and WT mice. The primary chondrocytes were isolated from *Col2a1-CreERT²;Fgfr1^{fl/fl}* mice and treated with 4OH-TM on culture day 3 to drive Cre-mediated recombination. We performed real-time PCR to quantify the expression of *Fgfr1*, *aggrecan*, *Fgfr3*, *Mmp13*, *Mmp3*, and *Adamts5* in WT and *Fgfr1*-deficient mouse primary chondrocytes.

When *Fgfr1* was deleted from the articular chondrocytes, expression of *aggrecan* and *Fgfr3* was increased significantly, while *Mmp13* levels were significantly decreased (Figure 5A). The expression levels of other critical proteinase genes, such as *Mmp3* and *Adamts5*, were not significantly changed in *Fgfr1*-deficient mouse chondrocytes compared to WT control mouse chondrocytes (Figure 5A).

Results of Western blotting showed that targeted deletion of the *Fgfr1* gene significantly abolished the IL-1 β -mediated up-regulation of MMP-13 expression (Figure 5B). We also found that overexpression of *Fgfr1* in mouse prechondrogenic ATDC5 cells led to up-regulation of *Mmp13* (Figure 5C). These results demonstrate that the deletion of the *Fgfr1* gene in mouse primary chondrocytes will lead to down-regulation of the catabolic enzyme MMP-13 and accumulation of proteoglycan, indicating that suppression of FGFR-1 may inhibit catabolic action and promote anabolic metabolism in primary chondrocytes.

We next performed immunohistochemical staining of the knee joints of mice at age 12 months. Results of immunostaining showed that the expression levels of FGFR-1, type X collagen, and MMP-13 were significantly decreased in the knee joints of *Fgfr1* cKO mice compared to WT mice (Figures 6A–C). These results indicate that inhibition of FGFR-1 signaling may delay the process of matrix degradation and articular chondrocyte hypertrophy.

FGFR-1 and FGFR-3 are considered to be the predominant FGFRs expressed in human articular chondrocytes (21), and these 2 receptors may play opposite functions in cartilage homeostasis. We thus determined the expression of FGFR-3 in the knee joints of WT and *Fgfr1* cKO mice that were subjected to either DMM surgery or sham operation.

Consistent with the results of messenger RNA (mRNA) quantification (Figure 5A), the expression of FGFR-3 in the sham-operated knee joints was increased in *Fgfr1*-deficient mice compared to WT mice (Figure 6D). At 4 weeks after DMM surgery, the expression of FGFR-3 decreased in both the WT and *Fgfr1* cKO mice compared to that in the respective

sham-operated control mice (Figure 6D). In the DMM groups, FGFR-3 expression was notably higher in the knee joints of *Fgfr1*-deficient mice compared to WT mice (Figure 6D). Our results suggest that FGFR-3 is down-regulated in the articular cartilage when OA is induced by DMM surgery. Deletion of the *Fgfr1* gene will lead to a relatively higher expression of FGFR-3, even under conditions of DMM-induced joint instability.

With regard to the expression of FGFR-1 in articular cartilage chondrocytes, we compared the *Fgfr1* mRNA levels in the chondrocytes between patients with OA and healthy control subjects, and found that *Fgfr1* mRNA levels were significantly increased in OA patients compared to healthy controls (results available from the corresponding author upon request). When we treated the OA human articular chondrocytes with the FGFR-1 selective inhibitor PD166866, we found that the chondrocytes expressed significantly lower levels of *MMP13* and *ColX* and higher levels of *aggrecan*. The *MMP13* mRNA levels were elevated by 6.9-fold after treatment of the articular chondrocytes with IL-1 β for 24 hours. However, treatment of the chondrocytes with PD166866 remarkably reversed the IL-1 β -induced up-regulation of *MMP13* (detailed data available from the corresponding author upon request). These results suggest that suppression of FGFR-1-mediated signaling could antagonize the up-regulation of MMP-13 induced by catabolic factors such as IL-1 β .

DISCUSSION

OA is one of the most prevalent aging-related joint diseases and is the leading cause of disability in the elderly. Currently, there is no effective way to prevent and treat cartilage destruction in OA, which is a reflection of the insufficient understanding of the molecular mechanisms of the initiation and progression of OA. In our study, we provide evidence, for the first time, to indicate that genetic inhibition of *Fgfr1* in mature adult mouse articular cartilage attenuated the development of OA.

Understanding of the physiologic role of FGFR-1 in the maintenance of articular cartilage homeostasis in vivo has been elusive, due to the lethal effects of conventional *Fgfr1* knockout on mouse embryos (32). Adult mice with conditional knockout of the *Fgfr1* gene in chondrocytes (*Col2-Cre;Fgfr1^{fl/fl}* mice) will remain alive, but these mice undergo significant skeletal changes, including an increased height of the hypertrophic chondrocyte zone and significantly disturbed bone homeostasis (33), which may cause intrinsic changes in the articular cartilage and affect the susceptibility of articular cartilage to OA at the adult stage.

To determine the specific function of FGFR-1 in articular chondrocytes, we specifically disrupted *Fgfr1* gene expression in articular cartilage of mice at an adult stage, in a tamoxifen-inducible and chondrocyte-specific manner, by crossing *Fgfr1*-floxed mice with *Col2-CreER^{T2}*-transgenic mice. *Fgfr1* cKO mice and their Cre-negative WT littermates were administered tamoxifen at the age of 8 weeks. Mice with the *Fgfr1* deletion exhibited no gross abnormalities of body size, body weight, skeletal structure, or histologic features when compared to WT mice. Thus, these *Fgfr1*-deficient mice with grossly normal skeletons, joint structures, and morphologic features were suitable for study of OA at an adult stage.

To investigate the biologic effect of FGFR-1 on cartilage degeneration, 3 different arthritis models, including aging-associated spontaneous OA, surgery-induced OA, and AIA, were used. Histologic examination of the knee joints of mice in these 3 models revealed that deletion of the *Fgfr1* gene resulted in a substantial protection against loss of aggrecan and against articular cartilage structural damage in the knee joint, demonstrating that down-regulation of FGFR-1 in articular cartilage has a chondroprotective effect, thereby slowing

the development of OA. These observations are consistent with those from a recent in vitro study showing that FGFR-1 mainly transmits an FGF-2-mediated catabolic signal in human articular chondrocytes (21).

The primary characteristic of OA is an imbalance between anabolic effects on extracellular matrix synthesis and catabolic effects on matrix degradation. These dual processes of reduced matrix synthesis and/or increased production of degradative proteinases will lead to generation of articular cartilage matrix that is unable to transmit normal mechanical stress. The proteolytic functions of the ADAMTS and MMP family members play important roles in the development of OA. *Adamts5*, a key member in the ADAMTS family, is responsible for aggrecan degradation in mouse models of OA (34,35), a feature that constitutes an early event in the development of OA (36). Furthermore, MMP-13 is considered the most active collagenase for type II collagen degradation, and greatly contributes to OA development (37). Genetically modified mice with constitutionally active MMP-13 expression showed development of OA, and *Mmp13* deficiency protected mice against OA cartilage damage (38,39).

FGF-2 has been shown to stimulate production of MMP-13 by activating the NF- κ B and MAPK pathways in human articular chondrocytes (40). Moreover, IL-1 β could stimulate the synthesis and secretion of multiple degradative enzymes (such as MMP-13) in cartilage, which contributes to OA development (41). In the present study, we found that deletion of the *Fgfr1* gene decreased MMP-13 expression and significantly reversed the IL-1 β -induced up-regulation of MMP-13 in primary chondrocytes. Consistent with this finding, we also found that inhibition of FGFR-1 with the selective inhibitor PD166866 antagonized the IL-1 β -induced up-regulation of MMP-13 in human articular chondrocytes. In contrast, we did not detect any significant changes in *Adamts5* expression in *Fgfr1*-deficient mouse chondrocytes. In addition, we observed that disruption of the *Fgfr1* gene decreased the expression of type X collagen, enhanced the expression of aggrecan, and increased the accumulation of proteoglycan in articular cartilage.

These changes may be at least partly responsible for the decelerated progression of cartilage degeneration in *Fgfr1*-deficient mice. Our results suggest that *Fgfr1* deficiency suppresses the expression of MMP-13 in articular chondrocytes, indicating that FGFR-1, as a critical regulator of MMP-13 expression, may be used as a therapeutic target for OA treatment.

The FGF family plays a critical role in cartilage development. In growth plate cartilage, FGFR-3 is mainly expressed in the proliferating and prehypertrophic chondrocytes. FGFR-3 suppresses the proliferation and differentiation of chondrocytes, while FGFR-1 functions as a potent mitogenic stimulator (42,43). However, few studies have explored the physiologic role of FGFR signaling in vivo in adult articular chondrocytes. It has been reported that FGFR-1 and FGFR-2 are the predominant receptors in healthy mature articular cartilage in mice (19). In contrast, the major FGFRs expressed in human articular chondrocytes are considered to be FGFR-1 and FGFR-3 (21).

During OA development, FGFR-3 expression is down-regulated in articular chondrocytes from patients with OA (21), and early development of OA in *Fgfr3*-KO mice could be attributed to an increase in MMP-13 expression (22). We also found that expression of FGFR-3 was decreased in the knee joints of mice with OA induced by DMM surgery. These results suggest that down-regulation of FGFR-3 is at least partially responsible for the development of OA, and that FGFR-3 plays a potentially chondroprotective role in the articular cartilage.

FGFR-3-transmitted signaling in articular chondrocytes may have an effect on cartilage metabolism that is opposite to that of FGFR-1. In this study, we found that the absence of

FGFR-1 in mouse articular cartilage not only could antagonize up-regulation of MMP-13 but also could lead to increased expression of FGFR-3 in articular chondrocytes. These results indicate that deletion of the *Fgfr1* gene may, in addition to antagonizing the catabolic action of certain proteinases such as MMP-13 on cartilage, also delay the progression of OA by elevating the expression of FGFR-3. It is conceivable that knockdown of *Fgfr3* in articular chondrocytes would attenuate the chondroprotective phenotype in *Fgfr1*-deficient mice. Studies involving chondrocyte-specific *Fgfr1/Fgfr3* double-KO mice would provide further solid evidence to support the opposing roles of FGFR-1 and FGFR-3 in the development and progression of OA; these studies are now under way.

Overexpression of FGFR-3 could inhibit the Indian hedgehog (IHH) signaling pathway in the growth plate, and inhibition of IHH signaling is able to attenuate the severity of OA (11,44). Whether IHH is involved in the chondroprotective effects of FGFR-3 requires further investigation. In addition, it has been reported that FGFR-3 is down-regulated by FGF-2 via the FGFR-1/ERK/MAPK pathway in human articular chondrocytes (21). Whether deletion of FGFR-1 in chondrocytes leads to up-regulation of FGFR-3 via down-regulation of the ERK/MAPK pathway needs further study.

In summary, these results show, for the first time, that conditional deletion of the *Fgfr1* gene in mature mouse articular chondrocytes protects the knee joint cartilage against destruction, probably by suppressing the production of MMP-13 and enhancing the expression of FGFR-3. Our study provides valuable insights into the potential roles of FGFR-1 in articular cartilage metabolism. Therefore, these findings suggest that targeting of FGFR-1 could be a potential therapeutic strategy in patients with OA.

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References

1. Bijlsma JW, Berenbaum F, Lefeber FP. Osteoarthritis: an update with relevance for clinical practice. *Lancet*. 2011; 377:2115–26. [PubMed: 21684382]
2. Burrage PS, Mix KS, Brinckerhoff CE. Matrix metalloproteinases: role in arthritis. *Front Biosci*. 2006; 11:529–43. [PubMed: 16146751]
3. Abramson SB, Attur M. Developments in the scientific understanding of osteoarthritis. *Arthritis Res Ther*. 2009; 11:227. [PubMed: 19519925]
4. Lin EA, Liu CJ. The role of ADAMTSs in arthritis. *Protein Cell*. 2010; 1:33–47. [PubMed: 21203996]
5. Wang M, Shen J, Jin H, Im HJ, Sandy J, Chen D. Recent progress in understanding molecular mechanisms of cartilage degeneration during osteoarthritis. *Ann N Y Acad Sci*. 2011; 1240:61–9. [PubMed: 22172041]
6. Lieberman J, Goldberg RL. Chondrocyte culture and assay. *Curr Protoc Pharmacol*. 2001; Chapter 12(Unit12.2)
7. Van den Berg WB. Osteoarthritis year 2010 in review: pathomechanisms. *Osteoarthritis Cartilage*. 2011; 19:338–41. [PubMed: 21324370]
8. Saito T, Fukai A, Mabuchi A, Ikeda T, Yano F, Ohba S, et al. Transcriptional regulation of endochondral ossification by HIF-2 α during skeletal growth and osteoarthritis development. *Nat Med*. 2010; 16:678–86. [PubMed: 20495570]
9. Yang S, Kim J, Ryu JH, Oh H, Chun CH, Kim BJ, et al. Hypoxia-inducible factor-2 α is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med*. 2010; 16:687–93. [PubMed: 20495569]

10. Xu L, Servais J, Polur I, Kim D, Lee PL, Chung K, et al. Attenuation of osteoarthritis progression by reduction of discoidin domain receptor 2 in mice. *Arthritis Rheum.* 2010; 62:2736–44. [PubMed: 20518074]
11. Lin AC, Seeto BL, Bartoszko JM, Khoury MA, Whetstone H, Ho L, et al. Modulating Hedgehog signaling can attenuate the severity of osteoarthritis [published erratum appears in *Nat Med* 2010;16: 129]. *Nat Med.* 2009; 15:1421–5. [PubMed: 19915594]
12. Ellman MB, An HS, Muddasani P, Im HJ. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. *Gene.* 2008; 420:82–9. [PubMed: 18565695]
13. Davidson D, Blanc A, Filion D, Wang H, Plut P, Pfeffer G, et al. Fibroblast growth factor (FGF) 18 signals through FGF receptor 3 to promote chondrogenesis. *J Biol Chem.* 2005; 280:20509–15. [PubMed: 15781473]
14. Moore EE, Bendele AM, Thompson DL, Littau A, Waggle KS, Reardon B, et al. Fibroblast growth factor-18 stimulates chondrogenesis and cartilage repair in a rat model of injury-induced osteoarthritis. *Osteoarthritis Cartilage.* 2005; 13:623–31. [PubMed: 15896984]
15. Vincent TL. Fibroblast growth factor 2: good or bad guy in the joint? *Arthritis Res Ther.* 2011; 13:127. [PubMed: 21996043]
16. Im HJ, Muddasani P, Natarajan V, Schmid TM, Block JA, Davis F, et al. Basic fibroblast growth factor stimulates matrix metallo-proteinase-13 via the molecular cross-talk between the mitogen-activated protein kinases and protein kinase C δ pathways in human adult articular chondrocytes. *J Biol Chem.* 2007; 282:11110–21. [PubMed: 17311929]
17. Schmal H, Zwillingmann J, Fehrenbach M, Finkenzeller G, Stark GB, Sudkamp NP, et al. bFGF influences human articular chondrocyte differentiation. *Cytotherapy.* 2007; 9:184–93. [PubMed: 17453970]
18. Sawaji Y, Hynes J, Vincent T, Saklatvala J. Fibroblast growth factor 2 inhibits induction of aggrecanase activity in human articular cartilage. *Arthritis Rheum.* 2008; 58:3498–509. [PubMed: 18975307]
19. Chia SL, Sawaji Y, Burleigh A, McLean C, Inglis J, Saklatvala J, et al. Fibroblast growth factor 2 is an intrinsic chondroprotective agent that suppresses ADAMTS-5 and delays cartilage degradation in murine osteoarthritis. *Arthritis Rheum.* 2009; 60:2019–27. [PubMed: 19565481]
20. Vincent T. Basic FGF mediates an immediate response of articular cartilage to mechanical injury. *Proc Natl Acad Sci U S A.* 2002; 99:8259–64. [PubMed: 12034879]
21. Yan D, Chen D, Cool SM, van Wijnen AJ, Mikecz K, Murphy G, et al. Fibroblast growth factor receptor 1 is principally responsible for fibroblast growth factor 2-induced catabolic activities in human articular chondrocytes. *Arthritis Res Ther.* 2011; 13:R130. [PubMed: 21835001]
22. Valverde-Franco G. Defects in articular cartilage metabolism and early arthritis in fibroblast growth factor receptor 3 deficient mice. *Hum Mol Genet.* 2006; 15:1783–92. [PubMed: 16624844]
23. Xu X, Qiao W, Li C, Deng CX. Generation of Fgfr1 conditional knockout mice. *Genesis.* 2002; 32:85–6. [PubMed: 11857785]
24. Chen M, Lichtler AC, Sheu TJ, Xie C, Zhang X, O'Keefe RJ, et al. Generation of a transgenic mouse model with chondrocyte-specific and tamoxifen-inducible expression of Cre recombinase. *Genesis.* 2007; 45:44–50. [PubMed: 17211877]
25. Zhu M, Chen M, Lichtler AC, O'Keefe RJ, Chen D. Tamoxifen-inducible Cre-recombination in articular chondrocytes of adult Col2a1-CreER^{T2} transgenic mice. *Osteoarthritis Cartilage.* 2008; 16:129–30. [PubMed: 17888690]
26. Zhu M, Tang D, Wu Q, Hao S, Chen M, Xie C, et al. Activation of β -catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult β -catenin conditional activation mice. *J Bone Miner Res.* 2009; 24:12–21. [PubMed: 18767925]
27. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage.* 2007; 15:1061–9. [PubMed: 17470400]
28. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, Yokoyama S, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev.* 2010; 24:1173–85. [PubMed: 20466812]

29. Glasson SS, Chambers MG, Van Den Berg WB, Little CB. The OARSI histopathology initiative—recommendations for histological assessments of osteoarthritis in the mouse. *Osteoarthritis Cartilage*. 2010; 18:S17–23. [PubMed: 20864019]
30. Little CB, Meeker CT, Golub SB, Lawlor KE, Farmer PJ, Smith SM, et al. Blocking aggrecanase cleavage in the aggrecan interglobular domain abrogates cartilage erosion and promotes cartilage repair. *J Clin Invest*. 2007; 117:1627–36. [PubMed: 17510707]
31. Ma HL, Blanchet TJ, Peluso D, Hopkins B, Morris EA, Glasson SS. Osteoarthritis severity is sex dependent in a surgical mouse model. *Osteoarthritis Cartilage*. 2007; 15:695–700. [PubMed: 17207643]
32. Xu X, Li C, Takahashi K, Slavkin HC, Shum L, Deng CX. Murine fibroblast growth factor receptor 1 α isoforms mediate node regression and are essential for posterior mesoderm development. *Dev Biol*. 1999; 208:293–306. [PubMed: 10191046]
33. Jacob AL, Smith C, Partanen J, Ornitz DM. Fibroblast growth factor receptor 1 signaling in the osteo-chondrogenic cell lineage regulates sequential steps of osteoblast maturation. *Dev Biol*. 2006; 296:315–28. [PubMed: 16815385]
34. Glasson SS, Askew R, Sheppard B, Carito B, Blanchet T, Ma HL, et al. Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis. *Nature*. 2005; 434:644–8. [PubMed: 15800624]
35. Stanton H, Rogerson FM, East CJ, Golub SB, Lawlor KE, Meeker CT, et al. ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro. *Nature*. 2005; 434:648–52. [PubMed: 15800625]
36. Bondeson J, Wainwright S, Hughes C, Caterson B. The regulation of the ADAMTS4 and ADAMTS5 aggrecanases in osteoarthritis: a review. *Clin Exp Rheumatol*. 2008; 26:139–45. [PubMed: 18328163]
37. Minond D, Lauer-Fields JL, Cudic M, Overall CM, Pei D, Brew K, et al. The roles of substrate thermal stability and P₂ and P₁' subsite identity on matrix metalloproteinase triple-helical peptidase activity and collagen specificity. *J Biol Chem*. 2006; 281:38302–13. [PubMed: 17065155]
38. Little CB, Barai A, Burkhardt D, Smith SM, Fosang AJ, Werb Z, et al. Matrix metalloproteinase 13-deficient mice are resistant to osteoarthritic cartilage erosion but not chondrocyte hypertrophy or osteophyte development. *Arthritis Rheum*. 2009; 60:3723–33. [PubMed: 19950295]
39. Neuhold LA, Killar L, Zhao W, Sung ML, Warner L, Kulik J, et al. Postnatal expression in hyaline cartilage of constitutively active human collagenase-3 (MMP-13) induces osteoarthritis in mice. *J Clin Invest*. 2001; 107:35–44. [PubMed: 11134178]
40. Muddasani P, Norman JC, Ellman M, van Wijnen AJ, Im HJ. Basic fibroblast growth factor activates the MAPK and NF κ B pathways that converge on Elk-1 to control production of matrix metalloproteinase-13 by human adult articular chondrocytes. *J Biol Chem*. 2007; 282:31409–21. [PubMed: 17724016]
41. Pelletier JP, Martel-Pelletier J, Abramson SB. Osteoarthritis, an inflammatory disease: potential implication for the selection of new therapeutic targets [review]. *Arthritis Rheum*. 2001; 44:1237–47. [PubMed: 11407681]
42. Kilkenny DM, Hill DJ. Perinuclear localization of an intracellular binding protein related to the fibroblast growth factor (FGF) receptor 1 is temporally associated with the nuclear trafficking of FGF-2 in proliferating epiphyseal growth plate chondrocytes. *Endocrinology*. 1996; 137:5078–89. [PubMed: 8895382]
43. Wang Q, Green RP, Zhao G, Ornitz DM. Differential regulation of endochondral bone growth and joint development by FGFR1 and FGFR3 tyrosine kinase domains. *Development*. 2001; 128:3867–76. [PubMed: 11585811]
44. Naski MC, Colvin JS, Coffin JD, Ornitz DM. Repression of hedgehog signaling and BMP4 expression in growth plate cartilage by fibroblast growth factor receptor 3. *Development*. 1998; 125:4977–88. [PubMed: 9811582]

Figure 1. Histologic changes related to aging in the articular cartilage of *Fgfr1* conditional knockout (cKO) mice compared to wild-type (WT) (Cre-negative) littermate control mice. **A**, Sagittal sections of whole knee joints from mice at ages 3, 6, and 12 months were stained with Safranin O-fast green to visualize gross morphologic differences in knee joint degeneration and loss of proteoglycan (**arrows**). All mice received treatment with the same amounts of tamoxifen. Representative images are shown (n = 5 samples per group). In WT mice at age 12 months, tidemark (**open arrowhead**) was still well preserved. Bar = 200 μ m. **B**, Summed scores (mean $\pm$ SD) for proteoglycan loss in the medial femoral condyle (MFC) and medial tibial plateau (MTP) were determined in the knee joints of *Fgfr1* cKO and WT mice at age 12 months. ** = $P < 0.001$ versus WT.

Figure 2. Characteristics of antigen-induced arthritis (AIA) in *Fgfr1*-deficient mice compared to wild-type (WT) control mice. **A** and **B**, The extent of proteoglycan loss (arrows) was examined in Safranin O-fast green-stained sections from WT mice (**A**) and *Fgfr1* conditional knockout (cKO) mice (**B**) after intraarticular injection of the knee joints with methylated bovine serum albumin (mBSA) (right) compared to saline control (left). In knee joints injected with mBSA, *Fgfr1* deficiency protected the articular cartilage against degeneration induced by AIA. Boxed areas in top panels (bars = 400 μ m) are shown at higher magnification in bottom panels (bars = 100 μ m). Representative images are shown (n = 7 samples per group). GP = growth plate. **C**, Summed and maximal scores (mean $\pm$ SD) for proteoglycan loss in the medial femoral condyle (MFC) and medial tibial plateau (MTP) were determined in the knee joints of *Fgfr1* cKO and WT mice. * = $P < 0.05$ versus WT.

Figure 3.

Histologic features and summed and maximal scores for structural damage in mouse articular cartilage at 4 weeks following destabilization of the medial meniscus (DMM) surgery. **A**, Whole knee joints from *Fgfr1* conditional knockout (cKO) and wild-type (WT) control mice were subjected to sham operation (left) or DMM surgery (right). The articular cartilage was stained with Safranin O–fast green at 4 weeks after surgery to assess the extent of articular cartilage degeneration in WT mice (**arrow**) and *Fgfr1* cKO mice (**arrowhead**). Representative images are shown (n = 10 mice per genotype). Bar = 200 μ m. **B**, Summed (top) and maximal (bottom) histologic scores for cartilage structure damage (mean $\pm$ SD) were determined in the medial femur and tibia of *Fgfr1* cKO and WT control mice at 4 weeks following sham operation or DMM surgery. * = $P < 0.05$; ** = $P < 0.001$ versus WT.

Figure 4.

Histologic features and summed and maximal scores for structural damage in the mouse articular cartilage at 8 weeks following destabilization of the medial meniscus (DMM) surgery. **A**, Whole knee joints from *Fgfr1* conditional knockout (cKO) and wild-type (WT) control mice were subjected to sham operation (left) or DMM surgery (right). The articular cartilage was stained with Safranin O–fast green at 8 weeks after surgery to assess the extent of articular cartilage degeneration. After DMM surgery, WT mice exhibited a loss of uncalcified cartilage and exposed subchondral bone (**arrow**), whereas *Fgfr1* cKO mice showed reduced damage in the articular cartilage (**arrowhead**). Sham-operated knees showed no cartilage damage. Representative images are shown (n = 10 mice per genotype). Bar = 200 μ m. **B**, Summed (top) and maximal (bottom) histologic scores of cartilage structure damage (mean $\pm$ SD) were determined in the medial femur and tibia of *Fgfr1* cKO and WT mice at 8 weeks following sham operation or DMM surgery. ** = $P < 0.001$ versus WT.

Figure 5.

Effects of *Fgfr1* deficiency on gene expression in primary chondrocytes from mouse articular cartilage. **A**, The fold change in expression levels of *Fgfr1*, *aggrecan*, *Fgfr3*, *Mmp13*, *Mmp3*, and *Adamts5* was assessed by real-time polymerase chain reaction in primary chondrocytes from *Fgfr1* conditional knockout (cKO) mice compared to wild-type (WT) control mice. * = $P < 0.05$ versus WT. **B**, Primary chondrocytes were treated with or without 4-hydroxytamoxifen (4OH-TM) ($1 \mu M$) for 48 hours, or stimulated with or without recombinant human interleukin-1 β (IL-1 β) (1 ng/ml) for 24 hours after incubation with 4OH-TM. Western blot results show that disruption of *Fgfr1* in the mouse primary chondrocytes can antagonize the IL-1 β -induced up-regulation of matrix metalloproteinase 13 (MMP-13); β -actin was used as a loading control. **C**, ATDC5 cells were transfected with pcDNA3.1 or pcDNA3.1-*Fgfr1* plasmids, and after 48 hours of incubation, the fold change in expression of *Fgfr1* and *Mmp13* was assessed. * = $P < 0.05$; ** = $P < 0.001$ versus pcDNA3.1-transfected cells. Bars in **A** and **C** show the mean $\pm$ SD.

Figure 6. Immunohistochemical staining for fibroblast growth factor 1 (FGFR-1), type X collagen, matrix metalloproteinase 13 (MMP-13), and FGFR-3 in the knee joints of *Fgfr1* conditional knockout (cKO) mice compared to wild-type (WT) control mice. **A–C**, The knee joints of WT mice and *Fgfr1* cKO mice at age 12 months were immunostained for FGFR-1 (**A**), type X collagen (**B**), and MMP-13 (**C**). Bar = 100 μ m. **D**, The knee joints of WT mice and *Fgfr1* cKO mice were immunostained for FGFR-3 at 4 weeks after either sham operation (left panels) or destabilization of the medial meniscus (DMM) surgery (right panels). Bar = 200 μ m. **Insets**, Higher-magnification views of the articular cartilage chondrocytes (original magnification $\times$ 400). **GP** = growth plate.