

Reduced Epidermal Growth Factor Receptor (EGFR) Signaling Enhances Cartilage Destruction in Mouse Osteoarthritis Model

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Introduction

Osteoarthritis (OA) is a degenerative joint disease primarily characterized by the destruction of articular cartilage. Growth factors, such as TGF- β , IGFs, BMPs and FGFs, regulate synthesis and maintenance of cartilage ECM and therefore, play important roles in cartilage homeostasis and OA development. Our recent work demonstrated that EGFR signaling is important for cartilage matrix degradation during endochondral ossification, and deficiency of EGFR activity either globally or specifically in chondrocytes causes expansion of the hypertrophic zone in the growth plate and delayed formation of secondary ossification center in long bones at early postnatal stage.^{1,2} We hypothesize that EGFR is important for cartilage homeostasis and OA development. In this study, we performed destabilization of the medial meniscus (DMM) to induce OA in mouse models with reduced EGFR activity.

Methods

Three mouse models with reduced EGFR activity were used in this study. In the first model, we compared OA development between Egfr^{Wa5/+} ($n = 9$) and their WT siblings ($n = 10$) after DMM surgery. Egfr^{Wa5} codes for a kinase-dead, dominant negative receptor. Mice homozygous for Wa5 are embryonic lethal but the heterozygotes are viable and show no major pathological changes. In the second model,

we compared OA development in WT mice treated with either vehicle ($n = 8$) or gefitinib (100 mg/kg, $n = 9$), an EGFR kinase inhibitor, once every other day, for 12 weeks. In the third model, we generated cartilage-specific EGFR inactivation mice (Col2-cre Egfr^{Wa5/flox}, $n = 5$) and their control siblings (Col2-Cre Egfr^{flox/+} and Egfr^{flox/+}, $n = 6$) by breeding Col2a1-Cre with Egfr^{Wa5/+} followed by breeding Col2-Cre Egfr^{Wa5/+} with Egfr^{flox/flox}.² In all these models, 3-month-old animals received DMM surgery in the right knees and sham operation in the left knees. Bilateral knee joints free of soft tissues were harvested 12 weeks post-surgery for histological and immunohistochemical analysis. OA changes were evaluated by Mankin's method.

Results

Histological analysis showed that total cartilage area and thickness, particularly the uncalcified area and thickness, at both medial femoral condyle and medial tibial plateau were reduced in Egfr^{Wa5/+} and gefitinib-treated mice in comparison with WT and vehicle-treated mice, respectively. Further scoring by Mankin's method revealed significant 19% and 36% increases of OA scores at medial femoral condyle and medial tibial plateau, respectively, in Egfr^{Wa5/+} mice compared to those in WT mice (Figure 1A). In gefitinib-treated mice, OA damage on the articular cartilage was relatively milder than those in Egfr^{Wa5/+} mice, but still more

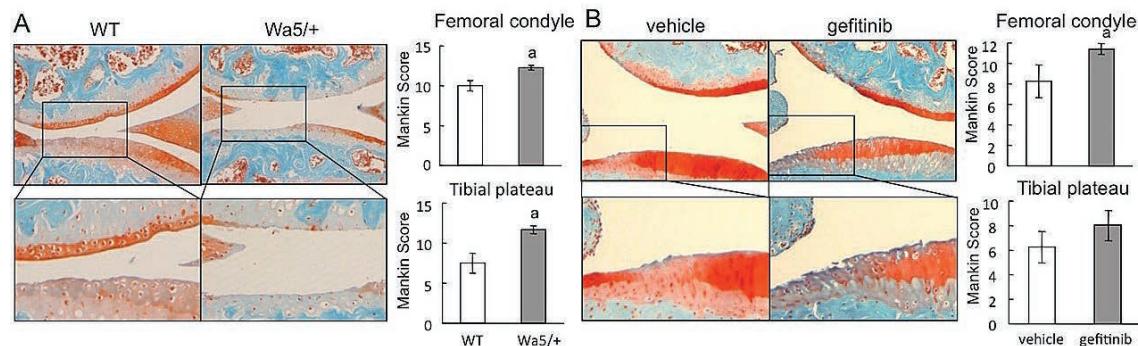


Figure 1. Egfr^{Wa5/+} and gefitinib-treated mice exhibit accelerated osteoarthritis progression after DMM surgery. Representative Safranin O/Fast Green staining images and Mankin scores of mouse knee joints show increased articular cartilage degradation in Egfr^{Wa5/+} and gefitinib-treated mice 3 months after DMM surgery. a: $p < 0.05$; c: $p < 0.001$.

severe than their vehicle-treated controls (Figure 1B). These results clearly demonstrate that mice with reduced EGFR activity exhibit more cartilage destruction and accelerated OA progression.

Chondrocyte apoptosis has been observed during OA progression and the apoptotic rate is positively correlated with the severity of cartilage damage. TUNEL assay revealed there were more than 60% increases of apoptotic chondrocytes in the articular cartilage of gefitinib-treated mice and *Egfr^{Wa5/+}* mice, suggesting that EGFR protects chondrocytes from OA-induced cell death (data not shown).

Proteolytic cleavage of aggrecan weakens the cartilage matrix and is a key event in OA pathogenesis. Immunohistochemistry showed that aggrecan degradation products generated by either aggrecanases or MMPs were significantly increased in both femoral and tibial articular cartilage areas in *Egfr^{Wa5/+}* mice and gefitinib-treated mice compared to their respective control mice. These results indicate that activation of EGFR signaling pathway suppresses cartilage matrix degradation during OA development. Further study revealed elevated amounts of ADAMTS5 and MMP13, the critical proteinases responsible for cartilage degradation during OA development, in articular cartilage after DMM surgery in both mouse models with reduced EGFR activity, compared to those in their respective control mice (Figure 2), which coincides with the increased aggrecan degradation in these mouse models. Interestingly, we found elevated expression of hif2a, a transcription factor highly expressed in OA development and essential for Mmp13 expression in chondrocytes, in the articular cartilage of those mouse models after DMM surgery, compared to their respective control mice (Figure 2).

To clarify whether global reduction of EGFR signaling by gefitinib treatment or a dominant negative allele *Egfr^{Wa5/+}* might act on cells other than chondrocytes to affect OA development, we specifically reduced EGFR activity in chondrocytes by generating Col2-cre *Egfr^{Wa5/flox}* mice (CKO). Our previous data has shown that chondrocytes from these CKO mice exhibit much lower EGFR activity than those from *Egfr^{Wa5/+}* mice. Interestingly, after DMM surgery, these mice developed a very sever OA phenotype with a complete loss of articular cartilage layers at femoral condyle and tibial plateau at 3 months post-operatively (Figure 3). In addition, the affected sites showed thickening of subchondral bone plate and contained less subchondral bone marrow in both femurs and tibiae.

Discussion

We provide the first direct evidence that chondrogenic EGFR signaling and its cognate ligands, most likely TGF β , is essential for articular cartilage homeostasis and is critical for OA development.³ Our data demonstrate that reduction in EGFR activity, particularly in articular chondrocytes, increases apoptosis and amounts of cartilage matrix degradation enzymes such as ADAMTS5 and MMP13, eventually leading to accelerated articular cartilage destruction. Further investigations are underway to understand how EGFR pathway regulates the crosstalk between articular cartilage and subchondral bone during OA development.

Significance

Our studies reveal a novel role of EGFR signaling in the protection of articular cartilage during OA development

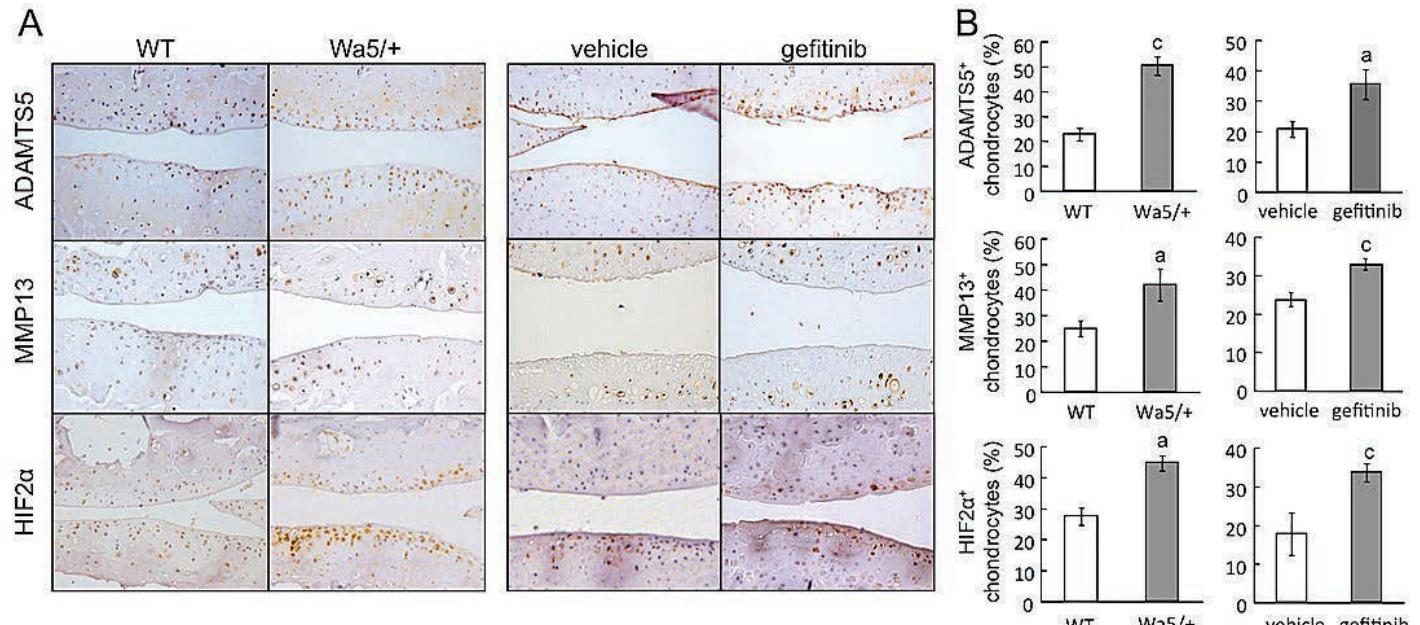


Figure 2. The protein amounts of ADAMTS5, MMP13 and HIF2 α are increased in osteoarthritic cartilage from mice with reduced EGFR activity. A: Immunostaining of ADAMTS5, MMP13 and HIF2 α in DMM-operated knee joints from *Egfr^{Wa5/+}* mice and gefitinib-treated mice and their respective controls. B: The percentages of ADAMTS5-, MMP13- and HIF2 α -positive articular chondrocytes were quantified. a: p < 0.05; c: p < 0.001.

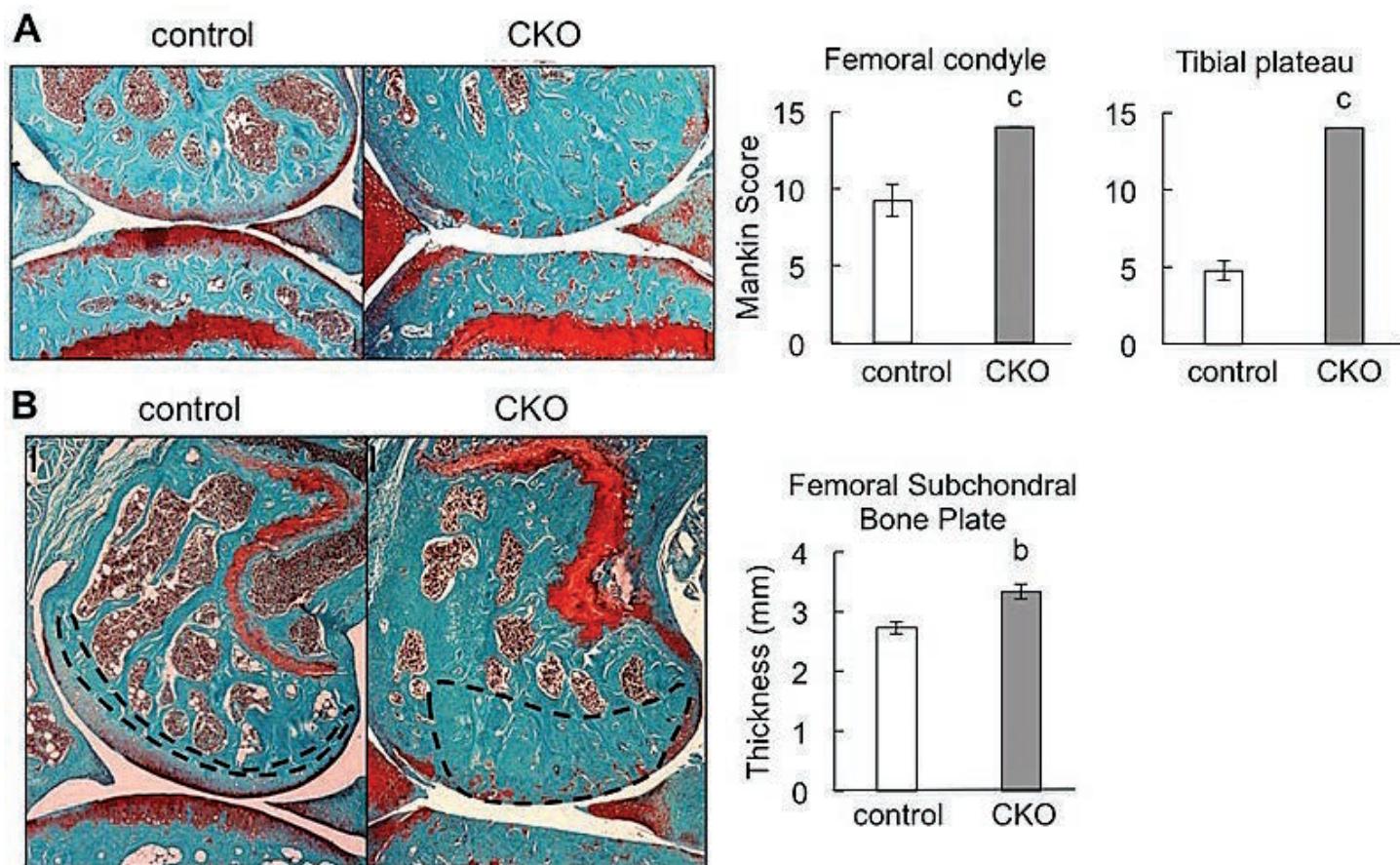


Figure 3. Mice deficient in chondrogenic EGFR activity exhibit severe osteoarthritis progression, characterized by deprivation of the cartilage layer and subchondral bone sclerosis, after DMM surgery. (A) Representative Safranin O/Fast Green staining images and Mankin scores of mouse knee joints. (B) The thickness of subchondral bone plate was measured by Safranin O/Fast Green staining images. b: p < 0.01; c: p < 0.001.

and therefore identify this signaling pathway as a potential therapeutic target for OA treatment.

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