Epidermal Growth Factor Receptor (EGFR) Signaling in Cartilage Prevents Osteoarthritis Progression

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Introduction

Osteoarthritis (OA) is the most common chronic condition of the joints affecting approximately 27 million adults in the United States alone. OA is a disease of mechanics but the molecular mechanism by which normal, healthy articular cartilage maintains its strength to resist mechanical loading thereby preventing OA progression is poorly understood. We recently demonstrated a pivotal role of epidermal growth factor receptor (EGFR) signaling in growth plate development1 and endochondral ossification2, indicating that this is a critical pathway regulating chondrocyte function. To understand the role of EGFR in maintaining articular cartilage and in OA development, we generated cartilage-specific *Egfr* null mice and characterized their knee joint phenotypes under physiological and pathological conditions.

Methods

All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. *Animals and surgery-* Cartilage-specific *Egfr CKO* (*Col2-Cre* $Egfr^{Wa5/f}$) mice and their *Wa5* ($Egfr^{Wa5/f}$) and *wild-type* (*WT, Col2-Cre* $Egfr^{f/+}$ and $Egfr^{f/+}$) siblings were generated by breeding *Col2a1-Cre,* $Egfr^{Wa5/+}$, and $Egfr^{f/f}$ mice. $Egfr^{Wa5}$ codes for a kinase-dead, dominant negative receptor. To induce OA, male mice at 3 months of age were subjected to destabilization of the medial meniscus (DMM) surgery of the right knees and sham surgery of the left knees. *Histology and immunohistochemistry (IHC)-* Mouse knee joints were harvested at indicated times for a serial of 6 μm-thick sagittal sections across the entire medial compartment of the joint for quantification of cartilage thickness, chondrocyte number, subchondral bone plate thickness, and Mankin score, as well as IHC staining for EGFR, phosphorylated-EGFR (p-EGFR), phosphorylated-Erk (p-Erk), osteocalcin, and sclerostin. *μCT-* The distal femurs and proximal tibias were scanned by μCT 35 (Scanco Medical AG) at a resolution of 6 μm to calculate trabecular bone structural parameters. *Nanoindentation-* Atomic Force Microscopy (AFM)-based nanoindentation was performed on the superficial zone of cartilage using a borosilicate colloidal spherical tip ($R \approx 5$ μm, nominal spring constant $k \approx 7.4$ N/m, AIO-TL tip C) and a Dimension Icon AFM with indentation depth of ~ 1 μm at 1 μm/s and 10 μm/s rates. Effective indentation modulus, E^{ind} (MPa), was calculated from the loading portion of indentation force-depth curves using the Hertz model. *Primary chondrocytes-* Primary epiphyseal chondrocytes were obtained from mouse newborn pups by enzymatic digestions. Cells were cultured in chondrogenic medium (DMEM/F12 medium with 5% fetal bovine serum, 50 μg/ml of L-ascorbic acid, and 1% glutamine). *Statistics-* Data are expressed as means±SEM and analyzed by unpaired, two-tailed Student's t-test.

Results

To delineate the function of cartilage EGFR signaling, qRT-PCR and IHC were first performed with articular cartilage in 2-month-old mice. We observed strong EGFR expression in all mice. p-EGFR and p-Erk were only detected in *WT* and *Wa5* mice but not in *CKO* mice, confirming that *CKO* mice were deficient in EGFR activity in articular cartilage. The most abundant EGFR ligand in cartilage is TGFα followed by epiregulin and betacellulin. While there was no histological changes in articular cartilage among *WT, Wa5*, and *CKO* mice at this developmental stage (Fig. 1A), cartilage surface E^{ind} was significantly decreased in *Wa5* mice (41.3% of *WT*) and further diminished in *CKO* mice (25.3% of *WT*) (Fig. 1B). Four months later, while the nanomechanical property remained low in *Wa5* and *CKO* mice, *CKO* mice had early OA symptoms, including partially depleted uncalcified zone, decreased Safarin O staining, and increased chondrocyte hypertrophy (Fig. 1). Wa5 cartilage had similar alterations but to a much lesser extent. Osteophytes in knee joints were observed in 66.7% of CKO mice but never in WT or Wa5. Primary chondrocyte culture confirmed that activating EGFR stimulates cell proliferation and survival and inhibits their terminal differentiation. DMM was performed in 3-month-old mice to induce OA. Three months later, *WT* developed mild-to-moderate OA (Makin score: 6.0 ± 0.5), *Wa5* exhibited advanced OA (11.5 ± 0.5), and *CKO* had the most severe OA (14.0 ± 0.0) with a complete loss of entire cartilage layer restricted at the medial side (Fig. 2A). Moreover, we observed a local and drastic

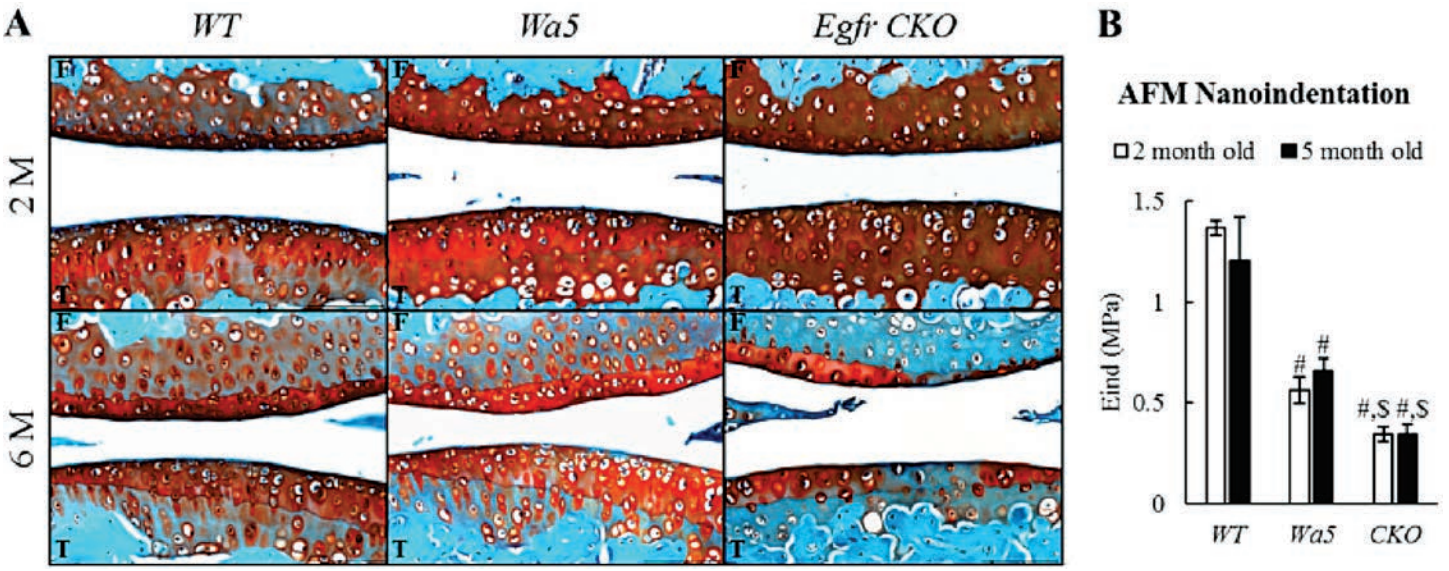

Figure 1. EGFR signaling is important for maintaining articular cartilage structure and mechanical strength. (A) Safranin O staining of knee joints in 2 and 6 month-old mice. F: femur; T: tibia. n = 6/genotype/time point. (B) Nanoindentation shows that EGFR deficiency decreases the stiffness of articular cartilage. n = 5/genotype/time point. #: $p < 0.05$ vs *WT*; $: $p < 0.05$ vs *Wa5*.

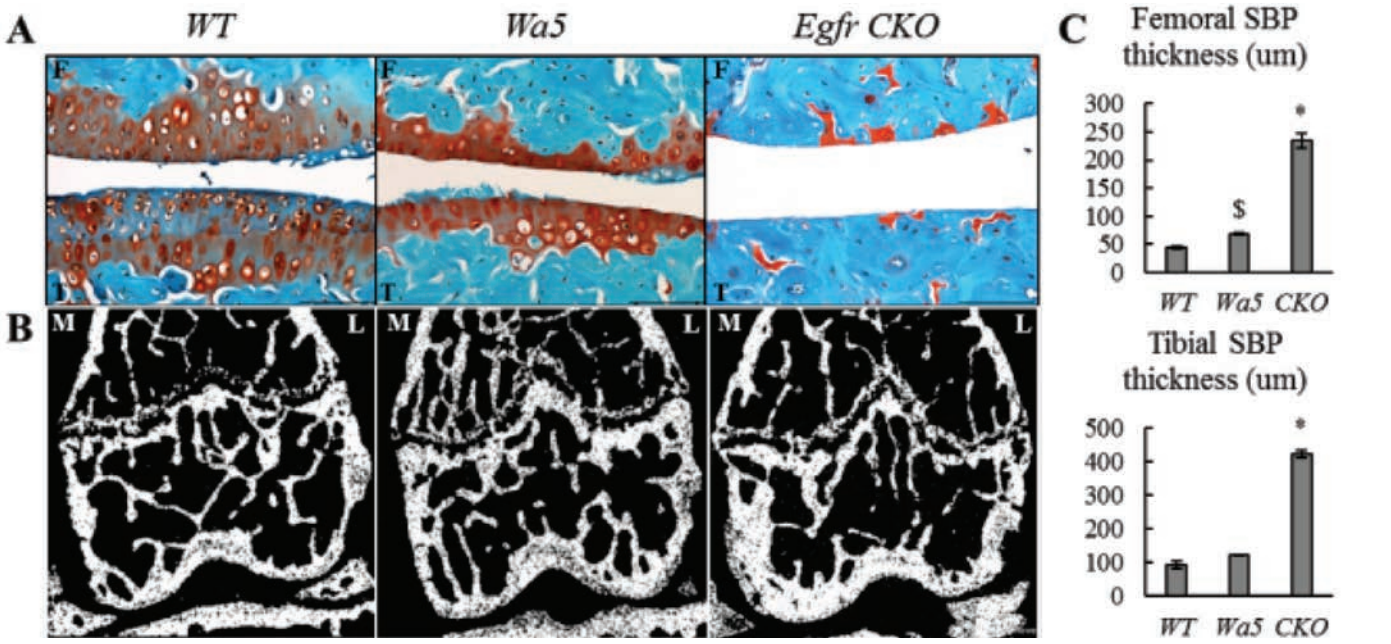

Figure 2. EGFR chondrogenic deficiency causes severe osteoarthritis after DMM. (A) Safranin O staining of knee joints in mice at 3 months post DMM surgery. (B) μCT images reveal local SBP thickening only at the medial site of *CKO* mouse joint. M: Medial; L: Lateral. (C) Quantification of SBP thickness. n = 6/genotype. $: $p < 0.01$; *: $p < 0.001$ vs *WT*.

subchondral bone plate (SBP) thickening (4.5-fold) only under the cartilage damage area (Fig. 2B, C). This was correlated with locally reduced sclerostin amount by osteocytes within SBP (Fig. 3) and increased number of osteoblasts lining SBP surface. Since subchondral and metaphyseal trabecular bone parameters were not altered, we reason that SBP sclerosis was caused by increased mechanical loading and decreased sclerostin amount after cartilage depletion.

Discussion

Our studies demonstrate that chondrogenic EGFR signaling and its cognate ligands, most likely TGFα, are essential for maintaining articular cartilage and are critical for OA development. *Egfr CKO* mice have much weaker articular cartilage and develop spontaneous OA at a much earlier stage compared to *WT*. We also demonstrate that *CKO* mice exhibit much more accelerated OA symptoms in a surgical OA

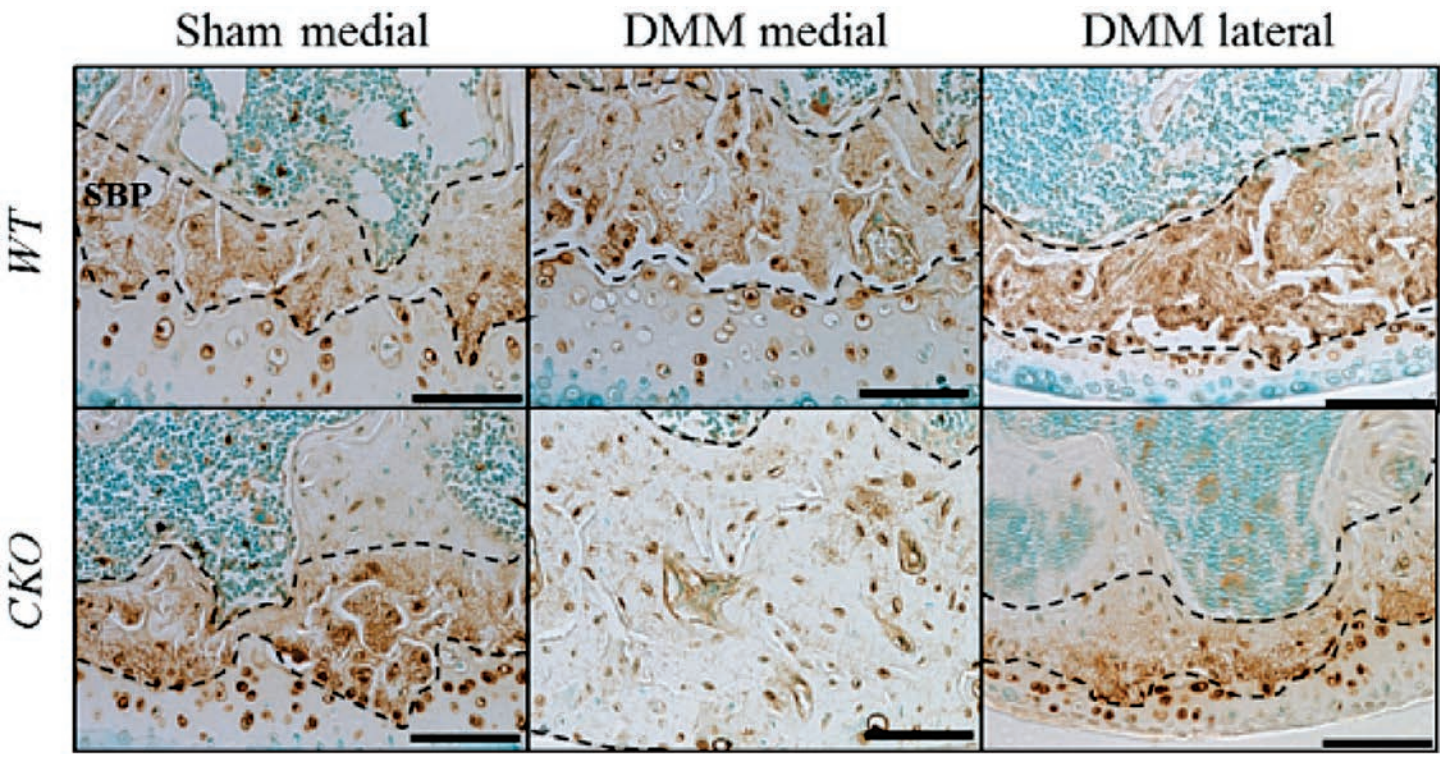

Figure 3. Increased bone formation in SBP in *CKO* mice is accompanied by decreased sclerostin amount. Scale < 100um.

model. The limitation of this study is that articular cartilage of *CKO* mice was already abnormal before surgery. Further investigation using an inducible system (*aggrecan-CreER*) to diminish EGFR activity at the same time of DMM is currently underway to delineate more precisely the role of EGFR in OA development.

Conclusion

We provide the first direct evidence that chondrogenic EGFR signaling is critical for articular cartilage homeostasis and OA development and that local crosstalk between cartilage and SBP plays an important role in accelerating OA progression.

References

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