Targeting Cartilage EGFR Pathway for Osteoarthritis Treatment

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Disclosures

A.T. is a founder of and owns equity in AlphaThera, Inc.

Introduction

Osteoarthritis (OA) is a widespread chronic joint disease characterized by cartilage degeneration. We previously discovered that EGFR signaling is critical for maintaining the superficial layer of articular cartilage and found that mice with cartilage-specific (*Col2-Cre*) EGFR deficiency develop spontaneous OA[1]. Here, we designed a two-pronged approach to investigate the effects of positively targeting the EGFR pathway on articular cartilage. First, we genetically enhanced EGFR activity by adopting a *Rosa-DTR* model. Originally identified as a receptor for bacterial diphtheria toxin (DT), DTR was later discovered to be human full-length HBEGF[2], a ligand for EGFR. Thus, it allows us to study the effect of cartilage-specific EGFR over-activation on OA progression. Second, we synthesized and characterized nanoparticles (NPs) conjugated with TGFα, another EGFR ligand, and tested their therapeutic efficacy in OA mice.

Methods

Animals

All animal work was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. *Col2-Cre Rosa-DTR* (*HBEGF Over^{Col2}*) and *Aggrecan-CreER Rosa-DTR* (*HBEGF Over^{AgcER}*) mice, and their *WT* (*DTR* or *Cre* only) siblings were generated. *HBEGF Over^{AgcER}* mice and *WT* received Tamoxifen (Tam, 75 mg/kg/day) injections for 5 days before surgery. Male mice at 3 months of age were subjected to destabilization of medial meniscus (DMM) or sham surgery at right knee. For treatment, *WT* mice received 10 µl of PBS, TGFα-DBCO (10 µM TGFα content), Ctrl-NP (no TGFα) and TGFα-NPs (10 µM TGFα content) intra-articularly once every 3 weeks starting from right after DMM surgery for 3 months.

TGFα-NP Synthesis

Bacteria-expressed human TGFα were labeled at the C-terminus with a constrained alkyne, dibenzocyclooctyne (DBCO), via sortase-tag expressed protein ligation (STEPL). TGFα-NPs were then prepared via copper-free click chemistry, by mixing TGFα-DBCO with azide-functionalized NPs made from 55mol% poly(ethylene glycol)-polycaprolactone (PEG-PCL)/20mol% poly(L-lysine-block-poly(α-caprolactone) (PLL-PCL)/25mol% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[azido(polyethylene glycol)-5000] (DSPE-PEG5K-N3) using the film hydration method.

Histology

Knee joints were processed for paraffin sections followed by HE, Safranin-O/fast green (SO/FG), p-EGFR, Ki67, TUNEL, and PRG4 staining.

µCT

Femurs were scanned from the epiphyseal end at a 6-µm resolution by µCT 35. The 3D images of the femoral distal end were reconstructed to generate a 3-D color map of thickness for the entire subchondral bone plate (SBP).

Cell Culture

Chondroprogenitors were harvested from articular cartilage of 5-month-old mouse knee joints by enzymatic digestion. Cells were then used for Western blots and CFU-F assays.

Statistics

Data are expressed as means±SEM and analyzed by one- or two-way ANOVA and unpaired, two-tailed Student's t-test.

Results

HBEGF Over^{Col2} mice displayed normal knee joints. No gross abnormality was detected. Long bone structure, including subchondral trabecular bone, subchondral bone plate (SBP), and metaphyseal trabecular bone, was also not affected. The most obvious change was cartilage. *HBEGF Over^{Col2}* mice displayed expanded growth plate and articular cartilage at

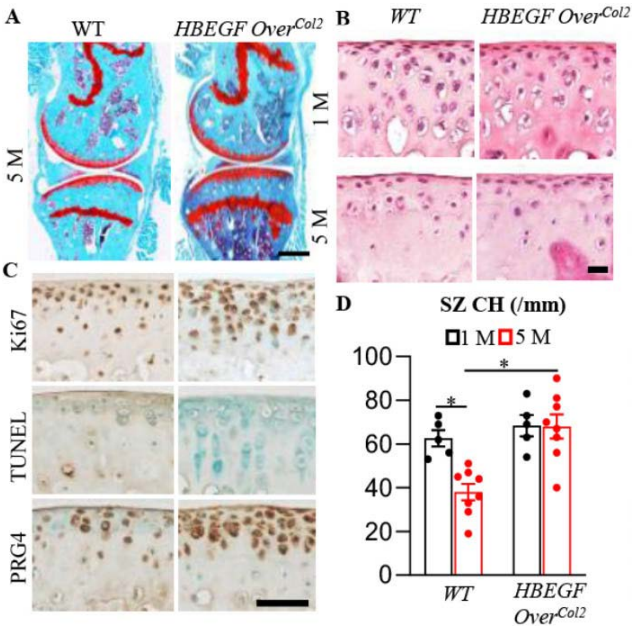

Figure 1. HBEGF overexpression in chondrocytes enlarges cartilage thickness and expands the chondroprogenitor pool. (A) SO/FG staining of *WT* and *HBEGF Over*^{Col2} knee joints at 5 months of age. Scale bar, 1 mm; **(B)** HE staining of *WT* and *HBEGF Over*^{Col2} knee joints at 1- and 5-month-old mice. Scale bar, 50 μm; **(C)** Ki67, TUNEL, and Prg4 staining in articular cartilage. Scale bar, 100 μm. **(D)** Quantification of superficial layer chondrocytes of (B). n = 8 mice/group. *: $p < 0.05$.

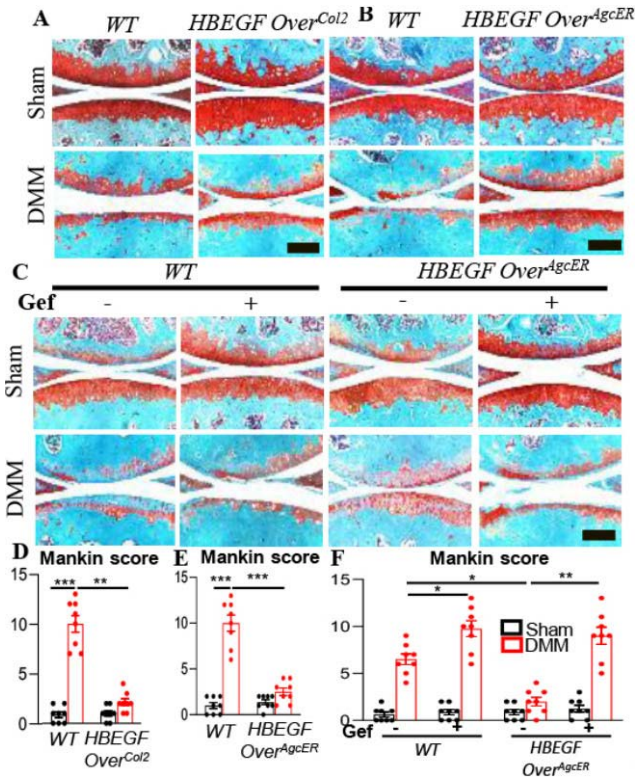

Figure 2. Cartilage-specific EGFR overactivation attenuates OA progression. A: *WT* and *HBEGF Over*^{Col2} joints at 4 months post sham or DMM surgery were stained with SO/FG. **B:** *WT* and *HBEGF Over*^{AgcER} mice received Tam injections right before DMM and their joints were harvest at 4 months post-surgery for SO/FG staining. **C:** *WT* and *HBEGF Over*^{AgcER} mice were subjected to sham or DMM surgery followed by vehicle or Gefinitib (100 mg/kg, once every other day) treatment for 3 months. Joints were harvested for histology analysis. Scale bars, 200 μm. **D:** The OA severity of (A) was measured by Mankin score, n = 8 mice/group. **E:** Mankin score of (B). n=8 mice/group. **F:** Mankin score of (C). n = 8 mice/group. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

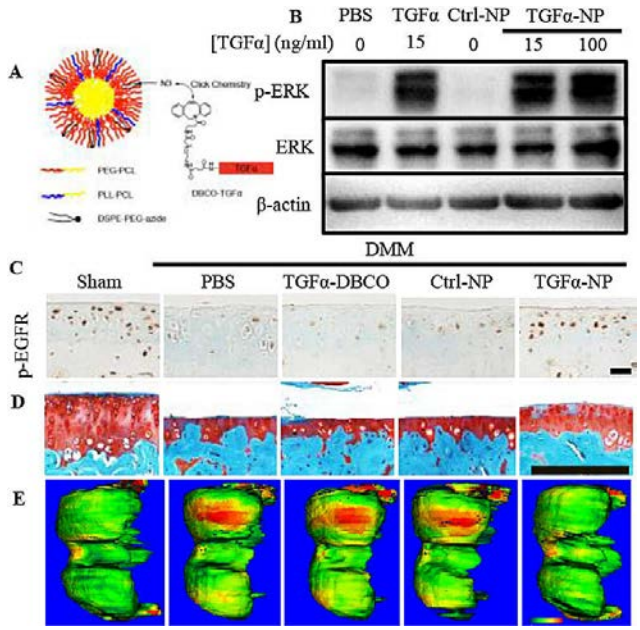

Figure 3. TGFα-NPs maintain EGFR signaling in chondrocyte and prevents cartilage damage after DMM surgery. A: Schematic diagram of TGFα-NPs. **B:** Western blots of EGFR activity (p-ERK) in primary chondrocytes with indicated treatment. **C:** EGFR activity (p-EGFR) staining in the articular cartilage of mice received intra-articular injections of PBS, free TGFα, Ctrl-NPs, and TGFα-NPs for 3 months after sham or DMM surgery. Scale bar, 100 μm. **D:** SO/FG staining. Scale bar, 200 μm. Mankin score: 0.63 ± 0.26 (Sham); 8.88 ± 0.74 (DMM PBS); 8.13 ± 0.81 (DMM TGFα-DBCO); 8.38 ± 0.82 (DMM Ctrl-NP); 3.25 ± 0.59 (DMM TGFα-NP). (TGFa-NP vs PBS: $p < 0.001$) **E:** Representative 3D color maps showing SBP thickness. Color ranges from 0 (blue) to 320 μm (red).

1 and 5 months of age (23.27% and 34.28% thicker than *WT* cartilage, respectively) (Fig. 1A). The superficial layer contains chondroprogenitors for articular cartilage. *HBEGF Over*^{Col2} articular cartilage had 1.79-fold more superficial chondrocytes (Fig. 1B, D) and formed 1.96-fold more CFU-F colonies than *WT* mice at 5 months of age, which was accompanied by enhanced Ki67 and Prg4 staining and reduced TUNEL staining (Fig. 1C). Interestingly, after DMM injury, articular cartilage degeneration was remarkably attenuated in *HBEGF Over*^{Col2} mice (Fig. 2A, D) and *Over*^{AgcER} mice (with Tam injections before the surgery, Fig. 2D, E). This cartilage protective action is mediated by EGFR signaling because it was completely abolished by co-treatment of EGFR inhibitor, Gefinitib (Fig. 2C, F). TGFα-NPs (Fig. 3A) were approximately spherical in shape with a hydrodynamic diameter of 25.93 nm. They activated EGFR signaling in primary chondrocytes as potent as free TGFα (Fig. 3B). Due to a positive charge, TGFα-NPs had superior cartilage uptake, penetration, and joint retention abilities compared to free TGFα. Strikingly, intra-articular delivery of TGFα-NPs effectively maintained EGFR activity (p-EGFR) in cartilage (Fig. 3C) and attenuated DMM-induced OA cartilage degeneration (Fig. 3D), SBP sclerosis (Fig 3E) and joint pain measured by von Frey assay. Free TGFα or NPs alone did not alter OA progression.

Discussion

Our study provides genetic evidence demonstrating that overactivation of EGFR signaling modestly thickens the

articular cartilage and completely blocks OA progression after DMM surgery. Other joint tissues, such as bone, synovium, and meniscus, as well as major vital organs, appeared normal in mice up to 12 months of age, suggesting that EGFR signaling could be precisely regulated in vivo to fulfill its anabolic actions without inciting catabolic, damaging effects. We also provided proof-of-principle evidence that administration of TGFα into mouse joints using an advanced nanoparticle delivery system is effective in preventing DMM-induced OA initiation and development.

Significance

Our studies uncover the critical role of EGFR signaling in cartilage homeostasis and demonstrate the feasibility of targeting EGFR signaling for OA treatment as a novel therapeutic approach using nanotechnology.

References

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