Targeting Cartilage EGFR Pathway for Osteoarthritis Treatment

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. 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, 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\textsuperscript{Col2}) and Aggrecan-CreER Rosa-DTR (HBEGF Over\textsuperscript{AgECER}) mice, and their WT (DTR or Cre only) siblings were generated. HBEGF Over\textsuperscript{AgECER} 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.

TGFC-NP Synthesis
Bacteria-expressed human TGFα were labeled at the C-terminus with a constrained alkyne, dibenzocyclooctyne (DBCO), via sortase-tag expressed protein ligation (STEP). TGFα-NPs were then prepared via copper-free click chemistry, by mixing TGFα-DBC0 with azide-functionalized NPs made from 55mol% poly(ethylene glycol)-polycaprolactone (PEG-PCL)/25mol% poly(L-lysine-block-poly(α-caprolactone) (PLL-PCL)/20mol% 1,2-dicarboxyl-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\textsuperscript{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\textsuperscript{Col2} mice displayed expanded growth plate and articular cartilage at...
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 OverCol2 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 OverCol2 mice (Fig. 2A, D) and OverAgcER 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). TGF\(_{\alpha}\)-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 TGF\(_{\alpha}\) (Fig. 3B). Due to a positive charge, TGF\(_{\alpha}\)-NPs had superior cartilage uptake, penetration, and joint retention abilities compared to free TGF\(_{\alpha}\). Strikingly, intra-articular delivery of TGF\(_{\alpha}\)-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 TGF\(_{\alpha}\) 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