Artificial receptors govern regenerative behaviors of engineered cells programmed to detect damaged cartilage

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INTRODUCTION: Osteoarthritis (OA) is a joint disease that affects over 32 million Americans, leading to pain and limited mobility [1]. Cartilage catabolism, which exposes a collagen II (CII)-rich extracellular matrix, is one biophysical hallmark of OA brought about by several factors including joint-resident inflammatory cytokines [1]. Current treatments are either palliative or involve surgical intervention; there are no clinically approved disease-modifying OA drugs. One approach towards bridging this gap is to use mesenchymal stromal cell (MSC) therapy for the delivery of biologic drugs, such as inflammatory antagonists and growth factors that promote neotissue synthesis. However, current cell therapy strategies do not offer feedback control over cell behaviors in response to their microenvironmental changes [2]. Without fine-tuning expression of these pleiotropic factors, they can negatively impact health when expressed for long durations or outside of pathologic target tissues. Synthetic biology tools have been developed to address these shortcomings by rationally assembling new biological systems that possess predictable and finely controlled properties. One such platform, the synthetic Notch (synNotch) platform, exploits the mechanism of juxtacrine transmembrane Notch receptors to regulate ligand-dependent transcriptional reprogramming [3]. By exchanging Notch's extracellular domain with an alternative recognition motif and the intracellular domain with a synthetic transcription factor, we can create synNotch receptors that produce user-defined sense/response behaviors. Here, we aimed to establish synNotch-programmed MSCs that conditionally respond to pathologic markers of OA progression, via targeting degraded cartilage, and exert therapeutic functions.

METHODS: We previously discovered a monoclonal antibody (mAbCII) that recognizes epitopes of CII exposed during cartilage matrix degradation [4]. By programming the recognition domain of synNotch with variable chains from mAbCII, we enable MSCs to directly probe for cartilage matrix degradation and, upon binding to CII, implement orthogonal responses based on this pathology-dependent biomarker assessment of cartilage status (**Fig. 1A**). Lentiviral vectors encoding the receptor and inducible payload transgenes were used to co-transduce mouse MSCs with gene circuit elements. For solid-phase CII experiments, 25 μg/ml CII was adsorbed to culture vessels overnight. The solution was then aspirated, and cells were plated for 72 hours to track transgene expression. Luciferase detection was performed using a BrightGlo assay. Protein concentrations were determined by performing ELISA. For primary cartilage experiments, *ex vivo* damaged porcine cartilage was seeded with mAbCII-synNotch MSCs, and synNotch-inducible mCherry was assessed by fluorescence microscopy. For trans-well assays, the lower chamber was populated with MSCs engineered to inducibly express transforming growth factor beta (TGFβ) or IL-1 receptor antagonist (IL-1Ra) on either untreated control surfaces or surfaces treated with 25 μg/ml CII for passive adsorption. ATDC5 mouse chondrocytes were plated in the upper chamber on a porous insert. In anti-inflammatory trans-well experiments, 0.1 ng/ml IL-1 was used to stimulate chondrocytes. Modulation of chondrocyte gene expression was assessed via qRT-PCR after one week and was normalized to a control group with no CII MSC activation and no IL-1 stimulation of chondrocytes. Statistical analysis was performed via two-way ANOVA (Fig. 1B and Fig 1F compared to control conditions) or Student's t-test.

RESULTS: mAbCII-synNotch MSCs selectively demonstrate robust (over 65-fold activation) on solid-phase CII. Cells engineered with a control, GFP-sensitive synNotch receptor do not activate (**Fig. 1B**) in response to CII. mAbCII-synNotch MSCs potently activate mCherry transgene expression when in contact with physically damaged (i.e., cryosectioned) CII-rich porcine cartilage explants; however, cells adjacent to the cartilage do not express high levels of the mCherry activation marker (**Fig. 1C)**, highlighting that the synNotch receptor enables activation only in the context of the arthritis-specific biomarker of cartilage damage. Engineered MSCs also achieve programmable secretion of anti-inflammatory IL-1Ra as well as pro-anabolic TGFβ **(Fig. 1D)**, demonstrating versatility in therapeutic transgene production. To assess pro-anabolic efficacy, activated TGFβ-expressing synNotch MSCs in a trans-well culture with chondrocytes showed significant upregulation of pro-anabolic genes *Sox9*, *Col2a1*, and *Acan* (**Fig. 1E**). In a similar trans-well culture, CII-induced expression of synNotch-driven IL-1Ra antagonized chondrocyte expression of IL-1-stimulated pro-inflammatory markers *Ccl5*, *Mmp13*, and *Il6* (**Fig. 1F**). All data plotted as mean of n=3 replicates with SEM. *p<0.05; **p<0.002; ***p<0.0002; ****p<0.0001.

DISCUSSION: This work demonstrates the first use of the synNotch platform for musculoskeletal engineering of MSCs. Programmed cells are capable of promoting OA-protective gene expression profiles in chondrocytes by both antagonizing inflammatory responses and promoting anabolic behaviors in chondrocyte co-cultures. Subsequent *in vitro* experiments will determine pro-restorative behaviors of engineered cells in a co-culture with primary human cartilage explants. The ability of engineered MSCs to activate in an *in vivo* murine mechanical loading PTOA model will also be assessed.

SIGNIFICANCE/CLINICAL RELEVANCE: No clinical DMOADs have been approved; using CII as an OA biomarker for synNotch transgene expression enables cells to be functional detectors of arthropathies and serve as living therapies. The regulation conferred by mAbCII-synNotch MSCs may circumvent off-target effects of biologic drugs by restricting expression to niches displaying direct signatures of OA pathology.

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IMAGES AND TABLES:

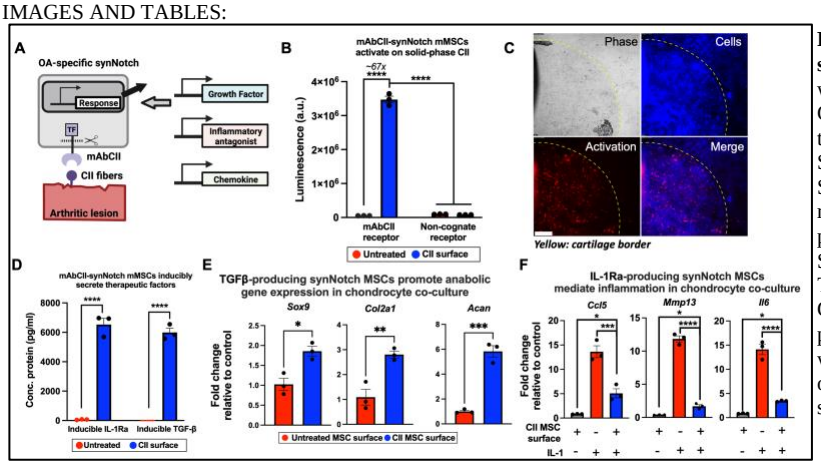

Figure 1: Development and validation of a mAbCII-synNotch cell-based therapeutic for OA. (A) SynNotch works by: 1) receptor recognition of exposed CII; 2) Cleavage at the transmembrane core; 3) release of a transcription factor that drives transgene expression. **(B)** SynNotch mMSCs activate ~67x on CII surface. (**C)** SynNotch cells (constitutive BFP) activate spatially restricted transgene expression (inducible mCherry) on physically damaged porcine cartilage samples (t=18 days). Scale = 500μm. **(D)** Anti-catabolic IL-1Ra and pro-anabolic TGFβ are selectively produced on solid-form CII. **(E-F)** Gene expression profiling of mouse chondrocytes reveals (E) pro-anabolic upregulation of chondrocytes when co-cultured with activated TGFβ-secreting MSCs and (F) antagonization of IL-1 stimulation when co-cultured with activated IL-1Ra-secreting MSCs.