A biotherapeutic circuit for the treatment of osteoarthritis

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Introduction: We describe a mRNA bio-therapeutic circuit platform capable of simultaneously producing ligand and restoring the minimum essential components of a natural signaling pathway in a tissue of interest. mRNA is uniquely suited for generation of these biotherapeutic circuits because it avoids risks of genomic integration and is temporally limited, which allows tissue homeostasis to return once the biotherapeutic circuit successfully treats the disease. To further demonstrate the versatility of this platform, we apply it to a disease with a significant lack of modern biotherapeutic options: osteoarthritis. OA is the most common musculoskeletal disease in the world, afflicting more that 220 million patients.[1] It is a progressive disease that manifests as the degeneration joints and cartilage. Clinically approved treatment options for OA are limited to pain and symptom management, with the exception of invasive surgical procedures, highlighting the fact that there are no highly-effective, non-surgical treatments for OA. An optimal treatment for OA would tackle the two primary drivers of degeneration: loss of GAG density and chondrocyte senescence and apoptosis.[2, 5] This requires a therapy capable of stimulating GAG deposition from surviving chondrocytes while simultaneous driving chondrogenesis and reversing the senescent phenotype. To address disease-state dependent dysregulation, we created an mRNA-based biotherapeutic circuit capable of simultaneously restoring the healthy signaling cascade and activating the pathway via production and secretion of the endogenous ligand (**Figure 1**). The OA circuit encodes IGF-1 and endogenous ligand that supports chondrogenesis, and PAPPA-2, a metalloproteinase responsible for cleaving IGFB-3, a protein upregulated in OA, responsible for sequestering IGF-1, preventing chondrogenesis.[5]

Methods: The proposed biotherapeutic circuit platform is based on the premise that an exogenous mRNA-expressed ligand and its receptor can auto-induce a natural signaling cascade. As visible in Figure 1, we have chosen to deliver different, yet necessary components of the therapeutic signaling cascade. Each component includes an endogenous signaling ligand, in addition to a component that has been demonstrated to be down-regulated in a disease-dependent state, exacerbating the disease itself and limiting the therapeutic potential of the ligand itself. To confirm the disease dependent overexpression of IGFBP-3 in OA, synovial fluid from patients with OA was harvested and IGFBP-3 was quantified via ELISA (Figure 2A-B). The biotherapeutic mRNAs were generated by first amplifying and then completing Gibson cloning to insert and each biomolecule into the relevant location, to be used for the production of mRNA via IVT. Both polycistronic constructs encoding both molecules and monocistronic mRNA combinations were assessed for efficacy. Biotherapeutic activity of the therapeutic mRNA of the circuit was assessed via stably expressing reporter cell lines, generated in HEK293T cells, using luminescent readouts related to cAMP production from the intact signaling circuit. In order to optimize the formulation of the LNPs, a screen of ionizable lipids used in LNP packaging was conducted and an optimized formulation was established. The IGF-1 and PAPPA-2 encoding therapeutics were assessed in primary human tissue, alongside 3D models of cartilage seeded with bovine chondrocytes and with and without the presence of intact IGFBP-3 to mimic OA conditions. GAG deposition was assessed via colorimetric assay and the determination of Young's modulus (in the 3D constructs). In vivo studies of IGF-1 and PAPPA-2 biotherapeutic delivery in OA mouse model (intra-articular injections) using optimized LNP formulations are ongoing.

Results: Disease dependent regulation of IGFBP-3 was demonstrated in primary human tissues and cell. Ligand expression of IGF-1 and PAPPA-2 mRNAs delivered via LNP were robust and sustained over at least 72 hours. Auto-activation of the anti-fibrotic circuit was detected as detected as measured via reduction of IGF-1 activity in an engineered cell line upon treatment with the RNA bio-circuit, similar to levels as when treated with recombinant protein. IGF-1 signaling was demonstrated to be abrogated upon the addition of IGFBP-3 (Figure 2C-D). IGF-1 signaling was significantly increased upon treatment the anti-osteoarthritic circuit. GAG concentration and Young's modulus was significantly increased in 3D cartilage models of bovine chondrocytes embedded in agarose.

Discussion: The addition of recombinant IGFBP-3 in addition to the demonstration of its upregulation in diseased tissues demonstrated a clear ability to abrogate the activity of IGF-1. The addition of PAPPA-2 has strong evidence to support its use in rescuing the signaling and chondrogenic effects of IGF-1, and its synergy as a potential dual therapeutic

Significance/Clinical relevance: Our work supports two important conclusions: 1) that osteoarthritis itself is responsible for abrogating the effects endogenous protein therapeutics are able to exert on their intended targets and tissues 2) that we can design robust biotherapeutic circuits, capable of successfully delivery mRNA to cells that can re-create the minimal necessary components of the system for therapeutic signaling and repair. This work demonstrates both a novel way of designing therapeutics, as well as a potential solution for treating OA.

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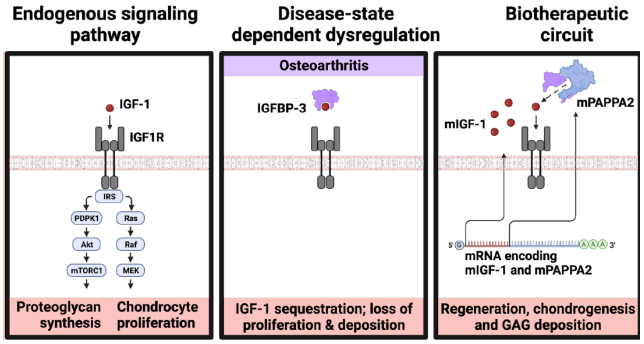

Figure 1: The signaling pathway of IGF-1 through IGF-1R (left). The upregulation of IGFBP-3 in osteoarthritis leads to sequestration of IGF-1, preventing IGF-1R activation (middle). A biotherapeutic circuit capable of simultaneously expressing mIGF-1 and mPAPPA2 to cleave IGFBP-3, increase synovial IGF-1 bioavailability, and restore cartilage health (right).

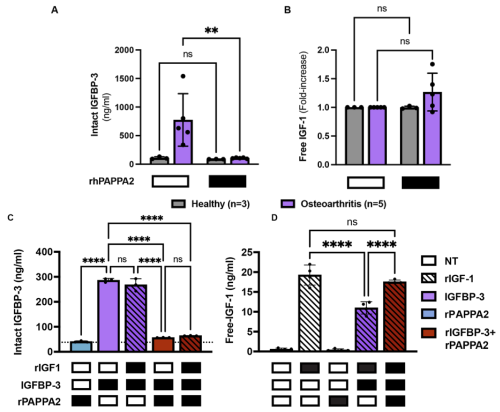

Figure 2: A) Intact IGFBP-3 and **B)** Free IGF-1 concentrations in synovial fluid from osteoarthritis patients (n=5), compared to synovial fluid from healthy cadaveric donors (n=3), before and after treatment with PAPPA2 from conditioned media. Concentration determined by sandwich ELISA.**C)** Concentration of intact IGFBP-3 before and after PAPPA2 treatment. **D)** Free IGF-1 concentrations with and without PAPPA2 after sequestration by IGFBP-3. PAPPA2 activity assays measured via sandwich ELISA.