Utilizing Synthetic Gene Circuits in Mouse Mesenchymal Stem Cell Derived Chondrocytes to Verify Cartilage Regeneration Outcomes In Vitro

Lauren Terry[1](Honors Capstone), Gurcharan Kaur[1], Rhima M. Coleman[1,2]

[1]Department of Biomedical Engineering, University of Michigan, Ann Arbor
[2]Department of Mechanical Engineering, University of Michigan, Ann Arbor

Introduction

- Articular cartilage, the specialized connective tissue at the end of long bones, is avascular, often causing injuries to progress into PTOA [1].
- Cartilage repair strategies using mesenchymal stem cells (MSCs) is hindered by our inability to regulate the phenotype of MSC-derived chondrocytes (MdChs) [2].
- After differentiation into chondrocytes, MdChs undergo hypertrophic maturation via endochondral ossification [3].
- The transcription factor RUNX2 mediates many of the phenotypic changes associated with MdCh hypertrophy, upregulating the expression of matrix degrading metalloproteinases (MMPs), resulting in degradation of accumulated cartilage extracellular matrix (ECM) [4-6].
- Previous work in our lab has shown that hypertrophy-specific suppression of RUNX2 in human MdChs can improve matrix accumulation, inhibit hypertrophy, and protect from inflammation-induced matrix catabolism [7].
- In order to move forward to animal studies using mouse models of PTOA, the efficacy of the circuit in mouse MSCs needs to be confirmed.
- We hypothesize that suppression of RUNX2 activity, via the engineered gene circuit used in vitro in human MdChs, will be equally successful in vitro in mouse MdChs.

Methods

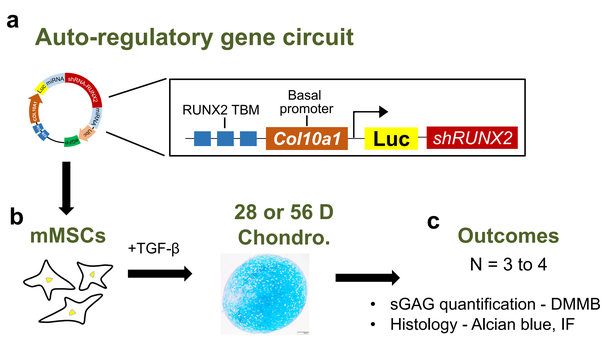
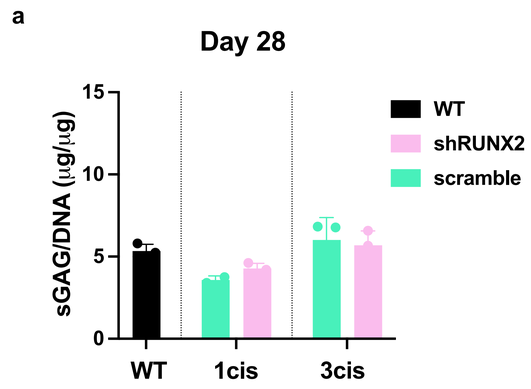


Fig 1. Experiment Design (a) Structure of gene circuit (b) Experimental methodology (c) Description of outcomes.

- Auto-regulatory gene circuits allowing varying levels of RUNX2 suppression were engineered. Mouse MSCs were modified with both low (20%) and high (70%) level RUNX2 suppressing gene circuit using lentiviruses.
- Wildtype (WT) mMSCs and mMSCs reprogrammed with gene circuits were placed in short term (28 days) or long term (56 days) chondrogenic culture
- Sulfated glycosaminoglycan levels (sGAG) were quantified using dimethylmethylene blue assay and visualized using Alcian Blue staining. RUNX2 protein expression analyzed using immunofluorescence (IF)

Results



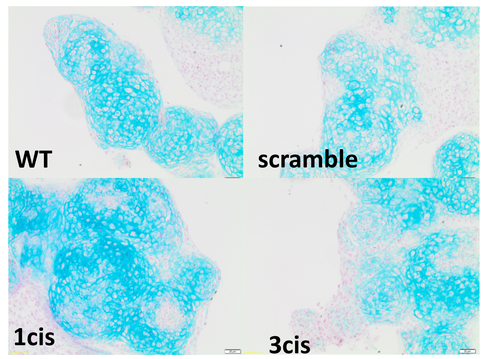
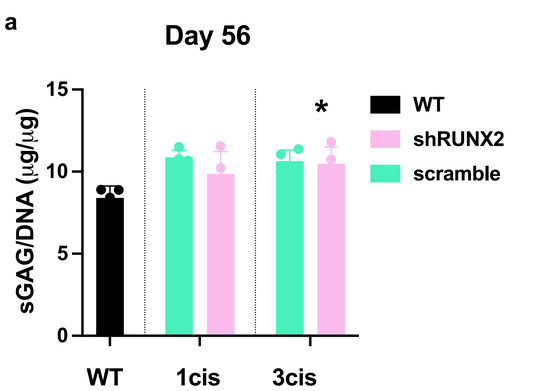


Fig 2. Quantification of sulfated glycosaminoglycan (sGAG) levels at day 28. (a) Dimethylmethylene blue assay results for WT, scramble (1cis & 3cis), and shRUNX2 (1cis & 3cis) mMdCh pellets. (b) Alcian blue staining for WT, scramble (1cis & 3cis), and shRUNX2 (1cis & 3cis) mMdCh pellets, scale bar: 100 μm.



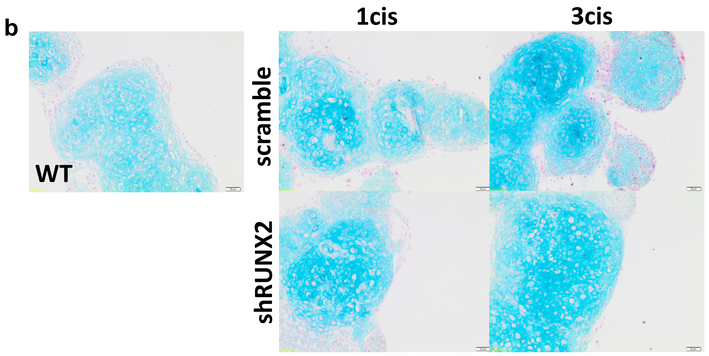


Fig 3. Quantification of sulfated glycosaminoglycan (sGAG) levels at day 56. (a) Dimethylmethylene blue assay results for WT, scramble (1cis & 3cis), and shRUNX2 (1cis & 3cis) mMdCh pellets. (b) Alcian blue staining for WT, scramble (1cis & 3cis), and shRUNX2 (1cis & 3cis) mMdCh pellets, scale bar: 100 μm.

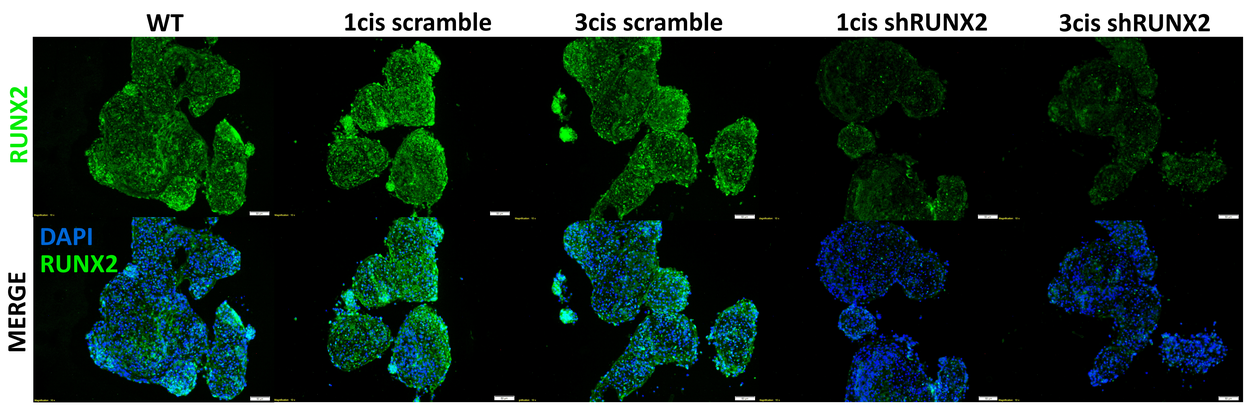


Fig 4. Successful suppression of RUNX2 activity in shRUNX2 modified mouse MdCh pellets. Immunofluorescence staining of RUNX2 (green) in mMdCh pellets. Merge images are superimposed RUNX2 (green) & nuclear stain DAPI (blue), scale bar: 50μm.

Results

- shRUNX2 and scramble gene circuit modified mMSCs successfully underwent chondrogenesis and produced comparable sGAG amounts to the WT in short-term culture by day 28 **(Fig 2a & 2b).**
- Increase in sGAG accumulation by WT, scramble and shRUNX2 modified mMdChs was observed from D28 to D56 **(Fig 2a & 3a).**
- 3cis shRUNX2 modified mMdChs produced a significantly higher amount of sGAG compared to the WT in a long-term culture **(Fig 3a).**
- 3cis shRUNX2 modified mMdChs exhibited more intense sGAG staining compared to WT in long-term chondrogenic culture by day 56 **(Fig 3b).**
- Immunofluorescence staining for RUNX2 protein expression demonstrated successful suppression of RUNX2 by both the 1cis and 3cis shRUNX2 gene circuits compared to WT and scramble controls **(Fig 4).**

Discussion & Conclusions

- In this study we confirmed successful suppression of RUNX2 expression in mouse MSCs in vitro.
- Short-term (D28) culture demonstrated that modifying the mMSCs with the RUNX2 suppressing gene circuit did not negatively impact chondrogenesis and matrix accrual.
- Long-term (D56) culture showed that modifying mMSCs with the RUNX2 suppressing gene circuit increased sGAG accumulation, indicating increase in the stability of mMdChs chondrogenic phenotype.
- The shRUNX2 gene circuit successfully suppresses RUNX2 in mMdChs without compromising chondrogenesis, therefore mMSCs can be used to test the efficacy of shRUNX2 gene circuit in mouse models of PTOA.

References

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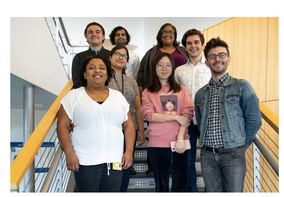
Acknowledgements

1R21AR07401
1P41EB021911 101
OREF Soft Tissue Repair And Regeneration Sports Medicine Grant In Honor Of Russell F. Warren, MD



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