Mesenchymal Stem Cells Modified with the Developmental Gene hMT1-MMP Improves Regeneration of the Tendon-Bone Insertion Site.

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INTRODUCTION:
The native tendon-bone insertion site is not recapitulated following rotator cuff repair surgery. Instead, scar tissue forms that has mechanical properties that are weaker than normal tissue and make repairs prone to failure. Studies have shown that the addition of mesenchymal stem cells (MSCs) can improve tendon healing in a bone tunnel (1,2). However, recent work in our laboratory has shown that these results cannot be extrapolated to rotator cuff tendon repair. MT1-MMP is a gene that is up-regulated during embryogenesis in areas that develop into tendon-bone insertion sites. Though its mechanism of action is unknown, its involvement in calcified cartilage formation suggests a role in formation of the enthesis. The purpose of this study was to determine if the application of Lewis MSCs transduced with Ad-hMT1-MMP can help regenerate a native tendon-bone insertion site in a rat rotator cuff repair model. Our hypothesis was that pluripotent stem cells in the presence of the developmental signal from MT1-MMP will drive the healing process towards regeneration and away from scar formation.

METHODS:
A total of 60 animals underwent the surgical procedure and an additional 10 were used for MSC harvest from bone marrow. The surgical procedure involved unilateral detachment and repair of the supraspinatus tendon. Animals were randomized into two groups (30 animals/group). The experimental group received 10⁶ bone marrow-derived MSCs in a fibrin sealant that had been transduced in vitro with Ad-hMT1-MMP. The second group received untransduced cells in the fibrin sealant carrier and served as control. Fifteen animals in each group were sacrificed at two and four weeks. At each time point, twelve animals were allocated for biomechanical testing, and three were allocated for histomorphometric analysis.

Ad-MT1-MMP Construction and Evaluation of Gene Expression: Ad-MT1-MMP was constructed using the AdEasy kit according to the manufacturer’s protocol (Stratagene, Inc) with cDNA from human MT1-MMP (Origene, Inc). In vitro gene expression was confirmed with rtPCR with the following primers: human MT1-MMP forward 5’-GCAGAAGTTTTTACGCGCTGC-3’; human MT1-MMP reverse 5’-TACCCGTCCTCTCAGAACAAT-3’; rat GAPDH forward 5’-TGCCACTCAGAAGACTTGCGG3’; and rat GAPDH reverse 5’-GGATGCAGGGATGATGTTCT-3’.

MSC Preparation and Transduction: Ten Lewis rats were euthanized and the bone marrow of their long bones was harvested by lavage with DMEM. Cells were cultured in DMEM/10%FBS/1%ABAM. They were passaged at 80% confluence a maximum of 3 times. For the Ad-MT1-MMP group, MSCs were transduced 24hrs prior to implantation at 10⁴pfu/s. On the day of surgery, 10⁶ cells were suspended in 50µl of Tissue Fibrin Sealant (Baxter, Inc) and added to the tendon-bone repair site (6,7). Animals in the MSC control group received the same number of cells in fibrin sealant.

Histomorphometric Analysis: The amount of new cartilage formation was determined by measuring the area of metachromasia on safranin-O stained slides using ImageJ (N.I.H.). The extent of collagen fiber organization was determined by the level of brightness (Gray Scale) under polarized light microscopy of picrosirius red stained slides, again with ImageJ.

Biomechanical Testing: Ultimate load-to-failure, the cross-sectional area, and the calculated stress required for failure were all determined using our established testing protocol(4).

Statistical Analysis: Statistical analysis was performed with Wilcoxon rank-sum test with significance set at p<0.05. A pre-study power analysis was performed for a primary outcome of ultimate load-to-failure.

RESULTS:
Gene Expression: Quantitative real-time PCR confirmed that the in vitro MT1-MMP gene expression was 223-fold greater in the transduced MSCs when compared to the untransduced MSCs.

Histomorphometric Analysis: At 4 weeks, there was more new cartilage formation as evidenced by the area of metachromasia in the Ad-MT1-MMP group as compared to the MSC group. There was no difference in collagen organization between the Ad-MT1-MMP group and the MSC group at either timepoint, and there were no differences in any outcome variable at 2 weeks (Data below).

Biomechanical Testing
At 4 weeks, the ultimate load-to-failure was greater in the Ad-MT1-MMP group as compared to MSC group (Data below).

DISCUSSION:
Mesenchymal stem cells genetically modified to overexpress the developmental gene MT1-MMP can help regenerate the tendon-bone insertion site based on the amount of new fibrocartilage formation at 4 weeks. Furthermore, MT1-MMP transduced cells also improved the ultimate load-to-failure when compared to specimens treated with untransduced MSCs at 4 weeks as well. This study introduces a novel paradigm for the augmentation of rotator cuff repair. By recreating some of the molecular events that occur during embryogenesis, we were able to improve healing in a small animal model. The mechanism(s) for these findings is not clear and warrant further investigation. Further studies are also needed to determine efficacy of this technique in larger animal models.

REFERENCES:

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