INTRODUCTION: Myogenic cell (muscle derived stem cell [MDSC], satellite cell or myoblast) transplantation is an approach that can introduce the normal dystrophin gene into the diseased muscle of Duchenne Muscular Dystrophy (DMD) patients. However, the transplanted myogenic cells usually do not migrate well within the muscle, and thus require many injections to enable graft success. Developing techniques that can promote and enhance myoblast transplantation is therefore of significant clinical value. Matrix metalloproteinase type 1 (MMP1), a naturally occurring collagen-digesting enzyme, can eliminate the existing fibrous scarring in different tissues including skeletal muscle. Additionally, MMP1 has been shown to enhance cell migration. Although MMP1 can degrade collagen, it also undergoes self-degradation resulting in a short biological half-life; therefore, fibrous scar tissue has the potential to reform following MMP1 degradation. Extending the function of MMP1 to control fibrosis back is necessary. The current experiment investigates whether MMP1 gene therapy can prolong MMP1 presence and increase myogenic cell migration through either local injection or systemic delivery, and enhance muscle cell fusion and regeneration, thereby improving myogenic cell transplantation in dystrophic skeletal muscle.

METHODS and RESULTS: 1. MMP-1 gene transduction stimulated myoblasts to increase migration. MMP1 gene transferred C2C12 cells and control C2C12 cells were cultured in medium containing Dulbecco’s Modified Eagle’s Medium (DMEM, Invitrogen, Carlsbad, CA), 10% fetal bovine serum, 10% horse serum, 0.5% chicken embryo extract, and 1% penicillin/streptomycin (P/S) at 37°C in a 5% CO2 atmosphere in 12 well plates. Cells were then placed in serum-free DMEM supplemented with 1% P/S alone. An artificial wound was created by disrupting the monolayer with a sterile plastic pipette tip. Cells were incubated for 6, 12, and 24 hours to allow for migration back into the wound area. Cells were then fixed in cold methanol, washed with PBS, and then stained with DAPI to help visualize migration distance. We observed that MMP-1 gene transduction (bottom row, Fig. 1) promoted C2C12 cell migration into an artificially created wound faster than control C2C12 (top row, Fig. 1).

2. Gene transfer of MMP1 enhances myoblast differentiation in vitro. We investigated whether MMP1 gene transduction has any effect on myogenic cell differentiation. MMP1 cloned myoblasts (C2C12), MDSCs, and their control non-gene-transferred cells were separately plated into 12-well plates and cultured with differentiation medium (2% H.S.) for up to 5 days. Results indicate that the MMP1 gene-transferred myogenic cells resulted in increased numbers of fused myotubes compared to control cells (Fig. 2). Myoblasts (C2C12) (Fig. 2A) and MDSCs (Fig. 2B) fused into myotubes in differentiation medium. At their usual levels, however, both MMP1 gene-transferred myoblasts (Fig. 2C, E) and MDSCs (Fig. 2D, F) increased their differentiation capacity (*P<0.05).

3. MMP1 gene therapy improves myoblast transplantation. To investigate whether these MMP1-expressing cells are able to produce a better muscle graft in the skeletal muscle of MDX/SCID (a dystrophic/immunodeficient mouse model) mice, we used a retrovirus vector to transfer the LacZ marker gene into genetically altered C2C12 cells that expressed MMP1 as well as control C2C12 cells that did not express MMP1. These cells were then separately transplanted into the skeletal muscle of MDX/SCID mice. Histological analysis was performed after cell transplantation, whereupon we detected both LacZ-positive and dystrophin-positive (brown; see asterisks; Fig. 3A-D) myoblasts in the host muscle at 2 weeks (Fig. 3A, B) and 4 weeks (Fig. 3C, D) after cell transplantation. However, we discovered that the MMP1-expressing C2C12 fused/differentiated into better muscle grafts (Fig. 3B, D) after transplantation into the skeletal muscle of MDX mice than the control C2C12-transplanted groups (Fig. 3A, C) at both 2 and 4 weeks after cell transplantation.

DISCUSSION: The limitations in myogenic cell transplantation for Duchenne Muscular Dystrophy (DMD) are quick death and poor migration of donor cells. Current treatment involves numerous injections, which not only result in pain and discomfort, but also the development of additional scar tissue. Therefore, the development of a novel therapeutic approach to enhance the migratory properties of the transplanted cells will help to significantly reduce the number of injection sites, promote widespread cell fusion, and improve muscle healing. MMP1 is an ideal collagen-digesting enzyme that can eliminate existing fibrous scar in different tissues including skeletal muscle tissues. However, its short biological half-life induces unpredictable fibrosis recovery limiting this application. Extending the function or prolonging lifetime of the MMP1 enzyme by gene therapy or other methods is essential. Current experiments use retrovirus vectors to transfer the MMP1 gene into myoblasts (C2C12) prior to transplantation into skeletal muscle of MDX mice. Our results demonstrated that MMP1 gene expression enhanced myoblast migration and fusion in the skeletal muscle of MDX mice in vivo. More importantly, our study has piloted investigations by using gene therapy to enhance systemic (blood stream) delivery of myogenic cells in the dystrophic muscle disease model of MDX mice and achieved intriguing results. Our overall goal is to identify a novel technique via gene therapy combining local and/or blood stream (systemic) injection that will stimulate transplanted cell migration in vivo, thereby improving dystrophin gene transfer into the dystrophic muscle fibers. We expect that this technical approach would increase the clinical applicability of myogenic transplantation therapy in injured and diseased skeletal muscles.

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