Abstract
Background: Over the past decade different stem cell (SC) based approaches were tested to treat Duchenne Muscular Dystrophy (DMD), a lethal X-linked disorder caused by mutations in dystrophin gene. Despite continued research efforts, there is no curative therapy for DMD. Allogeneic SC therapies aim to restore dystrophin in the affected muscles; however, they are challenged by immune rejection and limited engraftment. Thus, there is a need to develop new more efficacious SC therapies. Chimeric cells, created via ex vivo fusion of donor and recipient cells, represent a promising therapeutic option for tissue regeneration. Previously we have shown that by fusing normal myoblasts (dystrophin expressing) with the recipient’s myoblasts (no dystrophin expression) the immune system was bypassed. We have also shown that local delivery strengthens the gastrocnemius muscle for at least 90 days after injection.

Objectives: Investigate the ability of fused myoblasts to systemically engraft into and functionally benefit mdx mice.

Approach: We performed experiments using chemically fused human myoblasts. Mixed cell populations were obtained from a normal donor and a DMD patient by mixing normal myoblasts and DMD myoblasts at different ratios. After fusion, the mixtures were injected into mdx/scid mice. Efficacy of myoblast fusion was confirmed by flow cytometry and dystrophin immunostaining, while proliferative and myogenic differentiation capacity of chimeric cells were assessed in vitro. The mice were assessed 90 days after injection for dystrophin expression by immunofluorescence and western blot, and for dystrophin expression in muscle, diaphragm, and heart tissues by immunohistochemistry.

Results: Interosseous delivery of chimeric cells did increase multiple functional parameters 90 days after injection. Histology identified the presence of chimeric cells in multiple muscle tissues. The recipient mouse demonstrated increased cardiac function by echocardiography. Plethysmography also demonstrated significant benefits for the recipient mouse.

Conclusions: Our study confirmed feasibility and efficacy of dystrophin expressing chimeric cell therapy and represents a novel satellite cell based approach for treatment of MD.

Results

Figure 1. Dystrophin is expressed in gastrocnemius muscle after muscle direct injection of myoblasts cells. The fused cells engraft better and are most beneficial to muscle function. (N=6).

Figure 2. Dystrophin is expressed in hemorrhages, maybe diaphragm and possibly cardiac tissues by Wb. 60ug of protein/lane. Mandys antibody, Sigma.

Figure 3. Dystrophin is expressed in cardiac muscle tissue after interosseous DEC transplantation. Immunofluorescence with primary dystrophin antibody from Alcamb (ab15277, green) and DAPI (blue) counterstain. Magnification: 25x; Zeiss LSM 710 confocal microscope.

Figure 4. Dystrophin is expressed in hamstrings, maybe diaphragm and possibly cardiac tissues by Wb. 60ug of protein/lane. Mandys antibody, Sigma.

Figure 5. Reduced cardiac disease by echocardiography at 0, 30d and 90d post-transplant (Veo2100).

Figure 6. Reduced mdx-mediated respiratory disease assessed by plethysmography at 90d post transplant. Buxco Instrument (N=6).

Methods:

Seven different cell populations were analyzed after interosseous injections.

1. Vehicle
2. Myoblast normal mixed with myoblast DMD; 1 mill cells (MBN/MBDMD 1M)
3. Myoblast normal mixed with myoblast DMD; 500,000 cells (MBN/MBDMD)
4. Myoblast normal 1 mixed with normal 2; 500,000 cells (MBN1+MBN2)
5. Myoblast normal 1 mixed with dystrophin expressing DMD cell; 1 mill cells (MBN/MBDMD 1M)
6. Myoblast normal fused with myoblast DMD; 500,000 cells (MBN/MBDMD)
7. Myoblast normal fused with myoblast DMD; 1 mill cells (MBN/MBDMD 1M)
8. Vehicle

All cells were obtained from human normal or DMD patients (Lonza (Mapleton, UK), Lonza (Switzerland), Stem Cell Technologies (Canada)).

Figure 7. Dystrophin is detected in diaphragm, heart and skeletal tissues by immunohistochemistry.

Figure 8. Reduced skeletal muscle disease assessed by improved ex vivo muscle force, reduced fatigue and increased muscle weight (N=3, except MBN1+MBN2 N=2).