Myogenic Differentiation of Magnetically Labeled Mesenchymal Stem Cells

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Target Audience: Skeletal muscle physiologist

Introduction

Mesenchymal stem cells (MSCs) have great importance in the fields of regenerative medicine and immunology. Magnetic cell labeling of MSCs for the purpose of MRI based cell tracking has been widely performed. It has previously been established that the capacity of MSCs to differentiate down osteogenic, chondrogenic, and adipogenic lineages is largely unaffected by magnetic cell labeling[1]. A few papers have detailed the effect of magnetic labeling on muscle progenitor cell differentiation following labeling, but to our knowledge[2], there are no reports on whether MSCs maintain the capability to differentiate into muscle cells with magnetic particles. This verification is crucial for using MRI-based cell tracking of MSCs in skeletal muscle repair in regenerative medicine. Therefore, the purpose of this study was to investigate the ability of MSCs to differentiate into myogenic cells with varying magnetic labeling conditions.

Methods

MSCs were incubated in 6 well plates for 6 weeks with either control media (DMEM, 10% FBS, and 1% antibiotics) or muscle media (Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 10% heat-inactivated horse serum, 50uM hydrocortisone, and 1% antibiotics). 1.63 micro-sized iron oxide (MPIO) particles were introduced at week 0, 4, and 6 at high (33ul/10^6 cells) or low (13ul/10^6 cells) concentrations for 48 hours. Cells were plated at 10^6 cells/well to insure confluence prior to the addition of the MPIOs. After 48 hours, cells were passaged, a process that was continued over the duration of the 6 weeks as the cells continued to expand even with the muscle differentiation media. This eventually caused the particles in the week 0 and week 4 treatment groups to become dilute as the cells continually divided. Morpholocical differentiation was confirmed through optical fluorescent staining techniques of fast skeletal myosin. Cells were fixed and permeabolized allowing the primary and secondary antibodies to bind. Cells were then imaged under a fluorescent microscope to show the uptake in the myogenic cells in comparison to the control MSCs. Transmission electron microscopy (TEM) was used to confirm particle uptake within the cells. Since images only confirm uptake of particles and morphological changes, polymerase chain reaction (PCR) will be used in order to further confirm differentiation in comparison to control MSC cells and unlabeled myogenic cells using MyoD1, myogenin, myosin heavy chain (MHC)-β, IIA MHC, IIX MHC, and β-actin.

Results

Fluorescent images (Figure 1) show the uptake of the fast skeletal myosin in the differentiated myogenic cells in comparison to the MSCs. The morphological changes toward the myogenic lineage show elongation of cells in comparison to the standard fibrotic morphology seen in the MSCs. TEM images show the uptake of the particles, with the highest concentrations of particles at week 6, both low and high concentrations (images shown later). These data together confirm that MSCs are able to uptake particles without disruption to their differentiation toward the myogenic lineage.

Discussion

The importance of this study furthers our knowledge into muscle cell lineages and establishes feasibility for using these cells as important markers for muscle remodeling and repair. Cell therapy is emerging as an aspect of regenerative medicine and it will be pertinent to have the ability to monitor the transplanted cells *in vivo* with cell tracking through MRI. These cells differentiated from MSCs show myogenic potential and will enhance our understanding of the myogenic lineage and skeletal muscle repair via cell tracking. This is a novel tool to track skeletal muscle repair above what has previously been reported in satellite cells and myoblasts.

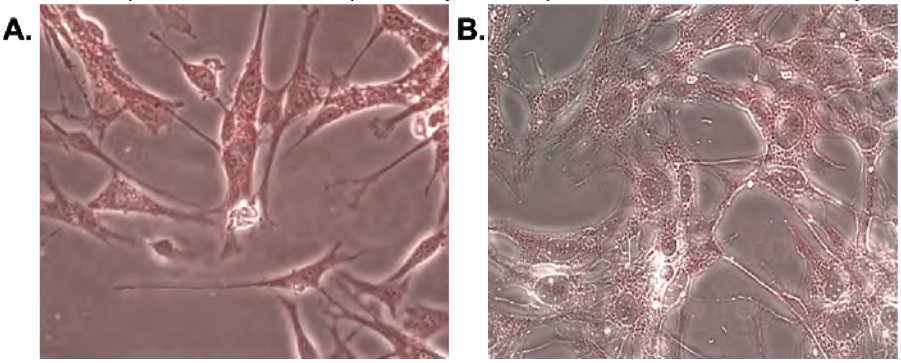

Figure 1. Flourescent staing for fast skeletal myosin. A. Representative myogenic cells (Week 6 with low particles), B. control MSCs.

References

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2. Elmi A, et al. Anal Sphincter Repair With Muscle Progenitor Cell Transplantation: Serial Assessment With Iron Oxide–Enhanced MRI. AJR 2014; 202:619–625.