MUSCLE-DERIVED STEM CELLS PROMOTE PERIPHERAL NERVE AND SKELETAL MUSCLE REPAIR BUT GENERATE TUMORS IN A NICHE DEPENDENT MANNER

**INTRODUCTION**

Our group has reported that muscle-derived stem cells (MDSCs) isolated from normal mice can differentiate into several cell types, including skeletal muscle cells, osteogenic progenitors, and vascular endothelial cells [1,2]. Furthermore, we have used these cells to regenerate both skeletal muscle and bone in vivo; however, the potential involvement of MDSCs in nerve regeneration remains undetermined. We observed that MDSCs isolated from newborn female mice (FN-MDSCs) express neuronal and glial markers without stimulation and can generate neurospheres following culture in neurogenic media. Furthermore, these cells can effectively regenerate skeletal muscle in a dystrophic muscle and peripheral nerve following a critical sized nerve defect injury. Though these cells can still be detected 17 weeks post engraftment into a skeletal muscle and regenerated peripheral nerve defects (by week 6), we found that they generated Triton tumors but only after 11-13 weeks post implantation into a sciatic nerve defect.

**MATERIALS AND METHODS**

Cell isolation and characterization: The modified preplate technique was used to isolate MDSCs from female newborn C57BL/10J mice and analyzed by flow cytometry for the expression of stem cell surface markers CD34, Sca-1, and CD45. Myogenic differentiation was assessed by switching to a low serum media (2% fetal bovine serum) and immunofluorescent staining for fast myosin heavy chain (IMHC). Undifferentiated MDSCs were characterized by immunofluorescent staining for the neuronal and glial markers: nestin, neurofilament (NF), and CNPase. Neuronal formation was performed and confirmed through immunofluorescence for the neuronal markers (nestin, Neu-N (neuronal nuclei antigen), β-Tubulin III, and NF), and the oligodendrocyte marker CNPase, and the astrocyte marker GFAP.

*In vivo analysis of nerve regeneration:* FN-MDSCs were transplanted with a retrovirus encoding a LacZ reporter gene containing a nuclear localization signal sequence (to enable donor cell tracking); limiting dilution then was used to clone the transduced cells. Muscle regeneration was determined via assessment of dystrophin restoration. FN-MDSC MDSCs (2-3x10⁵ cells) were transplanted into the gastrocnemius muscles of mdx (dystrophic) mice. Mice were sacrificed 2 weeks after transplantation, and the gastrocnemius muscles were harvested, frozen, and cryostat-sectioned. Neurogenic differentiation was examined by creating a Seven-millimeter sciatic nerve defects in SCID mice. Immediately thereafter, FN-MDSCs or phosphate buffered saline (PBS; control) was injected into the defects. Some of the mice from each group were sacrificed 6 weeks after transplantation, and the hind limbs, including the sciatic nerve and gastrocnemius muscles, were harvested, frozen, and cryostat-sectioned for further analysis. LacZ expression was revealed by histochemistry, and immunohistochemistry was used to colocalize donor cells and neuronal markers. The remaining mice were kept until later time points (up to 15 weeks) to check for possible tumor formation.

**RESULTS**

Undifferentiated FN-MDSCs had a high predilection towards expressing both neuronal and glial cell markers. FN-MDSCs showed high expression of neuroepithelia stem cell marker, nestin (98%), CNPase, a marker for oligodendrocytes (89%), with moderate expression of a postmitotic neuronal cell marker, neurofilament (NF) (53%). The FN-MDSCs were able to generate neurospheres (non-adherent 3-dimensional cell aggregates) in neurogenic media and the MDSC-derived neurospheres expressed β-tubulin III, CNPase GFAP, nestin, Neu-N, and NF (Figure 1).

FN-MDSCs can be detected in the skeletal muscle of an mdx mouse 17 weeks post-transplantation. Though FN-MDSCs have an innate predilection to undergo neurogenesis, they are still capable of undergoing myogenic differentiation. FN-MDSCs cultured in fusion media form IMHC-positive myotubes, and FN-MDSCs injected into a dystrophic skeletal muscle engraft regenerate lacZ [+] muscle fibers up to 17 weeks post injection (Figure 2).

FN-MDSCs can regenerate peripheral nerve following injury. FN-MDSCs were transplanted into a 7mm critical sciatic nerve defect, a complete regeneration was observed 6 weeks after injury only in those mice that received FN-MDSCs; the control (PBS) group exhibited minimal regeneration. The regenerated nerve contained many LacZ [+] cells and exhibited both NF and CNPase immunoreactivity. Colocalization of β-galactosidase with GFAP suggested possible differentiation of the MDSCs into glial cells. The cross section of regenerated nerve exhibited NF-positive axons encompassing with fluoromyelin-positive cells (Figure 3).

FN-MDSCs undergo niche induced transformation 11 weeks post transplantation into a sciatic nerve defect. Interestingly, between weeks 11-13, 11 out of 12 mice implanted with FN-MDSCs formed large neoplastic growths (Figure 4). The resulting tumors were classified as malignant peripheral nerve sheath tumors with rhabdomyoblastic differentiation (triton tumors). These neoplasias were highly invasive and positive for NF and fluoromyelin, as well as smooth muscle actin. In addition, they were composed almost entirely of Lac-Z [+] cells, and hence originated from our donor cells. Interestingly, cells isolated from these tumors grew as neurospheres in the absence of neurogenic medium and were positive for nestin, NF, β-tubulin III, CNPase, GFAP, but were negative for Neu-N marker and showed higher expression of myogenic marker, desmin (12%) compared to their parental counterparts (1.5%). They retained their ability to form myotubes in vitro, but had gained the property of anchorage independent growth (as determined by soft agar assays) and formed tumors when injected into the gastrocnemius muscle of mdx mice.

**DISCUSSION**

We have observed that cells isolated from skeletal muscle of newborn female mice can regenerate both skeletal muscle and peripheral nerve. These cells are not intrinsically oncogenic as no tumor formation was observed when these cells were implanted into a skeletal muscle for over 17 weeks. Furthermore, the tumorigenesis observed, though reproducible, was not a mutually exclusive event. The mice that developed triton tumors still displayed both complete sciatic nerve regeneration as well as engraftment into the skeletal muscle. We believe that by understanding the cause of our perceived niche induced oncogenesis we will be able to stop transformation and have a very effective cell for the regeneration of muscle and peripheral nerve.

**ACKNOWLEDGEMENTS**

This work was supported in part by funding from the Henry J. Mankin Endowed Chair for Orthopaedic Research at the University of Pittsburgh, the William F. and Jean W. Donaldson Chair at Children’s Hospital of Pittsburgh, the Hirtzel Foundation, and grants from the NIH (R01 AR049864) and the DOD (W81XWH-04-0003).

**REFERENCES**


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