IDENTIFICATION AND CHARACTERIZATION OF A NOVEL POPULATION OF MUSCLE STEM CELLS IN MICE: POTENTIAL FOR MUSCLE REGENERATION

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Introduction:

Recent investigations provide conclusive evidence demonstrating the existence of multiple types of pluripotent stem cells during the postnatal stage of life. These cells' capacity for differentiation into diverse lineages led to the therapeutic interest in somatic stem cells. Given the large amount of skeletal muscle in the body (30-40% of body weight), skeletal muscle is viewed as an accessible source from which to isolate pluripotent stem cells for cell replacement therapies (1-4). Although investigators have shown muscle derived cells can differentiate into muscle (1), hematopoietic (1,2) and osteogenic lineages (3), the cells' source, the extent of their differentiation, and their application in cell transplantation remain unclear. We recently isolated a rare purified population of muscle derived cells from normal newborn mice using the modified preplate technique (4). We characterized the basic biology of these cells: their phenotype, their pluripotency and their efficiency in cell transplantation. Our results suggest that these cells represent a novel population of muscle derived stem cells (MDSC) that will significantly improve muscle cell-mediated therapies.

Method:

1) Normal mice (C57 BL/6J) and dystrophin deficient (mdx) mice (C57BL/10ScSn DMD^{mdx}/J) used in this experiment were purchased from Jackson Laboratories (Bar Harbor, ME). All animal protocols used for these experiments were approved by the Children's Hospital of Pittsburgh's IACUC committee (protocol Nos. 2/00 and 7/00).

2) MDSC and satellite cell derived myoblasts (scdm) were isolated from normal newborn mice and assayed by flow cytometry for the expression of stem cell antigen Sca-1 and the major histocompatibility complex class 1 (MHC-1).

3) Normal muscle sections were used to colocalize Sca-1, m-cadherin (satellite cells markers) and laminin to investigate the source of these MDSC.

4) MDSC and scdm (3-4×10^5 cells), as well as MDSC transduced with retrovirus carrying the LacZ reporter gene, were injected into the gastrocnemius muscles of mdx mice.

5) The animals were sacrificed 10, 30 and 90 days post-injection; the injected muscles were harvested and either frozen for analysis of dystrophin by immunohistochemistry or stained with LacZ or ß-galactosidase to determine the fate of the transplanted cells.

6) Immunohistochemical staining for vWF, an endothelial cell marker, and CNPase, a Schwann cell marker, was used to investigate the nature of differentiated cells, while staining for CD4 was used to investigate immune-privileged behavior.

Results:

1) MDSC expressed stem cell markers Sca-1 and CD34 (Fig.1a). A subpopulation of Sca-1(+)/m-cadherin(-) satellite cells was observed in normal muscle cross-sections (Fig. 1b,c, arrows), suggesting a muscle origin of the MDSC.

2) MDSC significantly improved muscle regeneration and dystrophin delivery in dystrophic muscle of mdx mice 10-90 days post-transplantation (Fig. 2a,c), when compared with scdm (Fig. 2b,c).

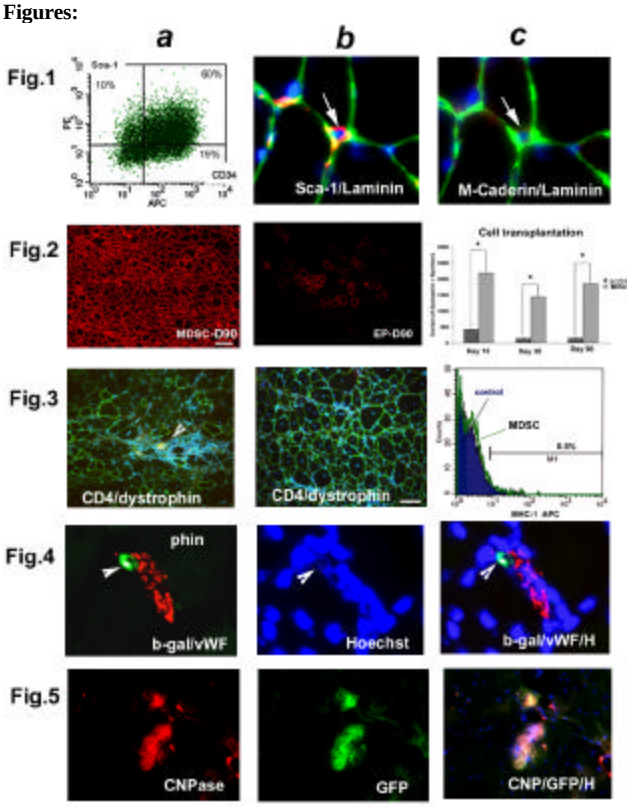
3) *Immune-privileged behavior displayed by MDSC:* Immune rejection of donor cells was observed in all of the scdm transplanted muscles (Fig. 3a), but in only some of the MDSC transplanted muscles by 30 days post-transplantation (Fig. 3b). Over 99% of the MDSC did not express MHC-1 as assessed by flow cytometry (Fig. 3c).

4) *Multipotency:* MDSC labeled with ß-galactosidase (green) differentiated into blood vessels (vWF positive, red; Fig. 4a-c); MDSC isolated from GFP transgenic mice (labeled with GFP, green) differentiated into peripheral nerve (CNPase positive, red; Fig. 5a-c). Hoechst staining was used to reveal nuclei (Fig.4b,c; Fig. 5c).

Discussion:

Two populations of myogenic cells have been isolated using a modified version of the preplate technique. The early preplate (EP) cells are derived from the main population of satellite cells and are a type of committed muscle precursor. The MDSC, derived from the subpopulation of later preplate cells, are characterized by myogenic lineage(-/+), Sca-1(+), CD34(+/-), c-kit(-) and CD45(-). The MDSC can differentiate into muscle, endothelial and nerve lineages both in vitro and in vivo, suggesting that they represent a population of pluripotent stem cells in skeletal muscle tissue. The use of MDSC can circumvent hurdles facing myoblast transfer therapy and, consequently, improve the efficiency of muscle regeneration and dystrophin delivery to dystrophic muscle. The unique features of the MDSC-including 1) their long-time proliferation ability, 2) their high self-renewal capacity, 3) their multipotent differentiation, and 4) their post-implantation immune-privileged behavior, reveal—at least in part—the basis for the improved transplantation capacity of this novel population of muscle-derived stem cells in skeletal muscle.

Figures:



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