

PARTIAL RESTORATION OF DYSTROPHIN IN MDX MICE FOLLOWING SYSTEMIC ADMINISTRATION OF GENETICALLY ENGINEERED MUSCLE DERIVED STEM CELLS

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Introduction: Duchenne Muscular Dystrophy (DMD) is a muscle disease characterized by a lack of dystrophin expression resulting in severe muscle weakness and early death due to cardiac and respiratory failures. Various approaches have been used to deliver dystrophin to the diseased muscle including myoblast transplantation (MT), gene therapy (GT), and ex vivo approach. The poor survival and the limited spread of transplanted myoblasts are two major hurdles facing the overall application of MT (1). We have shown previously that a population of highly purified muscle-derived stem cells obtained via the preplate technique express markers of myogenic and stem cells and displays increased survival in skeletal muscle following injection (2,3). The aim of this study was to further characterize the muscle derived cells by the microarray analysis and to investigate their ability to be systemically delivered to dystrophic muscles.

Materials and Methods: The policies and procedures of the animal laboratory are in accordance with those detailed by the USA Department of Health and Human Services. **Genetic engineering of muscle derived stem cell (mc13):** Muscle cells were isolated by the preplate technique (2). Highly purified muscle derived cells isolated by the preplate technique (pp6) obtained from mdx mouse was cloned and then transfected with a plasmid encoding the LacZ gene, neomycin resistance gene and mini dystrophin gene. Colonies that survived in G418-supplemented media were selected and tested for LacZ and dystrophin staining. Positive cells were expanded and used for the study.

Microarray analysis of mc13 gene expression: In order to define differential gene expression of our clone mc13 with a known myoblast cell line (C2C12), poly-A RNA was isolated from mc13 cells and C2C12 cells and submitted to IncyteGenomics company. IncyteGenomics utilizes a microarray chip known as Mouse GEM™. The microarray "contains more than 8,700 clones representing known genes which are mapped to the National Center for Biotechnology Information's (NCBI) UniGene database".

Intravenous and intramuscular injection of mc13 cells: 0.5×10^6 mc13 cells were injected intramuscularly or through the tail vein of adult mdx mice. Intravenous injection was also performed in mice pretreated with intramuscular injection of cardiotoxin to trigger local muscle regeneration. Hindlimb muscles were isolated 7 days post-injection and cryosectioned. Sections were stained for LacZ and dystrophin in serial sections.

Results: Stably transfected mc13 were found capable of expressing LacZ (Fig 1A, C; B: negative colony) and dystrophin *in vitro* (Fig 1D). The gene chip results have demonstrated potential differential expression in approximately 154 genes between mc13 and C2C12 cells. Of particular interest are the following genes that were screened because of their myogenic, osteogenic and stem cell involvement: **1)** Mus musculus myosin light chain 2, was expressed 8.2 times higher in mc13 than C2C12. This gene was found expressed in early embryonic stages especially during cardiogenesis (4,5). **2)** Mus musculus osteoblast specific factor 2, OSF-2, which was expressed 5.9 times higher in mc13 than C2C12. The OSF-2 gene encoded for a 90 kDA protein which is selectively expressed in bone (6). **3)** Catenin beta, a protein involved in somitogenesis and myotome compartment was expressed 4.3 times higher in mc13 than C2C12 (7). **4)** Follistatin-like gene (FLiK) was expressed 3.2 times higher in mc13 than C2C12. The FLiK gene encodes for a protein highly expressed on muscle precursor cells during avian somite compartmentalization and myogenesis (8) and **5)** Hematopoietic progenitor cell antigen CD34 precursor, which has been used as the hallmark of murine and human hematopoietic stem cells (9), was expressed at a lower level in mc13 than C2C12.

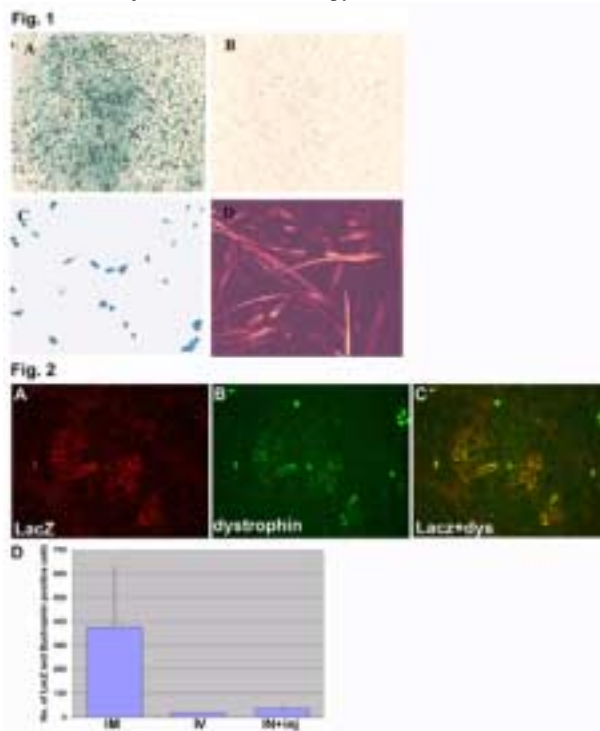
Systemic delivery produced encouraging results. 7 days following intravenous injection, LacZ and dystrophin positive myofibers were found in the gastrocnemius muscles of the injected mdx mice. However, the number of LacZ and dystrophin positive myofibers were found lower than the intramuscular injection of the mc13 cells (Fig 2 A,B,C). Pre-treatment of the

gastrocnemius muscle with cardiotoxin before systemic injection of mc13 increased the number of LacZ and dystrophin positive myofibers (Fig 2D).

Discussion: By the preplate technique we isolated a population of muscle derived stem cells which express some markers similar to those found on hematopoietic stem cells (3). We hypothesize that these cells are muscle derived early progenitor cells.

It is interesting that GEM™ microarray hybridization technology further confirmed our preliminary results showing that mc13 expresses a very low level of CD34 (3), and displays the expression of early progenitor cell markers and osteoblastic factors which further validates earlier results from our lab which show the ability of these cells to differentiate into osteogenic lineages *in vitro* and *in vivo* (3).

Systemic delivery of MC13 cells are capable of muscle regeneration although the efficiency is low compared to intramuscular injection. It is encouraging that with some pretreatment (injury) the number of injected cells migrating to muscle was increased. Further improvement of the migratory capacity may overcome a major hurdle for cell therapy to skeletal muscle.



Acknowledgements: Marcelle Pellerin (technician), Arvydas Usas (technician), Megan Mowry (secretary), Funding support: National Institute of Health (NIH PO1 AR 45925-01), Muscular Dystrophy Association (USA), Parent Project(USA)

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