Defective Proliferation and Myogenic Differentiation potential of Muscle-Derived Stem Cells Isolated from Dystrophin/Utrophin-/- mice

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a deadly genetic disease mainly characterized by progressive weakening of the skeletal, cardiac and diaphragmatic muscles. It is critical to find a successful therapy that will improve the histopathology of the muscles of DMD patients and restore their normal function. Most of the current treatments tested so far, including cell, gene and protein therapies, have been centered on the restoration of dystrophin within the dystrophic muscle; however, these technologies have faced serious limitations, including immunological reactions, which have hindered their success. Despite the lack of dystrophin at birth, the initiation of any signs of muscle weakness does not occur until 6-10 years of age. Hence, it has been posited that muscle weakness corresponds to the exhaustion of muscle progenitor cells which leads to the accumulation of fibrosis and fatty deposits that further exacerbates the muscle wasting process in DMD patients. In this study we isolated muscle derived stem cells (MDSCs) from dystrophin/utrophin deficient (dys/-/utro-/-, dKO-homo) and dystrophin deficient mice utrophin expressing (dys/-/utro+/-, dKO-hetero) mice. The phenotype of dKO-hetero mice is similar to MDX, so we compared MDSCs from these two animal models. We found that MDSCs isolated from the skeletal muscle of 6 week old dKO-homo mice have a reduced ability to proliferate and differentiate compared to MDSCs isolated from dKO-hetero mice. These observations suggest that blocking the exhaustion of muscle progenitor cells and stem cell-mediated therapy may represent a potential strategy to prevent or delay the onset of debilitating DMD disease-related changes. Moreover, this study also provided additional evidence that dKO is a better animal model of DMD than MDX.

MATERIALS AND METHODS

Cell isolation: MDSCs were isolated from dKO-homo (dys/-/utro-/-) and dKO-hetero (dys/-/utro+/-) mice 6 weeks of age, as previously described via a modified preplate technique [1]. After 7 days, MDSCs were obtained and cultured in proliferation medium (DMEM supplemented with 10% fetal bovine serum, 10% horse serum, 0.5% chicken embryo extract and 1% penicillin-streptomycin).

Cell proliferation: Proliferation behavior of the MDSCs isolated from dKO-homo and dKO-hetero mice was examined by using a robotic time-lapsed microscopic live-cell imaging system (LCI). Data was obtained for the cell population doubling time, total number of population doublings, and other measures of growth kinetics.

Myogenic differentiation assay: Myogenic differentiation capacity of the MDSCs was assessed by switching the proliferation medium into fusion medium (DMEM supplemented with 2% fetal bovine serum). After 3 days, the cells were stained for fast myosin heavy chain (MyHC), which is a marker of terminal myogenic differentiation. Myogenic differentiation levels were quantified as the percentage of the number of nuclei in MyHC positive myotubes relative to the total number of nuclei and compared between the two cell types.

Cell survival: Both populations were exposed to 250 μM hydrogen peroxide in proliferation medium containing propidium iodide (PI), a DNA-binding dye. LCI analysis was performed as described above. By identifying the number of PI+ cells per field of view per total cell number, we calculated the percentage of cell survival over time.

Single fiber isolation: Skeletal muscle tissue isolated from 6 week old dKO-homo and 9 week old WT control mice (C57BL/6) and then incubated the muscle in a solution of 0.2% of collagenase type I for 40 minutes at 37°C. When the muscle fibers were sufficiently loosened by the collagenase solution the muscle was triturated with heat polished glass pipettes to liberate single fibers. The muscle fibers were then transferred to a matrigel coated 12 well plate with proliferation medium.

RESULTS

MDSCs isolated from aged dKO mice display limited proliferation ability. MDSCs were isolated from 6 wk-old dKO-homo and dKO-hetero mice. The proliferation kinetics of both populations was examined in vitro using an automated live cell imaging system (LCI). We observed a significant reduction in the proliferation of the dKO-homo MDSCs compared to the MDSCs completely deficient for dystrophin and heterozygote for utrophin expression (Figure 1).

DISCUSSION

It is interesting to note that despite the lack of dystrophin at birth, the initiation of any signs of muscle weakness do not occur in DMD patients until late childhood and coincide with the exhaustion of muscle progenitor cells. In this study we demonstrated that MDSCs isolated from the skeletal muscle of 6 week old dKO-homo mice have a reduced ability to proliferate and differentiate compared to dKO-hetero MDSCs. Since dKO-homo mice can only live 6-8 weeks, stem cells exhaustion could represent the main mechanism for the rapid progress of this disease. Blocking the exhaustion of muscle progenitor cells and stem cell-mediated therapy may represent a potential strategy for treating these kinds of diseases. We have also shown that the dKO model is superior than mdx for testing the therapeutic effectiveness of various treatment modalities.

SIGNIFICANCE

This study suggested that the exhaustion of stem cells contributes to the histopathology associated with DMD and that blocking the exhaustion of muscle progenitor cells and stem cell-mediated therapy could be used as a potential clinical strategy to treat muscle disease.

REFERENCE


Figure 1

Figure 2

Figure 3

Figure 4

Isolated muscle fibers from dKO mice show a reduction in muscle progenitor cells. These single muscle fibers were isolated from 6 weeks old dKO-homo mice and 9 week old WT control mice. All single fibers attached on the surface of matrigel-coated plate after 24 hours. There are more cell nuclei in WT muscle fibers compared to dKO-homo muscle fibers. In addition, 5 days post-culturing, the WT muscle fibers were able to release myogenic progenitor cells forming new myotubes, in contrasts to that observed with dKO-homo muscle fibers. These results support both a reduction in the number and myogenic potential of the MDSCs derived from dKO-homo mice when compared to WT MDSCs (Figure 4).