Isolation of slow adhering stem cells from injured skeletal muscle of mouse

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ABSTRACT INTRODUCTION:

Isolation of muscle derived stem cells (MDSCs) has been popularly conducted with pre-plate technique according to their slow adhering characteristics on collagen-coated surface, and the transplantation of MDSCs has been verified to be greatly efficient for stem-cell based therapy of various tissues (1,2). However, most of previous studies on muscle stem cells have been focused on the cells from normal muscle without injury, in which the population of MDSCs is in fact very limited, and the isolation of slow adhering cells containing MDSCs from injured muscle has not been described yet. The tissue of skeletal muscle is highly regenerative and injuries to the muscle can result in the activation, proliferation, and even profound phenotypic modification of multiple cell types, including MDSCs (3, 4). In this study, with pre-plate technique, a greater population of slow adhering cell-like cells was isolated from injured muscle compared to normal muscle of mouse, and some of their characteristics have been compared.

METHODS:

Muscle injury: lacerations were performed on the gastrocnemius (GM) muscle of one leg of wild type mice through 50% of its width and 100% of its thickness at 60% of its length. And the GM muscle of the other leg serves as control.

Isolation of slow adhering cells: The slow adhering skeletal muscle cells containing muscle derived stem cells (MDSC) were isolated from the skeletal muscle of mice (C57BL/6J) with the preplate technique (1). Cells were cultured in the growth medium for stem cells [DMEM supplemented with 20% Fetal Bovine Serum (FBS), 10% Horse Serum (HS), 1% Penicillin-Streptomycin antibiotics, and 0.5% chicken essential extract (CEE)], and incubated in 5% CO2 at 37 °C.

Immunofluorescent staining of cells or tissue sections: Cells were fixed with 4% parafomaldehyde and frozen tissue sections were fixed with 4% formalin. The primary antibodies CD34 (BD), Sca-1 (BD), P21 (Santa Cruz), Pax7 (DHSB), dystrophin (sigma), myosin heavy chain (sigma), and CD31 (Abcam) were applied at 1:200. Fluorescent microscopy (Leica Microsystems Inc., IL) were used to visualize all of the immunofluorescence results and capture photographic imagines.

RESULTS:

1. Activation and proliferation of muscle stem cells in the lacerated-injured muscle 4 days after muscle injury

2. Cell isolation with pre-plate technique, and improved capacity in proliferation and migration of PP6 slow-adhering stem cells.

DISCUSSION:

This study took advantage of the efficient pre-plate technique and isolated a population of slow adhering stem cells which contains a larger population of Sca-1+/CD34+ stem-cell like cells compared to those from normal muscle. We have also proven that slow adhering stem cells from injured muscle demonstrated improved migration ability, proliferation rate, myogenic capacity, and was able to effectively repair dystrophic muscle in MDX mice. Our results can be a confirmative evidence for isolating highly regenerative stem cells from injured muscle, and further verifies a potential value of the stem cells from injured muscle in improving the regeneration of multiple tissues.


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