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PURIFICATION AND DIFFERENTIATION OF HUMAN MUSCLE DERIVED STEM CELLS FOR THE MANAGEMENT OF URINARY INCONTINENCE AND BLADDER RECONSTITUTION

Hypothesis / aims of study

To test the feasibility of isolation, purification, characterization and differentiation of the human muscle derived stem cells (MDSCs) for the management of urinary incontinence and bladder reconstitution.

Study design, materials and methods

Isolation of human muscle derived stem cells with modified preplate technique, CD 34-positive stem cell isolation, in vitro differentiation of MDSCs, myogenic, adipogenic and osteogenic induction of CD 34+ cells, immunolabeling procedures for flow cytometry, flow cytometry analysis, immunohistochemical staining, lipid droplet staining with Oil Red O, Alkaline phosphatase staining, immunofluorescence study, and RT-PCR were done.

Results

The MDSCs were isolated using modified preplate technique and were purified using Dyna-bead cell selection system. The growth doubling time of MDSCs was about 36 hours (Figure 1). Immunohistochemical staining showed positive for several CD markers, VCAM, VEGFR-2, CXCR4, CD56, and Desmin staining. Using special growth factors, the MDSCs could be differentiated into smooth muscle, skeletal muscle, adipocyte, and osteocyte. The differentiation was proved by immunohistochemical study and RT-PCR in protein and gene level.

Interpretation of results

The growth doubling time mimics that of the stem cells previously reported. The result of flow cytometric analysis confirmed the purification. The adipogenic, osteogenic, myogenic, and smooth muscle cell differentiation were feasible which were justified by immunohistochemical study and RT-PCR in protein and gene level (Figure 2).

Concluding message

The isolation, purification, characterization and differentiation of MDSCs were successfully conducted. The MDSCs may provide another novel way for the management of urinary sphincter deficiency and bladder reconstitution.

Figure 1. The growth doubling time of MDSCs was about 36 hours after purification and 24 hours before purification.

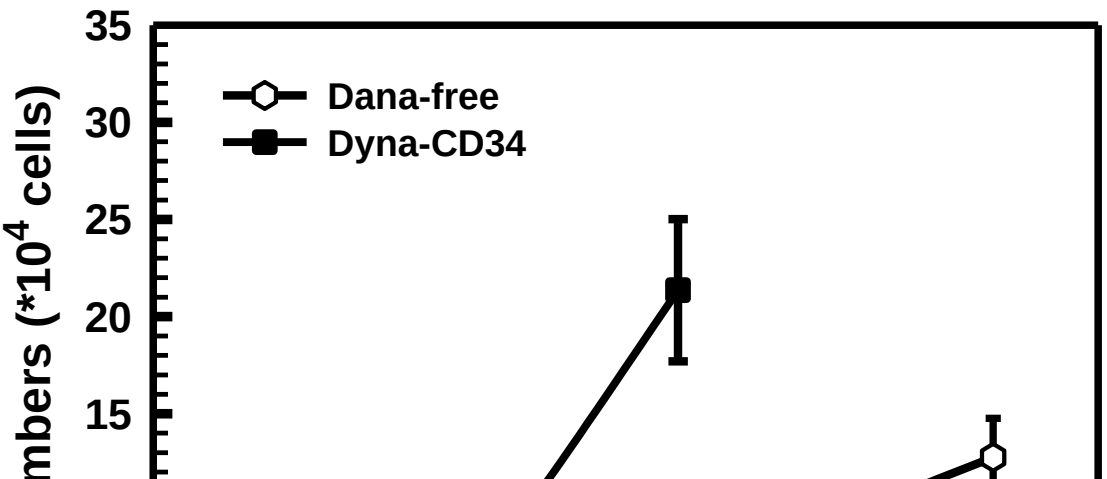
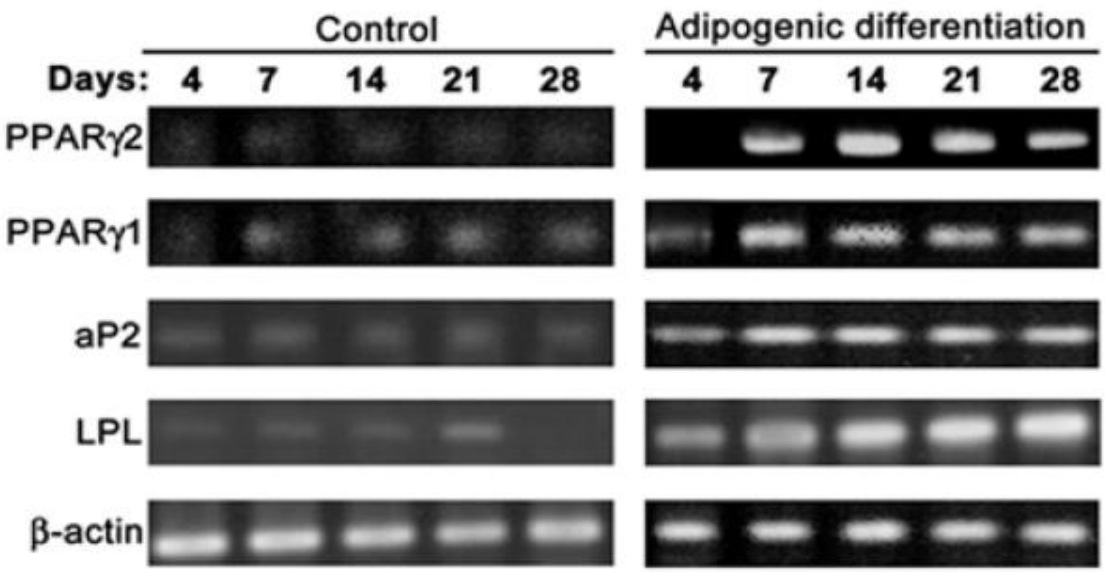


Figure 2. Adipogenic differentiation of MDSC tested using RT-PCR



Specify source of funding or grant	**The study was granted IRB approval from Taipei-Veterans General Hospital (VGHIRB No: 92-11-07A)**
Is this a clinical trial?	**No**
What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
Specify Name of Ethics Committee	**The study was granted IRB approval from Taipei-Veterans General Hospital (VGHIRB No: 92-11-07A)**
Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**