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| | | Clone | Adipogenic Differentiation (Oil Red O stain) | Osteogenic Differentiation (Alizarin Red stain) | Myogenic Differentiation |
|---|---|---|---|---|---|
| Dilution cloning | | 1 clone(CA1) | + | + | MyoD1-positive cells in vitro<br>Dystrophin-positive cells in vivo |
| | | 1 clone | + | + | MyoD1-positive cells in vitro |
| | | 10 clones | + | + | ND |
| Automated cloning | Retroviral marking | 3 LacZ-marked clones | + | + | ND |
| | | 3 GFP-marked clones sorted by FACS | + | + | ND |

**Table S1.** Results of cell differentiation assays performed with expanded clones. Clones were isolated by dilution cloning or automated cloning from wild-type or transduced hMADS-2 cells. Criteria were as follows: adipogenic differentiation: microscopic detection of Oil red O-positive cells; osteogenic differentiation: microscopic detection of bone nodules after Alizarin red staining; and myogenic differentiation: detection by RT-PCR of expression of MyoD1 after in vitro differentiation and detection of dystrophin by immunohistochemistry after transplantation in mdx muscle.

### Cloning of hMADS cells.

Cell cloning was performed by limiting dilution of wild-type cells, by limiting dilution of LacZ genetically marked cells, and by automated cell sorting of GFP-positive cells. For limiting dilution, cells were seeded at a nominal cell density of 0.5 cell per well into 24-well plates in DMEM supplemented with 10% FBS and 5 ng/ml$^{-1}$ hFGF-2. Cultures were examined daily by careful microscopic examination for the appearance of a single colony per well. Starting after 2 wk of culture, differentiation of several clones has been investigated. In a separate experiment, hMADs cells have been transduced with a lentiviral virus expressing nlsLacZ. At a multiplicicity of infection of 3, ~25% of the cells expressed the β-galactosidase (Fig. S2 A). After limiting dilution as indicated, 25% of the clones (3 clones out of 14 clones analyzed) were positive for β-galactosidase. More importantly, 100% of the cells of these three clones were β-gal positive (i.e., not

| Cells injected | Number of mdx mice injected | | Time of euthanasia (days) |
|---|---|---|---|
| | Dystrophin positive | Dystrophin negative | |
| hMADS-2 | 10 | 2 | 10 |
| | 2 | 0 | 50 |
| | 1 | 1 | 80 |
| | 1 | 0 | 180 |
| hMADS-2-CA1 clone | 1 | 0 | 10 |
| hMADS-3 | 3 | 0 | 10 |
| hMADS-1 | 1 | 0 | 10 |

**Table S2.** Results of transplantation of hMADS cells in the tibialis anterior muscle of immunocompetent mdx mice. The number of mice injected with hMADS cells is indicated. The result is considered as dystrophin positive when on two serial sections >5% of myofibers expressed human dystrophin. When detected, revertant myofibers represented <1% of the fibers on the section.

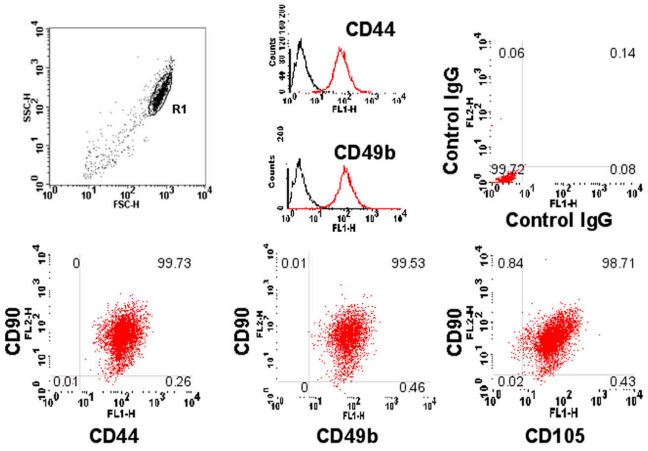


**Figure S1. Co-expression of cell surface markers by hMADS.** The R1 gate was established on forward versus side scatter parameters to exclude dead cells and debris from subsequent analysis. hMADS-2 cells cultured for 160 PDs and stained for CD44 and CD49b. The signals were amplified by adding FITC-labeled rabbit anti–mouse IgG after CD44 and CD49b staining. Double labeling were performed with CD90 and CD105 or CD90 and CD105, or CD90 and CD49b. The percentages of gated cells coexpressing the surface antigens are shown.

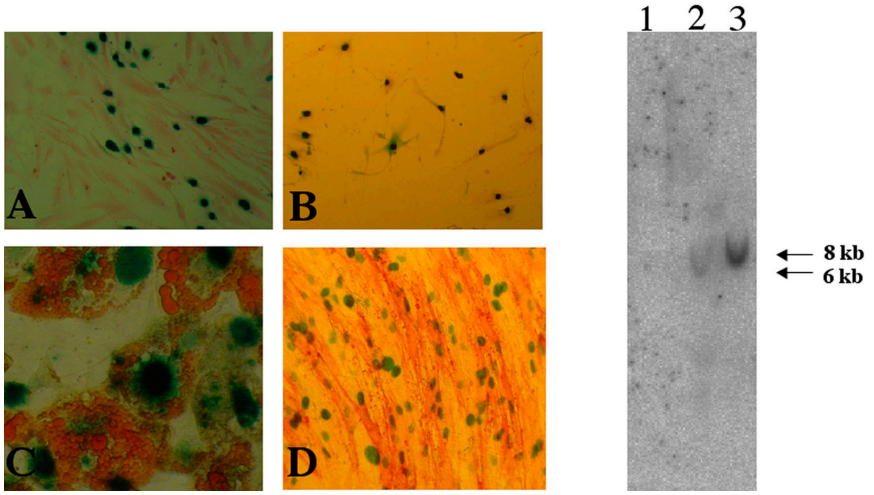


**Figure S2. Single cell origin of hMADS clones.** (A) hMADS cells were transduced with a lentiviral vector expressing LacZ. Cells were stained for β-galactosidase and counterstained with eosin; (B) one clone expressing β-galactosidase and isolated by limiting dilution is shown; (C) Differentiation of one β-galactosidase expressing clone is shown. Clonal cells were induced to undergo differentiation into adipocytes containing lipid droplets stained with Oil red O or (D) into osteoblasts expressing alkaline phosphatase. (E) DNA of two clones was extracted, digested with Not1 (cut only once in the retrovirus vector) and Southern blot was hybridized with a LacZ probe. A single retroviral insert at 6 kb and 8 kb for clone 2 and clone 3, respectively, is shown. 1, DNA of wild-type hMADS cells; 2, DNA of clone 2; 3, DNA of clone 3.

contaminated with wild-type cells representating 75% of the population before cloning). This observation indicated that, under our conditions, clones were likely formed from a single cell. LacZ-expressing clones were able to undergo differentiation into adipocytes and osteoblasts as shown in the Fig. S2. Southern blot analysis of LacZ marked clones has been performed and indicated that the integration of the marker was different for each clone. In a third series of experiments, hMADS cells have been transduced with a lentivirus expressing GFP. GFP-expressing cells were selected (FACSCalibur; Becton Dickinson) and a single cell was automatically deposited in each well of 96-well plates. From 192 wells, three clones were expanded and maintained in adipogenic and osteogenic conditions. Data are summarized in Table S1.

**Proliferation of OF1 and mdx splenocytes.**

OF1 and Mdx splenocytes were purified and stimulated at $10^6$ cells/ml with an equivalent number of irradiated (500 rad) hMADS cells (at 120 PDs) or with human peripheral blood lymphocytes (hPBLs), or with mouse BALB/c splenocytes. On day 4, 1 mCi/ml tritiated thymidine was added and cells were harvested 24 h after.

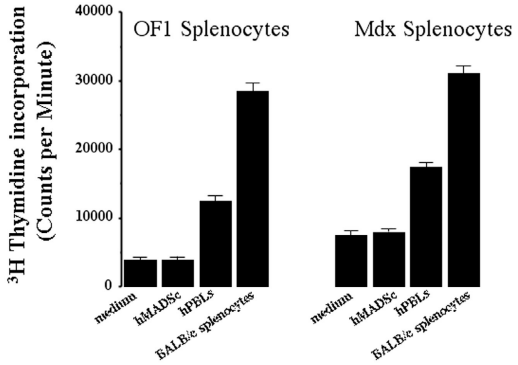


**Figure S3. The effect of hMADS cells on the proliferation of OF1 and mdx splenocytes.** OF1 and Mdx splenocytes were stimulated with an equivalent number of irradiated (500 rad) hMADS cells (at 120 PDs) or with human peripheral blood lymphocytes (hPBLs) or with mouse BALB/c splenocytes. Thymidine incorporation, determined 24 h after 1 μCi/ml tritiated thymidine, is expressed as counts per minute. Data are the mean of triplicate cultures.