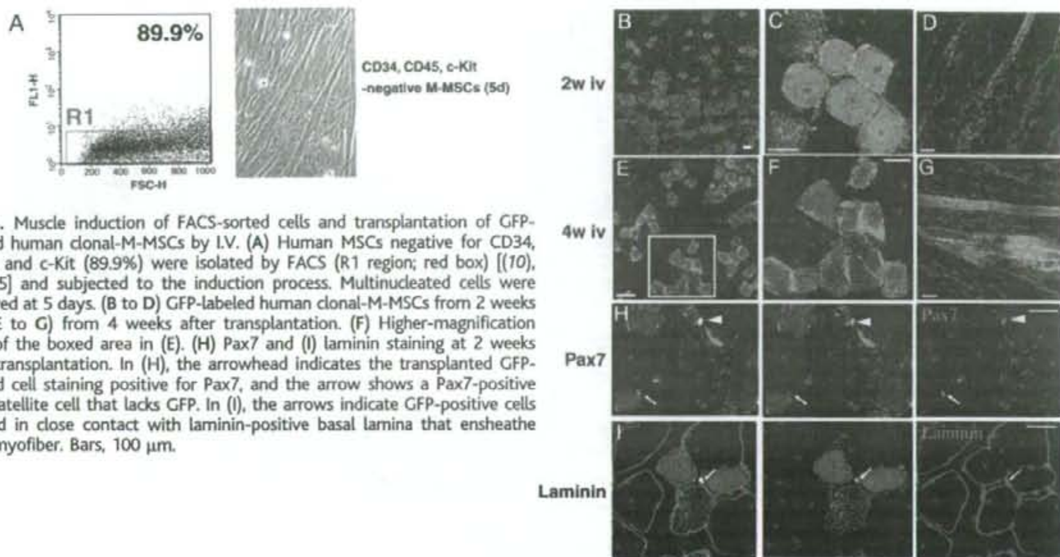

Fig. 3. Muscle induction of FACS-sorted cells and transplantation of GFP-labeled human clonal-M-MSCs by I.V. (**A**) Human MSCs negative for CD34, CD45, and c-Kit (89.9%) were isolated by FACS (R1 region; red box) [(*10*), Note 5] and subjected to the induction process. Multinucleated cells were observed at 5 days. (**B** to **D**) GFP-labeled human clonal-M-MSCs from 2 weeks and (**E** to **G**) from 4 weeks after transplantation. (**F**) Higher-magnification view of the boxed area in (E). (**H**) Pax7 and (**I**) laminin staining at 2 weeks after transplantation. In (H), the arrowhead indicates the transplanted GFP-labeled cell staining positive for Pax7, and the arrow shows a Pax7-positive host satellite cell that lacks GFP. In (I), the arrows indicate GFP-positive cells located in close contact with laminin-positive basal lamina that ensheathe each myofiber. Bars, 100 μm.

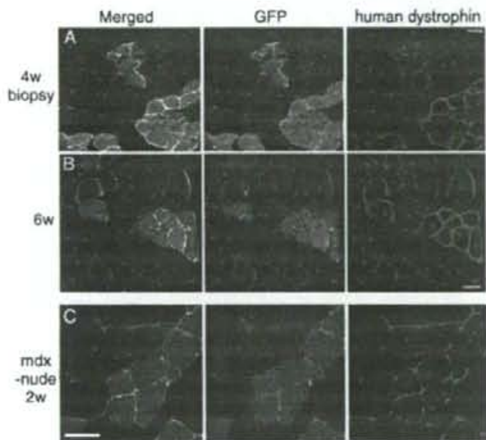

Fig. 4. Regeneration of human clonal-M-MSC transplanted rat (**A** and **B**) and mdx-nude mouse (**C**) gastrocnemius muscles after cardiotoxin treatment. (A) Specimen obtained from biopsy at 4 weeks by I.V. transplantation. Human dystrophin (red) is expressed by GFP-labeled transplanted cells. (B) After biopsy, cardiotoxin was administered and 2 weeks later (6 weeks after human clonal-M-MSC transplantation), gastrocnemius muscles were examined. Human dystrophin could be detected in GFP-labeled regenerating muscle fibers with centrally located nuclei. (C) Expression of human dystrophin in GFP-labeled cells in mdx-nude mouse gastrocnemius muscle after 2 weeks. Bars, 100 μm.

muscles of mdx-nude mice, which genetically lack dystrophin expression. Immunohistochemistry revealed the incorporation of transplanted cells into newly formed myofibers, which expressed human dystrophin 2 weeks after transplantation (Fig. 4C).

Compared to the various stem cell systems that have been reported (*1*, *20*–*22*), our MSCs offer several important advantages. First, MSCs can easily be obtained from patients or bone marrow banks and can be expanded efficiently in vitro. In the case of MSCs derived from inherited muscle dystrophy patients, genetic manipulation is possible after the isolation and expansion of MSCs. Second, transplantation of MSC-derived cells should encounter fewer ethical problems, because the use of these cells avoids the embryonic stem cell controversy and is in theory similar to bone marrow transplantation, which is currently in wide use for patients with leukemia, refractory anemia, etc. Third, autologous transplantation of MSC-derived muscle cells or transplantation of these cells with the same HLA (human leukocyte antigen) subtype from a healthy donor should minimize the risks of rejection. Because our induction system does not depend on a rare stem cell population, but can use the general population of adherent MSCs, which can be easily isolated and expanded, functional skeletal muscle cells can be obtained within a reasonable time on a therapeutic scale. At present, there are no effective therapeutic approaches for muscle dystrophy. Although the mechanism of muscle induction by NICD introduction remains to be clarified, we believe that our MSC differentiation system may contribute substantially to a major advance toward eventual cell-based therapies for muscle disease.

References and Notes

1. J. Rohwedel *et al.*, *Dev. Biol.* **164**, 87 (1994).
2. P. A. Dreyfus *et al.*, *Am. J. Pathol.* **164**, 773 (2004).
3. D. Suva *et al.*, *J. Cell. Physiol.* **198**, 110 (2004).
4. A. Polesskaya, P. Seale, M. A. Rudnicki, *Cell* **113**, 841 (2003).
5. Y. Torrente *et al.*, *J. Cell Biol.* **162**, 511 (2003).
6. A. Asakura, P. Seale, A. Girgis-Gabardo, M. A. Rudnicki, *J. Cell Biol.* **159**, 123 (2002).
7. J. R. Beauchamp *et al.*, *J. Cell Biol.* **151**, 1221 (2000).
8. G. Ferrari *et al.*, *Science* **279**, 1528 (1998).
9. M. Dezawa *et al.*, *J. Clin. Invest.* **113**, 1701 (2004).
10. Materials and methods are available as supporting material on *Science* Online.
11. N. Yoshida, S. Yoshida, K. Koishi, K. Masuda, Y. Nabeshima, *J. Cell Sci.* **111**, 769 (1998).
12. V. Andres, K. Walsh, *J. Cell Biol.* **132**, 657 (1996).
13. Y. Jiang *et al.*, *Nature* **418**, 41 (2002).
14. P. Seale *et al.*, *Cell* **102**, 777 (2000).
15. D. D. Cornelison, B. J. Wold, *Dev. Biol.* **191**, 270 (1997).
16. E. Bober *et al.*, *J. Cell Biol.* **113**, 1255 (1991).
17. M. Dezawa *et al.*, data not shown.
18. S. Fukada *et al.*, *J. Cell Sci.* **115**, 1285 (2002).
19. R. Bischoff, in *The Satellite Cell and Muscle Regeneration*, A. G. Engel, C. Franszini-Armstrong, Eds. (McGraw-Hill, New York, 1994), pp. 97–118.
20. M. A. LaBarge, H. M. Blau, *Cell* **111**, 589 (2002).
21. M. R. Wada, M. Inagawa-Ogashiwa, S. Shimizu, S. Yasumoto, N. Hashimoto, *Development* **129**, 2987 (2002).
22. Z. Qu, J. Huard, *Gene Ther.* **7**, 428 (2000).

23. We are grateful to N. Hashimoto and T. Partridge for providing the mdx-nude mice, R. Kageyama for pCI-neo Hes6, R. Yu and M. M. Taketo for critical reading of the manuscript, and M. Yoshida for technical assistance. This work was supported by Health and Labor Sciences Research Grants for "Research on Psychiatric and Neurological Diseases and Mental Health" and The Research Grant (16-8) for Nervous and Mental Disorders, both from the Ministry of Health, Labor and Welfare.

Supporting Online Material
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References

28 January 2005; accepted 13 May 2005
10.1126/science.1110364