human M-MLCs, cardiotoxin was re-administered into the same muscles without additional transplantation. Two weeks after the second cardiotoxin treatment (6weeks after initial transplantation), many regenerating GFP-positive myofibers with centrally-located nuclei were observed. This implies that, upon transplantation of M-MSCs to muscles of patients, those retained as satellite cells should be able to continue to contribute to future muscle regeneration[10].

Compared to the various muscle stem cell systems that have been reported, this system offers several important advantages. Since our induction system does not depend on a rare stem cell population, but can utilize the general population of adherent MSCs, which can be easily isolated and expanded, functional skeletal muscle cells can be obtained within a reasonable time course on a therapeutic scale. In case of MSCs derived from inherited muscle dystrophy patients, genetic manipulation is possible after the isolation and expansion of MSCs. Moreover, transplantation of MSC-derived cells should encounter fewer ethical problems.

General conclusions

While ES cells and tissue stem cells have great potential, MSCs also provide hopeful possibilities for clinical application, since they can be efficiently expanded *in vitro* and we could acquire a therapeutic scale of induced cells. In addition, transplantation of MSC-derived cells should pose fewer ethical problems by preventing stem cell controversy, since bone marrow transplantation has already been widely performed. As MSCs are easily obtained from patients or marrow banks, autologous transplantation of induced cells or transplantation of induced cells with the same HLA subtype from a healthy donor may minimize the risks of rejection. Needless to say, bone marrow should at least be 'normal and healthy' for transplantation.

Although we showed the high ratio and specific induction of Schwann cells, neurons and skeletal muscle cells, we still have to solve the following problems[8-10]. Although there have been so far few reports referring to tumor formation after transplantation of untreated MSCs, further studies are needed to ensure safety, tumor formation and efficacy of manipulated MSCs over a long-term period using primates. In fact, recent reports raised the possibility of transformation in the long term cultivation of MSCs[25,26]. Second, as the potential of differentiation would differ by age, individual, race, and sexes, each of these must be investigated in the future. Third, MSCs have been shown to be heterogeneous in terms of growth kinetics, morphology, phenotype and plasticity. With the development of specific markers and detailed characterization of heterogeneous general adherent MSCs, their properties and plasticity can be studied and defined with more certainty.

Notch-Hes signaling are known to inhibit neuronal and myogenic differentiation in conventional development[16]. However, in our system, NICD introduction accelerated the induction of neuronal and skeletal muscle cells from MSCs. Although our results appear inconsistent with previous work, they do not refute the known role of Notch-Hes signals during development. In the previous report, JAK/STAT inhibitor administration and constitutive active STAT1/3 transfection showed that down regulation of STATs was tightly associated with NICD-mediated neuronal induction, whereas Hes, down stream of Notch, was not involved in the induction event[9]. Skeletal muscle induction was also revealed to be independent of Hes1/5. Thus, our results suggest the distinct cellular responses to Notch signals; for example, the repertoire of second messengers and active factors may well be different between conventional neural stem cells and/or neural progenitor cells and MSCs. It might be possible that unknown signaling pathway downstream of Notch may be involved in these events, and thus further studies are needed to identify the factor involved in this phenomenon.

Since MSCs can be obtained from patients, it is possible to establish a "self-regenerative system" using MSCs. To realize this ideal, it is necessary to develop the regulatory system of differentiating MSCs into cells with a purpose. Our method would be one of possible way to regulate MSC differentiation into functional Schwann cells, neurons and skeletal muscle cells which will be applicable to neurodegenerative and muscle degenerative diseases.

References

1) Prockop DJ: Marrow stromal cells as stem cells for nonhematopoietic tissues. Science, 276: 71-74, 1997.
2) Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science, 284: 143-147, 1999.
3) Pittenger MF, Mosca JD, McIntosh KR: Human mesenchymal stem cells: progenitor cells for cartilage, bone, fat and stroma. Curr Top Microbiol Immunol, 251: 3-11, 2000.
4) Makino S, Fukuda K, Miyoshi S, Konishi F, Kodama H, Pan J, Sano M, Takahashi T, Hori S, Abe H, Hata J, Umezawa A, Ogawa S: Cardiomyocytes can be generated from marrow stromal cells in vitro. J Clin Invest, 103: 697-705, 1999.

5) Wang PP, Wang JH, Yan ZP, Hu MY, Lau GK, Fan ST, Luk JM: Expression of hepatocyte-like phenotypes in bone marrow stromal cells after HGF induction. Biochem Biophys Res Commun, 320: 712-716, 2004.
6) Choi KS, Shin JS, Lee JJ, Kim YS, Kim SB, Kim CW: In vitro trans-differentiation of rat mesenchymal cells into insulin-producing cells by rat pancreatic extract. Biochem Biophys Res Commun, 330: 1299-1305, 2005.
7) Wang G, Bunnell BA, Painter RG, Quiniones BC, Tom S, Lanson NA Jr, Spees JL, Bertucci D, Peister A, Weiss DJ, Valentine VG, Prockop DJ, Kolls JK: Adult stem cells from bone marrow stroma differentiate into airway epithelial cells: potential therapy for cystic fibrosis. Proc Natl Acad Sci USA, 102: 186-191, 2005.
8) Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H: Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci, 14: 1771-1776, 2001.
9) Dezawa M, Kanno H, Hoshino M, Cho H, Matsumoto N, Itokazu Y, Tajima N, Yamada H, Sawada H, Ishikawa H, Mimura T, Kitada M, Suzuki Y, Ide C: Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest, 113: 1701-1710, 2004.
10) Dezawa M, Ishikawa H, Itokazu Y, Yoshihara T, Hoshino M, Takeda S, Ide C, Nabeshima Y: Bone marrow stromal cells generates muscle cells and repair muscle degeneration. Science, 309(5732): 314-317, 2005.
11) Dubovy P: Schwann cells and endoneurial extracellular matrix molecules as potential cues for sorting of regenerated axons: a review. Anat Sci Int, 79: 198-208, 2004.
12) Edgar JM, Garbern J: The myelinated axon is dependent on the myelinating cell for support and maintenance: molecules involved. J Neurosci Res, 76: 593-598, 2004.
13) Dezawa M, Adachi-Usami E: Role of Schwann cells in retinal ganglion cell axon regeneration. Prog Retin Eye Res, 19: 171-204, 2000.
14) Mimura T, Dezawa M, Kanno H, Sawada H, Yamamoto I: Peripheral nerve regeneration by transplantation of bone marrow stromal cell-derived Schwann cells in adult rats. J Neurosurg, 101: 806-812, 2004.
15) Kamada T, Koda M, Dezawa M, Yoshinaga K, Hashimoto M, Koshizuka S, Nishio Y, Moriya H, Yamazaki M: Transplantation of bone marrow stromal cell-derived Schwann cells promotes axonal regeneration and functional recovery after complete transection of adult rat spinal cord. J Neuropathol Exp Neurol, 64: 37-45, 2005.
16) Lundkvist J, Lendahl U: Notch and the birth of glial cells. Trends Neurosci, 24: 492-494, 2001.
17) Yamasaki M, Yamada K, Furuya S, Mitoma J, Hirabayashi Y, Watanabe M: 3-Phosphoglycerate dehydrogenase, a key enzyme for l-serine biosynthesis, is preferentially expressed in the radial glia/astrocyte lineage and olfactory ensheathing glia in the mouse brain. J Neurosci, 21: 7691-7704, 2001.
18) Shibata T, Yamada K, Watanabe M, Ikenaka K, Wada K, Tanaka K, Inoue Y: Glutamate transporter GLAST is expressed in the radial glia-astrocyte lineage of developing mouse spinal cord. J Neurosci, 17: 9212-9219, 1997.
19) Kawasaki H, Mizuseki K, Nishikawa S, Kaneko S, Kuwana Y, Nakanishi S, Nishikawa SI, Sasai Y: Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron, 28: 31-40, 2000.
20) Akerud P, Alberch J, Eketjall S, Wagner J, Arenas E: Differential effects of glial cell line-derived neurotrophic factor and neurturin on developing and adult substantia nigra dopaminergic neurons. J Neurochem, 73: 70-78, 1999.
21) Yoshida N, Yoshida S, Koishi K, Masuda K, Nabeshima Y: Cell heterogeneity upon myogenic differentiation: down-regulation of MyoD and Myf-5 generates 'reserve cells'. J Cell Sci, 111(Pt 6): 769-779, 1998.
22) Seale P, Sabourin LA, Girgis-Gabardo A, Mansouri A, Gruss P, Rudnicki MA: Pax7 is required for the specification of myogenic satellite cells. Cell, 102: 777-786, 2000.
23) Fukada S, Miyagoe-Suzuki Y, Tsukihara H, Yuasa K, Higuchi S, Ono S, Tsujikawa K, Takeda S, Yamamoto H: Muscle regeneration by reconstitution with bone marrow or fetal liver cells from green fluorescent protein-gene transgenic mice. J Cell Sci, 115: 1285-1293, 2002.
24) Bischoff R: The satellite cell and muscle regeneration. New York, McGraw-Hill, 1994, pp97-188.
25) Serakinci N, Guldberg P, Burns JS, Abdallah B, Schrodder H, Jensen T, Kassem M: Adult human mesenchymal stem cell as a target for neoplastic transformation. Oncogene, 23: 5095-5098, 2004.
26) Rubio D, Garcia-Castro J, Martin MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A: Spontaneous human adult stem cell transformation. Cancer Res, 65: 3035-3039, 2005.