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Dezawa M, Nabeshima Y-I	Transdifferentiation systems in bone marrow stromal cells and its application to muscle dystrophy: Insights into cell-based therapy.	*"Recent Advances of Skeletal Muscle Differentiation" in Research Signpost*			in press
Dezawa M	Induction system of neuronal and muscle cells from bone marrow stromal cells and applications for degenerative diseases.	*Inflammation and Regeneration*	27(2)	96-101	2007

Dezawa M	The unexpected discovery of neuronal and muscle induction systems in bone marrow stromal cells; insights into auto-transplantation therapy.	*Medical Molecular Morphology*			in press
Kidata M & Dezawa M	Differentiation system of neural and muscle lineage cells from bone marrow stromal cells; a new strategy for tissue reconstruction in degenerative diseases	*Histology and Histopathology*			in press
国府田正雄、出澤真理	骨髄間葉系細胞を用いた脊髄損傷修復の試み	*脳２１*	10(2)	144-147	2007
石川裕人、出澤真理	視神経移植とは	*視覚と眼球運動のすべて*		216-221	2007
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出澤真理	胚葉をこえた多分化能と自己細胞移植治療への可能性	*蛋白質核酸酵素シリーズ「幹細胞技術の現状と展望」*	52(2)	158-165	2007
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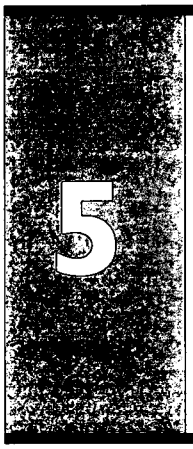

Transdifferentiation system in bone marrow stromal cells and its application to muscle dystrophy: Insights into cell-based therapy

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Abstract

Many kinds of cells, including embryonic stem cells and tissue stem cells, have been considered candidates for cell transplantation therapy for muscle-degenerative diseases. Bone marrow stromal cells (MSCs) also have great potential as therapeutic agents since they are easily isolated and can be expanded from patients without serious ethical or

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technical problems. Recently, new methods for the highly efficient and specific induction of functional skeletal muscle cells have been found in MSCs. Induced cells differentiate into muscle fibers upon transplantation into degenerated muscles of rats and mdx-nude mice. Furthermore, the induced population contained Pax7-positive cells that contribute to subsequent regeneration of muscle upon repetitive damage without additional transplantation of cells. Here we describe the discovery of these induction systems and focus on the potential use of MSC-derived cells for cell-based therapy in muscle-degenerative diseases.

Introduction

Muscle degenerative diseases, such as muscle dystrophy, are responsible for a decline in muscular function, which limits life span. While transplantation of the liver, kidney, and bone marrow has already been performed on thousands of patients, transplantation of the general muscle tissue has faced many limitations. Thus, it is hoped that effective therapeutic strategies will be developed. As for muscle tissue, satellite cells are considered stem cells in adult muscle tissue, although the difficulty in isolating a sufficient number of pure satellite cells has precluded their use in cell-based tissue repair [1-3]. Furthermore, there is a need to establish cell therapies based on healthy donors since muscle dystrophies are inheritable diseases.

Recently, ES cells and tissue stem cells have aroused a great deal of interest because of their potential for treating degenerative diseases. ES cells are known to differentiate into various kinds of cells including skeletal muscle cells, either by spontaneous differentiation or by certain induction methods [4, 5].

Tissue specific stem cells are identified in various tissues of more advanced developmental stages. Stem cells and satellite cells isolated from adult and prenatal muscle tissue [1-3] and myogenic stem cells from the bone marrow [6, 7] are considered to be sources of cell replacement, and there have been several attempts to ameliorate muscle degeneration by transplantation of these muscle stem cells [6]. Although tissue stem cells have great potential, they face limitations inherent in procurement from fetal tissue, including problems of histocompatibility and of ethical concerns. Recently mesangioblast, one type of adult mesenchymal stem cell, has generated particular interest and expectation since it offers sufficient myogenic cells for use in therapy [8].

The bone marrow contains a category of nonhematopoietic mesenchymal cells that can be cultivated *in vitro* as plastic adherent cells, namely bone marrow stromal cells (MSCs) [9]. MSCs are mesenchymal elements that normally provide structural and functional support for hematopoiesis and express mesenchymal markers [10, 11]. The great benefit of MSCs is that they are easily accessible through aspiration of the bone marrow from patients. This strategy avoids ethical issues, enabling us to use them for "auto-cell transplantation therapy". Other than this, MSCs with same HLA subtype is obtainable from

healthy donors in marrow bank or from relatives. They are also easily expanded in a large scale; for example, 20-100 ml of bone marrow aspirate provides 10^7 cells within two to three weeks, a plentiful number of cells for transplantation.

At the present time, the efficacy of MSCs for transplantation therapy is twofold. First, the transient trophic effect of MSCs can delay cell death and restore the tissues [12-14]. Second, the multipotency of MSCs gives rise to "cells with a purpose" for cell-based transplantation therapy. According to a hierarchical paradigm, MSCs differentiate into mesenchymal lineage cells such as osteocytes, chondrocytes and adipocytes [9, 15, 16]. Recently, however, the unorthodox plasticity of MSCs has been described as they have the ability to cross oligolineage boundaries, which were previously thought to be impenetrable. In fact, it has been suggested that various kinds of cells are inducible from MSCs both *in vivo* and *in vitro*. The possibility of MSC plasticity and transdifferentiation into muscle cells was initially described in *in vivo* experiments, where transplanted donor bone marrow-derived cells integrated into the recipient tissue and supported regeneration [6]. While this study suggested the plasticity of MSCs because of the expression of donor markers and cell specific markers, however, the clonality and functions of these transdifferentiated cells were not clearly estimated in some cases. Moreover, these phenomena have been suspected to be based on cell fusion or spontaneous trans-differentiation with extremely rare frequency [17, 18].

Apart from these *in vivo* experiments, there have been several *in vitro* attempts to induce MSCs into purposeful cells such as cardiomyocytes with cardiac muscle properties, hepatocytes, insulin-producing cells and airway epithelial cells. However, some of these reports had lower induction efficiency [19-22]. Indeed, the potential of MSCs to transdifferentiate from mesenchymal lineages to other lineages is now of great interest. It is clear that MSCs will represent good candidates for practical cell-based therapy if their differentiation into target cells can be controlled with high efficiency and purity.

Recently, a method was developed which systematically induced skeletal muscle cells from human and rat MSCs on a therapeutic scale [23]. This review describes the process of discovery of systemic induction, the properties of induced cells, and finally their potential, advantages and disadvantages for clinical application in muscle-degenerative diseases.

I. The process of discovery

The finding of muscle induction system from MSCs owes its properties to the fruit of an unexpected discovery. The initial goal of this MSC study was to develop an efficient Schwann cell induction system from MSCs for application to spinal cord injury. As described previously, induction of Schwann cells was finally established using a reducing reagent, retinoic acid, and trophic factors related to Schwann cell development (see other review) [24, 25] (Fig. 1).

However, Dezawa et al first tried to induce Schwann cells from MSCs by introducing glial instructive factor Notch gene. The Notch gene encodes a 300 KD single transmembrane cell-surface receptor protein that is activated by Delta/Serrate/Lag-1 ligands presented by neighboring cells [26]. Upon ligand binding, the intracellular portion of the Notch receptor is cleaved and enters the nucleus, where it influences the expression of numerous transcription factors related to progenitor pool maintenance, cell fate, and, in the case of the nervous system, terminal specification as glial cells [26-28]. In fact, a series of studies have shown that when Notch signaling is activated, astrocytes and Schwann cells differentiate from neural stem cells (NSCs) and neural crest stem cells, respectively [27, 28]. However, it was very surprising to see neuronal cells induced in the final product by introducing Notch gene followed by trophic factor treatment related to neurogenesis such as basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF) and forskolin known to upregulate intracellular camp [29]. While it was quite accidental, this method was found to induce functional post-mitotic neurons without containing glial cells from MSCs (Fig.1).

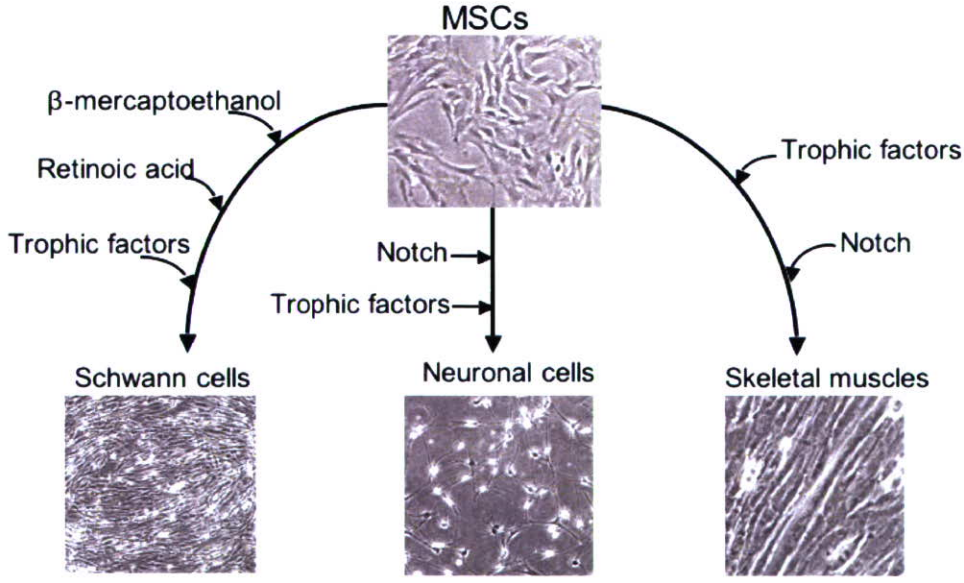

Figure 1. Schematic diagram of induction system from MSCs. Schwann cells could be induced by treatment with beta-mercaptoethanol, retinoic acid followed by trophic factor administration of bFGF, forskolin, PDGF and neuregulin. In the final step, MSCs became similar to Schwann cells, and express Schwann cell markers of p75. Neurons are induced by Notch intracellular domain gene transfer followed by trophic factor administration of bFGF, FSK and CNTF. The final population is consisted mostly of neurons immunopositive to neuronal markers such as neurofilament. Skeletal muscle lineage cells could be obtained by trophic factor treatment of bFGF, FSK, PDGF and neuregulin, followed by Notch gene transfer.

During the experiment of neural induction, the order of treatment was just reversed for the control experiment (Fig.1). Again, the surprising phenomenon of muscle differentiation, small number of slender cells containing two to three nuclei, could be recognized in the culture dish. Considering the advantages of MSCs, this phenomenon was expected to develop the large-scale induction system of skeletal muscle cells from patient's own MSCs. Thus, the induction experiment was repeated, and finally a new method to systematically and efficiently induce skeletal muscle lineage cells with high purity from large population of MSCs was established [23].

II. Induction systems of skeletal muscle cells from MSC

Human and rat MSCs were passaged at least for three times, and then plated on plastic dishes at 1,700~1,900 cells/cm^2. They were first treated with the trophic factors bFGF, FSK, platelet-derived growth factor (PDGF) and neuregulin for three days. After this treatment (C-MSCs), Pax7 expression could be recognized in MSCs (Fig.2). They were then transfected with a plasmid expression vector containing constitutive active form of Notch gene (The mouse Notch1 intracellular domain (NICD) cDNA was subcloned into

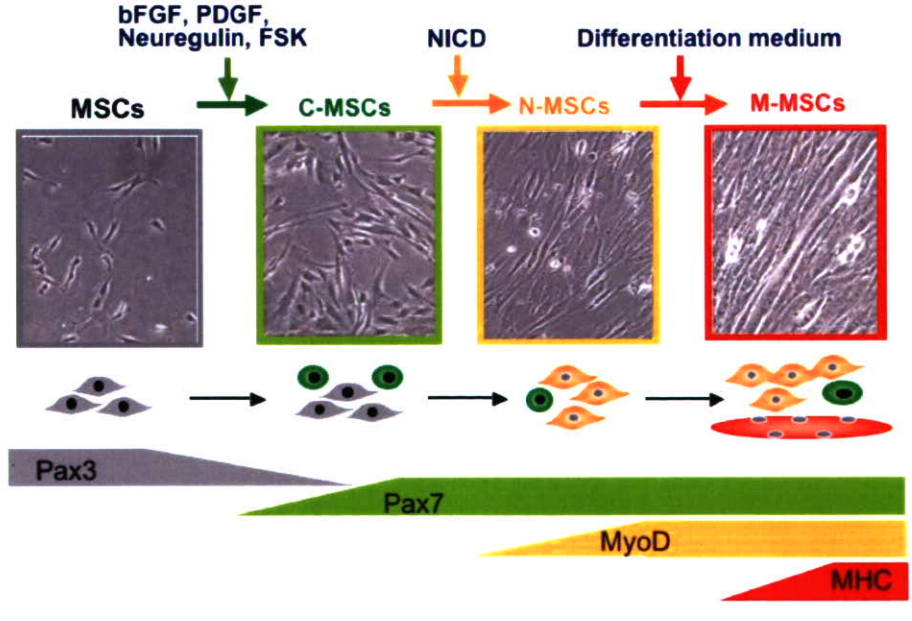

Figure 2. Induction of skeletal muscle cells from MSCs (human). MSCs originally express Pax3 become positive to Pax7 after trophic factor stimulation (C-MSCs). NICD transfection induced MyoD- and myogenin expression in N-MSCs. These N-MSCs fuse to form multinucleated myotubes by differentiation medium, expressing the marker of maturity, such as myosin heavy chain (MHC).

pCI-neo, a cytomegalovirus promoter-containing mammalian expression vector) by lipofection followed by G418 selection, and allowed to recover to 100% confluency. At this stage (N-MSCs), a large majority of MSCs developed into mononucleated myogenic cells expressing MyoD and myogenin, while a small population of Pax7 (+) satellite-like cells also existed (Fig.2). Cells were then supplied with a differentiation medium of either 2% horse serum, Insulin-Transferrin-Selenite (ITS)-serum free medium or the supernatant of the original untreated MSCs [23], and the final muscle lineage population (M-MSCs) was acquired (Fig.2). M-MSCs contained three kinds of muscle-lineage cells. The first population included post-mitotic multinucleated myotubes, which expressed myogenin, Myf6/MRF4 (a marker for mature skeletal muscle) and contractile proteins of skeletal myosin, myosin heavy chain, and troponin, all related to skeletal muscle characteristics. In fact, some multinucleated cells exhibited spontaneous contraction *in vitro*. They are also positive for p21, a marker for post-mitotic muscle lineage cells. The second group was mononucleated myoblasts which expressed MyoD and myogenin. The third group was composed of satellite-like cells and were immunopositive for Pax7 and c-Met, both markers for muscle satellite cells [23].

However, it is critical to determine if these MSC-derived skeletal muscle cells integrate into the host tissue and are genuine muscle cells. In the following sections, the effectiveness of these induced cells is verified by a transplantation experiment using animal models of muscle degeneration and dystrophy.

III. Mechanism of induction

To examine the induction events leading from MSCs to M-MSCs, we investigated the expression of genes related to myogenesis in these cells by RT-PCR [23]. Before trophic factor treatment, MSCs expressed Pax3, Six1 and Six4 while Pax7, MyoD and myogenin were not. After treatment with trophic factors bFGF, FSK, PDGF and neuregulin (C-MSCs), Pax3 was down-regulated instead Pax7 expression was recognized which persisted after NICD introduction (N-MSCs) and final population of M-MSCs. Expression of MyoD and myogenin was firstly detectable in N-MSCs and persisted in the M-MSCs. These results were also confirmed by Western analyses. Myf6/MRF4, a marker for mature skeletal muscle, was detectable only in the final MSC-M population. While expression of Six1 and Six4 persisted for the entire period, another myogenic factor, myf5 was not detected in any induction step. In this way, the induction process mimicked some aspects of conventional skeletal muscle development since Pax3, Pax7, MyoD, Myogenin and Myf6/MRF4, all of which are related to muscle development [30-33], could be detected in a sequential manner. However, as MSCs used in this induction system possess different characteristics from the conventional myogenic progenitor cells, it is

possible that some of mechanisms should differ, especially in the initial step converting MSCs to MyoD-positive N-MSC population. For this initial step, cytokine pre-treatment and the subsequent NICD transfection are critical and required for MSC-derived cells to acquire competence for myogenic induction. In fact, when we reversed the order of cytokine treatment and NICD transfection, muscle-lineage markers were not detected nor were multinucleated cells observed.

It is well established that Notch signaling inhibits myogenic differentiation; Delta1/Jagged1 inhibits MyoD expression, blocks the differentiation of myoblasts, and prevents the formation of myotubes [34, 35]. Hes 1/5, downstream effectors of Notch, are reported unrelated to the inhibition of the myogenic pathway in C2C12 myoblasts, while others report that Hes1 up-regulation results in the prevention of myogenesis [36, 37].

We examined the expression of Hes family members to judge whether conventional Notch pathway was activated in our induction process [38-40]. The expression of Hes 1/5 was not significantly upregulated by NICD transfection (N-MSCs). The forced expression of Hes 1/5 in place of NICD failed to induce skeletal muscle lineage cells, suggesting that Hes 1/5 signaling is not involved in the muscle induction event in MSCs. Hes 6, another Hes family member known to induce the myogenic differentiation program, was slightly up-regulated, while muscle induction by the forced expression of Hes6 in place of NICD could barely elicit muscle lineage cells.

In our induction system, NICD transfection up-regulated MyoD while it has been shown to inhibit myogenic differentiation in cultured muscle cells and in the embryo [34, 35]. We re-expressed NICD in rat N-MSCs and analyzed MyoD expression. N-MSCs were transfected with pCI-neo-NICD by lipofection, followed by G418 selection, and were brought to RT-PCR. Interestingly, the down-regulation of MyoD was recognized after re-expression of NICD in N-MSCs as well as in C2C12 cells. Furthermore, after the re-expression of NICD, cells were subjected to differentiation medium containing 2% horse serum to analyze myotube formation. The differentiation into multinucliated myotubes was significantly suppressed by re-expression of NICD in N-MSCs as well as C2C12 cells. These results collectively suggest that cellular response to NICD in MSCs is different from that of conventional myogenic progenitor cells, but once they differentiate into myogenic lineage cells by this induction system, they behave like real myogenic cells such as C2C12 cells [34, 35].

Our results showing that NICD introduction accelerates the induction of skeletal muscle cells from MSCs are surprising from the viewpoint of conventional Notch signaling in myogenesis. We consider our results do not refute the known role of Notch-Hes signals in myogenesis, but rather reflecting the distinct cellular responses of MSCS to Notch signals; for example, the

repertoire of proteins, second messengers and other active factors may well be quite different between conventional myogenic progenitor cells and MSCs. Notably, as described above, we observed the induction of neuronal cells from MSCs by NICD introduction. A yet unknown signaling pathway downstream of Notch may be involved in these events. Further studies are nevertheless needed to identify the factor involved in this phenomenon.

Bone marrow (mostly hematopoietic cells) contains a small population of myogenic stem cells known to express c-Kit, CD45 and CD34 [1-3, 7, 41, 42]. Hematopoietic cells are generally non-adherent and cells we used were adherent MSCs. However, even though we used adherent MSCs, several percent of cells are positive to above markers. To exclude the possibility that the production of muscle-lineage cells was due to the vast proliferation of myogenic stem cells contained in MSCs, human MSCs negative for c-Kit, CD45 and CD34 were isolated by FACS and subjected to the induction process [43]. We confirmed that isolated cells could also be driven to be muscle-lineage cells as efficiently as the unsorted MSCs. Therefore, in our system, it appears that it is not a small fraction of bone-marrow-derived myogenic stem cells, but rather the major population of MSCs contribute to the production of muscle lineage cells.

IV. Application of M-MSCs to muscle degenerative disease model

As induced multinucleated myotubes in M-MSCs are already post-mitotic, single cells of MyoD-positive myoblasts and Pax7-positive satellite cells were subjected to clonal culture (clonal M-MSCs) to exclude non-muscle cells and transplanted into muscle degenerative disease models [43]. To estimate how workable these clonally-cultured M-MSCs are in the repair of degenerated muscles, human cells were transplanted into immunosuppressed rats whose gastrocnemius muscles were damaged with cardiotoxin pretreatment [43]. Cells were labeled by means of a GFP-encoding retrovirus and then transplanted by local injection (L.I.) into muscles or by intravenous injection (I.V.). Two weeks after transplantation, GFP-labeled cells incorporated into newly formed immature myofibers, exhibited centrally located nuclei in both L.I. and I.V. treated animals. The ratio (%) of GFP (+) fibers in total fibers (1500 fibers with centrally located nuclei were counted for each sample) was 37.1±9.9 % in L.I. and 22.6±7.9 % in I.V. Four weeks after transplantation, GFP-positive myofibers exhibited mature characteristics with peripheral nuclei just beneath the plasma membrane. Functional differentiation of grafted human cells was also confirmed by the detection of human dystrophin in GFP-labeled myofibers. These findings indicate that clonal-M-MSCs are able to incorporate into damaged muscles and contribute to regenerating myofiber formation, regardless of the transplantation method [43].

Clonal M-MSCs contained Pax7-positive satellite cells which integrated into the satellite cell position after transplantation, namely the plasma membrane and the basal lamina inbetween [43]. The ratio of Pax7/GFP (+) cells in total Pax7-positive cells at 2 weeks was 17.2±4.2 % in L.I. and 5.9±2.8 % in I.V. In general, muscle satellite cells are known to contribute to the regeneration of myofiber formation upon muscle damage [44]. To confirm the contribution of transplanted satellite cells to muscle regeneration as *in vivo* satellite cells, the following experiment was performed. Four weeks after the initial transplantation of human clonal-M-MSCs intraveneously, cardiotoxin was re-administered into the same muscles without additional transplantation. Two weeks after the second cardiotoxin treatment (6 weeks after initial transplantation), many regenerating GFP-positive myofibers with centrally-located nuclei were observed. This implies that, upon transplantation of clonal-M-MSCs to the muscles of patients, those retained as satellite cells should be able to contribute to future muscle regeneration [23].

Transplantation of muscle lineage cells is a potential therapeutic approach for muscle degenerative disorders such as Duchenne muscular dystrophy (DMD), a severe progressive muscle wasting disease that results from a mutation in the dystrophin gene. The *mdx*-mouse, an animal model for DMD, was used for this experiment. The *mdx*-mouse is characterized by the absence of the muscle membrane associated protein, dystrophin. We locally injected GFP-labeled human clonal-M-MSCs into cardiotoxin-pretreated muscles of *mdx*-nude mice. Immunohistochemistry revealed the incorporation of transplanted cells into newly formed myofibers which expressed human dystrophin after transplantation as same as in case of above rat experiment [23].

V. Perspective

Cell transplantation therapy also offers hope for the treatment of intractable muscle degenerative disorders. Indeed, ES cells, stem cells derived from adult and prenatal muscle tissues, and myogenic stem cells from bone marrow are powerful candidates for transplantation therapy [1-5, 41]. Compared to these sources, the MSC system offers several important advantages. Firstly, 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. MSCs provide hopeful possibilities for clinical application, since they can efficiently expand *in vitro* and a therapeutic scale of induced cells are available. Thus functional skeletal muscle cells can be obtained within a reasonable time course on a therapeutic scale. Secondary, transplantation of MSC-derived cells should pose fewer ethical problems than ES cells and other kinds of stem cells, since bone marrow transplantation has already been widely performed. Hopefully, this MSC differentiation system may contribute substantially to eventual cell-based therapies for muscle disease.

Transplantation of untreated MSCs is reported to be effective to various kinds of degenerative models. In these reports, MSCs or cells derived from bone marrow are sometimes observed to penetrate into host tissue and thereby differentiate as mature neurons and skeletal muscle cells and so on [45, 46]. However, the ratio of so called "spontaneous differentiation" or "transdifferentiation" is extremely low and thus cannot be expected to the clinical application. Rather, transplantation of MSCs may contribute to the functional recovery in degeneration models by trophic supply, since they are known to produce various kinds of cytokines and trophic factors [47]. Needless to say, substantial supply of lost cells is crucial to the cell based therapy in degenerative diseases such as muscle dystrophy. Therefore, it is desirable to develop a systematic induction system to obtain large amounts of purposeful cells those confirmed to be morphologically and physiologically functional. Moreover, the practical application to human degenerative diseases depends on the ability to control their differentiation into functional cells with high efficiency and purity. As mentioned, 10^7 MSCs can be harvested from 20-100 ml of bone marrow aspirate within two to three weeks. If an induction procedure takes the shortest and most perfect course, 10^7 MSCs give rise to nearly 10^7 skeletal muscle cells within 5-7 weeks when taking into account the term necessary for NICD introduction, G418 selection and trophic factor administration. Therefore these induction systems may be useful since large amounts of purposeful cells can be obtained from the bone marrow for transplantation therapy within a reasonable time course.

Considering the advantages of MSCs, we can expect the possibility of establishing "auto-cell transplantation system" in muscle dystrophy (Fig.3). Nevertheless, the major matter is that how to replace the mutated gene in patient's MSCs. Probably, genetic manipulation is possible after the isolation and expansion of MSCs. Without resolution of this matter, our system will not lead to the fundamental "auto-cell transplantation therapy" in such hereditary disease. Another way is to utilize MSCs with the same HLA subtype from a healthy donor, namely allo-cell transplantation. This method may minimize the risks of rejection and be more realistic way for the clinical application. Needless to say, the bone marrow should at least be 'normal and healthy' for transplantation (Fig.3).

There are several problems that need to be solved in the future. First, while there have been few reports of tumor formation after transplantation of untreated MSCs, further studies are needed to ensure the safety and efficacy of manipulated MSCs over a long period using primates and nude-mice/rats. In fact, recent reports have raised the possibility of transformation in the long term cultivation of MSCs [48, 49]. Furthermore, yet we introduced NICD by plasmid but not by retrovirus or lentivirus vectors, the safety of induced cells should carefully be estimated. Although the expression of introduced NICD

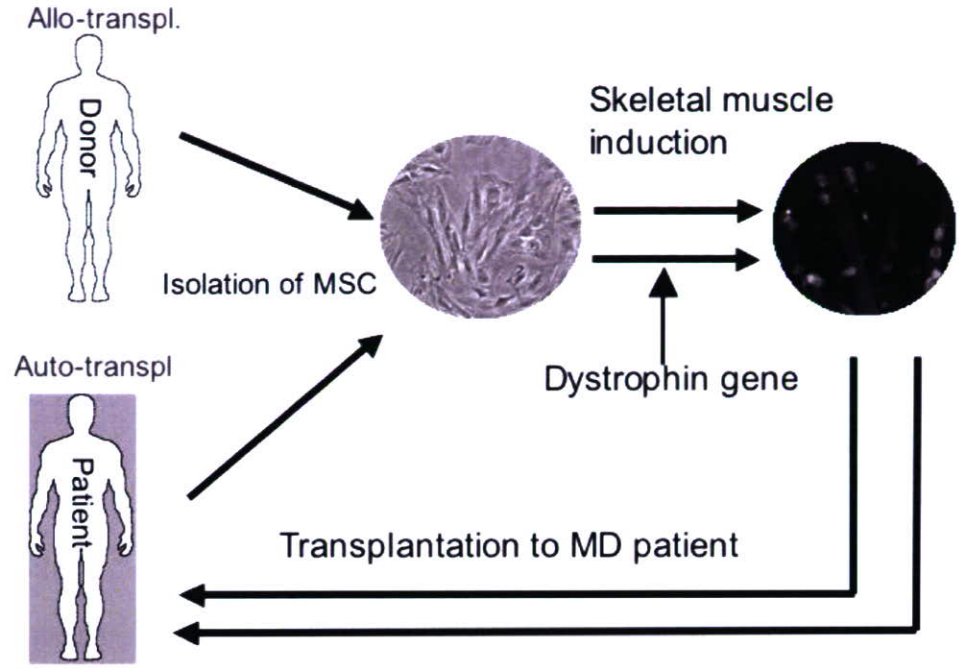

Figure 3. Schematic diagram of Allo- and Auto-transplantation therapy based on MSC-derived skeletal muscle cells. MSCs are isolated either from healthy donor with same HLA subtype or from patient and are subjected to the muscle induction. Those cells are transplanted back to the muscle dystrophy patient. "Auto-transplantation" system escapes not only from ethical problem but also from immuno-rejection. However, the replacement of the mutated gene is necessary in this case.

was very faint by RT-PCR in clonal-M-MSCs probably due to the diluting out of the transfected NICD plasmid, it would be more desirable to establish alternative system using protein introduction or signal activation. Second, as the potential differentiation may differ with age, individual, race, and sex, each of these characteristics must be examined in the future. Finally, 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 accuracy. Finally, the efficiency and safety of this system need to be examined using primate and higher mammal models such as dystrophy dog.

From the point view of basic research, the role of NICD in myogenic differentiation of MSCs needs to be clarified. As this induction was also suggested to be independent of Hes1/5 actions and the conventional Notch signaling pathway, it will be reasonable to consider that distinct cellular responses to Notch signals; for example, the repertoire of second messengers and active factors in MSC may well be different from conventional myogenic

precursor cells, or the susceptibility of MSCs to the Notch signal is probably different from that of known myogenic precursor cells. Thus further studies are needed to identify the factor involved in this phenomenon.

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Neuroprotective Effect of Bone Marrow–Derived Mononuclear Cells Promoting Functional Recovery from Spinal Cord Injury

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ABSTRACT

Neural cell transplantation, a new therapeutic strategy for replacing injured neural components and obtaining functional recovery, has shown beneficial effects in animal models. Use of this strategy in human patients, however, requires that a number of serious issues be addressed, including ethics, immunorejection, and the therapeutic time window within which the procedure will be effective. Bone marrow–derived mononuclear cells (BM-MNC) are attractive for transplantation because they can be used as an autograft, can be easily collected within a short time period, and do not have to be cultured. In a rat model of spinal cord injury (SCI), we transplanted BM-MNC at 1 h after SCI at Th 8–9 by injecting them into the cerebrospinal fluid (CSF), and investigated the effect of this on neurologic function. In the acute stage of injury, we found a neuroprotective antiapoptotic effect, with an elevated concentration of hepatocyte growth factor in CSF. At 1 week after transplantation, the Basso–Beattie–Bresnahan locomotor score had increased significantly over its baseline value. In the chronic stage of injury, we observed suppressed cavity formation and functional improvement. We conclude that transplantation of BM-MNC after SCI has a remarkable neuroprotective effect in the acute stage of injury, suppressing cavity formation, and contributing to functional recovery. Our results suggest that transplantation of BM-MNC via the CSF is a potentially effective means of enhancing functional recovery after SCI in humans.

Key words: bone marrow–derived mononuclear cell; cell transplantation; cerebrospinal fluid; neuroprotection; spinal cord injury

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