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Review

Insights into autotransplantation: the unexpected discovery of specific induction systems in bone marrow stromal cells

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Abstract. Many kinds of cells, including embryonic stem cells and tissue stem cells, have been considered candidates for transplantation therapy for neuro- and 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 technical problems. Recently, new methods for the highly efficient and specific induction of functional neurons and skeletal muscle cells have been developed for MSCs. These induced cells were transplanted into animal models of stroke, Parkinson's disease and muscle degeneration, resulting in the successful integration of transplanted cells and improvement in the behavior of the transplanted animals. Here I describe the discovery of these induction systems and focus on the potential use of MSC-derived cells for 'autocell transplantation therapy' in neuro- and muscle-degenerative diseases.

Keywords. Mesenchymal cell, transdifferentiation, regenerative medicine, cell therapy, transplantation, Schwann cell, neuronal differentiation, myogenic differentiation.

Introduction

Neurodegenerative diseases, such as Parkinson's disease and brain ischemia, and muscle-degenerative diseases, such as muscular dystrophy, are responsible for a decline in neuronal and muscular function which often limits the life span. While transplantation of liver, kidney, and bone marrow has already been performed on thousands of patients, transplantation of the nervous system and general muscle tissue has faced many limitations. Effective therapeutic strategies still need to be developed. In the central nervous system (CNS), where neurons become post-mitotic after birth, neural cell transplantation is one potential treatment of such neurologic disorders. As for muscles, satellite cells are considered stem cells in adult muscle tissue, although the difficulty isolating a sufficient number of pure satellite cells has precluded their use in cell-based tissue repair [1, 2] Furthermore, there is a need to establish cell therapies using healthy donors since muscle dystrophies are inheritable diseases.

Recently, embryonic stem (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 neurons and skeletal muscle cells, either by spontaneous differentiation or following certain induction methods [3–5].

Tissue-specific stem cells have been identified in various tissues at more advanced developmental stages. Neural precursors and/or progenitors have been identified in developing and adult CNS tissues [6–10]. These cells have the ability to self-renew and the potential to differentiate into neurons, astrocytes and oligodendrocytes. For neuronal cell replacement, transplantation of neural stem cells (NSCs) has been attempted in a wide range of animal models of diseases and injuries such as Parkinson's disease, Huntington's disease, stroke, and spinal cord injury,

and functional improvement has often been reported [11–15]. Stem cells and satellite cells isolated from adult and prenatal muscle tissue [16–19] and myogenic stem cells from bone marrow [20, 21] are considered to be sources of cell replacement, and several attempts have been made to ameliorate muscle degeneration by transplantation of these muscle stem cells [20]. Although tissue stem cells have great potential, they face limitations inherent in procurement from fetal tissue, including problems of histocompatibility and ethical concerns.

Bone marrow contains a category of nonhematopoietic cells that can be cultivated *in vitro* as plastic adherent cells, namely bone marrow stromal cells (MSCs) [22]. MSCs are mesenchymal elements that normally provide structural and functional support for hematopoiesis and express mesenchymal markers but lack hematopoietic surface markers [23, 24]. 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'. They are also easily expanded on a large scale; for example, 20–100 ml of bone marrow aspirate provides 10^7 cells within 2–3 weeks, a plentiful number for transplantation.

At the present time, the benefits of MSCs for transplantation therapy are twofold. First, the transient trophic effect of MSCs can delay cell death and restore the tissues [25–29] and, 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 [22, 30, 31]. 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 was initially described in *in vivo* experiments, where transplanted donor bone marrow-derived cells differentiated into glial cells in the recipient brain [32]. In the case of muscle, infused bone marrow cells integrated into host muscles and supported regeneration [20]. While these studies suggested the plasticity of MSCs because of the expression of donor markers and cell-specific markers, the clonality and functions of these transdifferentiated cells were not clearly evaluated in some cases. Moreover, there has been the suspicion that these phenomena are based on cell fusion or spontaneous transdifferentiation at a very low frequency [33, 34].

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 a low induction efficiency [35–38]. Nevertheless, 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 neurons, skeletal muscle cells (Fig. 1) and Schwann cells from human and rat MSCs on a therapeutic scale [39–41]. This review describes the discovery of systemic induction, the properties of induced cells, and finally their potential, advantages, and disadvantages for clinical application in neurodegenerative and muscle-degenerative diseases. Schwann cells, peripheral glia known to support axonal regeneration both in the peripheral nervous system (PNS) and CNS, are also inducible from human and rodent MSCs [39, 42, 43]. MSC-derived Schwann cells elicited axonal regeneration and functional recovery in spinal cord injury. The utility of these induced Schwann cells has been reviewed elsewhere [44, 45].

Systems for inducing neurons and skeletal muscle cells from MSCs; the fruit of unexpected discovery

Specific induction of neurons from MSCs

Recently, my research team established a new method to induce neurons systematically from human and rat MSCs. Highly efficient and specific induction of post-mitotic, functional neuronal cells, without glial differentiation, can be achieved by gene transfer of Notch1 intracellular domain (NICD) followed by the administration of certain trophic factors [40] (Fig. 1). However, all these findings are 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. A series of experiments demonstrated that transplanted Schwann cells can delay nerve cell death and promote regeneration of nerve fibers and functional recovery when supplied to the damaged spinal cord [46]. However, it is difficult to obtain a sufficient amount of Schwann cells. To cultivate Schwann cells for autologus transplantation in humans, for example, another PNS must be sacrificed. Furthermore, there are other technical difficulties in harvesting and expanding Schwann cells from PNS. Therefore, it would be more desirable to establish cells with Schwann cell characteristics from sources other than the PNS that are easily accessible and capable of rapid expansion. MSCs were thought to be a good candidate.

As described previously, induction of Schwann cells was finally established using a reducing reagent, retinoic acid, and trophic factors related to Schwann cell development [44, 45]. However, I first tried to induce Schwann cells

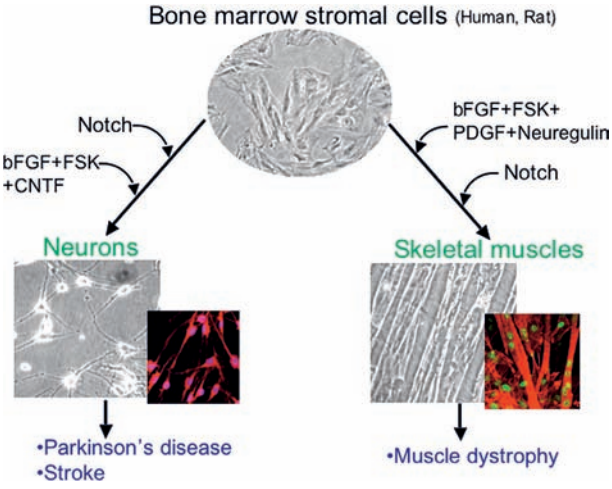

Figure 1. Schematic diagram of the induction system for neurons and skeletal muscle cells. Neurons are induced by Notch intracellular domain gene transfer followed by administration of trophic factors basic fibroblast growth factor (bFGF), forskolin (FSK) and ciliary neurotrophic factor (CNTF). The final population consisted mostly of neurons immunopositive for neuronal markers such as neurofilament. Skeletal muscle cells could be obtained by the reverse treatment, namely trophic factor treatment with bFGF, FSK, Platelet-derived growth factor (PDGF) and neuregulin, followed by Notch gene transfer.

from MSCs by Notch transfection. The Notch gene encodes a 300-kDa single transmembrane cell surface receptor protein that is activated by Delta/Serrate/Lag-1 ligands presented by neighboring cells [47]. 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 [47–49]. In fact, a series of studies have shown that when Notch signaling is activated, astrocytes and Schwann cells differentiate from NSCs and neural crest stem cells, respectively [48, 49]. Initially, it was expected that MSCs would shift from mesenchymal to Schwann cell characteristics by Notch introduction when combined with administration of trophic factors related to Schwann cell development, such as basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF) and forskolin (FSK), known to upregulate intracellular cyclic AMP [50]. After such treatment, however, it was very surprising to see a small population of neuron-like cells induced in the final product. The experiment was repeated and the original method was improved to establish the neuronal induction system from MSCs and to examine the properties of induced cells.

The mouse NICD cDNA was subcloned into pCI-neo, a cytomegalovirus (CMV) promoter-containing mammalian expression vector, and transfected into human and rat MSCs by lipofection followed by G418 selection [40]. After transfection with NICD, the MSCs substantially upregulated markers related to NSCs and/or neuronal progenitor cells (NPCs), such as the glutamate transporter GLAST, 3-phosphoglycerate dehydrogenase (3-PGDH) and nestin [51, 52]. This suggested that MSCs may acquire some of the characteristics of NSCs/NPCs when NICD is introduced (Fig. 2).

Next, cells were subcultured once (60–70% confluence) with administration of the trophic factors bFGF, FSK, and CNTF for 5 days, which resulted in a highly efficient and specific induction of cells with neuronal characteristics (Fig. 2) [40]. It was crucial that the cell density of NICD-transfected MSCs be reduced by subculture just before the administration of trophic factors. Some cells already started to extend neurite-like processes 6 h after trophic factor administration. However, if the cell density was too high, neurites attached to the neighboring cells soon after their extension, thereby retracting their neurites and preventing the differentiation of a neuron-like morphology. Therefore, adequate intercellular distance and timing of trophic factor stimulation are crucial for the MSCs to become neurons. Nontransfected as well as control vector-transfected MSCs could not be induced to neurons by trophic factors, indicating that NICD transfection is necessary for MSCs to acquire neuronal potential [40].

These MSC-derived neuronal cells (MSC-Ns) extended neurite-like processes with abundant varicosities and expressed neuronal markers such as MAP-2ab, neurofilament-M, and beta-tubulin isotype3. Approximately 96% of cells were immunopositive for MAP-2ab, although nearly 2% of nestin-positive cells could also be recognized. MSC-Ns did not proliferate when subcultured after trypsin treatment. Indeed, Brd-U incorporation performed 5 days after trophic factor administration showed

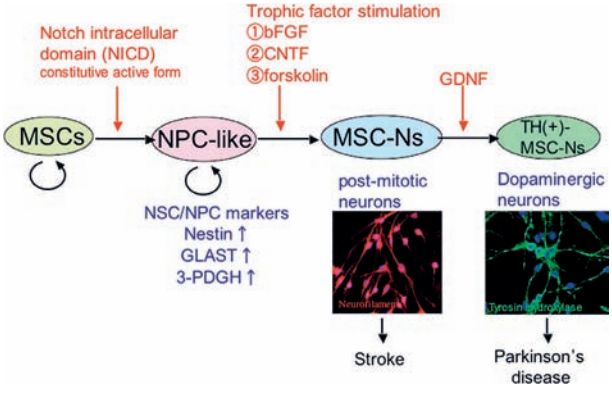

Figure 2. An outline of the neuronal induction system. After NICD transfection, MSCs become similar to NSCs/NPCs (NPC-like), since they express nestin, GLAST and 3-PGDH. After trophic factor stimulation, cells became post-mitotic neurons (MSC-Ns) expressing neuronal markers such as neurofilament. These neurons are effective in the stroke rat model. After administration of GDNF, post-mitotic neurons became dopamine-producing cells [TH(+)-MSC-Ns], useful in the Parkinson's disease model.

that few MAP-2ab-positive cells incorporated Brd-U. In addition, less than 1% of MAP-2ab-positive cells were immunoreactive to an intrinsic proliferation associated marker, Ki67, suggesting that the majority of MSC-Ns were post-mitotic [40].

MSC-Ns were evaluated physiologically using the voltage clamp method. Seven days after trophic factor induction, an outwardly rectified K^+ current was elicited by positive voltage steps in MSC-Ns, which was dramatically higher than in untreated MSCs. Concomitantly, resting membrane potential was lowered. However, the voltage-gated fast sodium currents, which represent functional neuron characteristics, could not be observed up to 14 days after trophic factor induction, suggesting that although MSC-Ns exhibit a neuron-like morphology and express several neuronal markers, they are not fully mature neurons but rather are in a process of maturation. Neurotrophins such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) which were administered to MSC-Ns to promote their maturation, resulted in the generation of sodium currents and action potentials in the small population of MSC-Ns. These results indicated that, just after trophic factor induction, MSC-Ns are neuronal cells in a premature state and can be induced to become functionally mature neurons with further administration of neurotrophins [40].

The final population of MSC-Ns are devoid of glial development. In fact, few cells positive for glial fibrillary acidic protein (GFAP, a marker for astrocytes), galactocerebroside, or O4 (markers for oligodendrocytes) were detected in the final MSC-N population by immunocytochemistry, Western blot or RT-PCR [40]. NSCs/NPCs are known to differentiate into GFAP-positive glial cells when the gliogenic factors Hes1/5 and STAT1/3 are activated, while they differentiate into neuronal cells with activation of proneural genes Mash1, Math1, and neurogenin (Fig. 3a) [53–55]. To examine the induction event from MSCs to MSC-Ns, the expression of those genes was examined by RT-PCR. MSCs initially expressed both neurogenic (Mash1, Math1, and neurogenin1) and gliogenic factors (Hes1/5 and STAT1/3), but during the induction procedure, gliogenic factors were sequentially inhibited and thus finally converged on neuronal factors (Fig. 3b). In fact, STAT1/3 was suppressed after the introduction of NICD and, following trophic factor administration, suppressed Hes1/5 expression (Fig. 3b) [40].

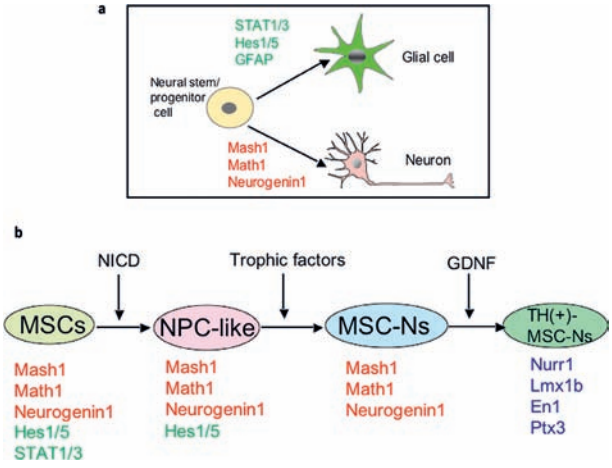

Figure 3. Neurogenic and gliogenic factors in neuronal induction. (*a*) Summary of neurogenic factors and gliogenic factors in conventional neural development. (*b*) Expression of factors during the neuronal induction system.

While it was quite accidental, this method was found to induce functional post-mitotic neurons without glial cells from MSCs. Identification of the molecular mechanism played by NICD in the neuronal induction in MSCs is underway The application of MSC-Ns to stroke and Parkinson's disease is discussed further on in this review.

Specific induction of skeletal muscle cells from MSCs

During the experiment of neural induction, I reversed the order of treatment in the control experiment (Fig. 1). Again, the surpising phenomenon of muscle differentiation could be recognized in the culture dish. The induction experiment was repeated, and finally a new method to systematically and efficiently induce skeletal muscle lineage cells with high purity from a large population of MSCs was established (Fig. 1) [41].

Human and rat MSCs were first treated with the trophic factors bFGF, FSK, PDGF, and neuregulin for 3 days and then transfected with an NICD expression plasmid by lipofection followed by G418 selection, and allowed to recover to 100% confluency (Fig. 4). At this stage, a large majority of MSCs developed into mononucleated myogenic cells expressing MyoD and myogenin, while a small population of Pax7(+) satellite cells also existed. 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 [41], and the final muscle lineage population (termed MSC-Ms) was acquired. MSC-Ms contained three kinds of muscle lineage cells (Fig. 4). 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 were also positive for p21, a marker for post-mitotic muscle lineage cells. The second group comprised mononucleated myoblasts which expressed MyoD and myogenin. The third group was composed of satellite cells which were immunopositive for Pax7 and c-MetR, both markers for muscle satellite cells [41].

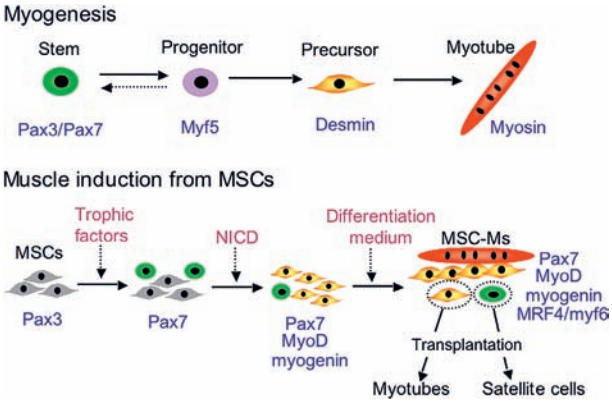

Figure 4. Factors related to myogenesis and the muscle induction system. There is some similarity between conventional myogenesis and the muscle induction system. MSCs generate Pax7-positive precursor cells after trophic factor stimulation and, after NICD transfection, induce MyoD- and myogenin-positive myoblasts. Myoblasts fuse to form multinucleated myotubes in differentiation medium, expressing the marker of maturity, MRF4/myf6. Single myoblast (yellow) and satellite (green) cells were subjected to clonal culture. Clonally isolated myoblasts differentiated into myotubes after transplantation, and clonally isolated satellite cells integrated as muscle stem cells which continued to contribute to muscle regeneration in the host muscle tissue.

However, it was critical to determine if these MSC-derived neuronal and skeletal muscle cells could integrate into host tissue and function as genuine neurons and muscle cells. The effectiveness of these induced cells was verified by a transplantation experiment using animal models of stroke, Parkinson's disease and muscle dystrophy.

Application of MSC-Ns to a stroke model

MSC-Ns were transplanted into the infarction area in a left middle cerebral artery occlusion (MCAO) rat model. The MCAO procedure was somewhat modified in our study, and circling to the right and adduction of the right forelimb when lifted up by the tail were used as signs of successful left MCAO. Seven days after reperfusion, MSC-Ns were transplanted into the nonnecrotic brain parenchyma by stereotaxical injection into the left cerebrum at three locations. The total number of transplanted cells was approximately 40,000–50,000. The control group received only PBS without cell transplantation [56].

MSC-Ns-transplanted rats showed significant recovery, compared with controls, of gross vestibulomotor function (beam balance test), sensorimotor function (limb-placing test), and cognitive function (Morris water maze test) ($p < 0.01$) after 28 days. Histologically, there was no statistical difference in the mean infarct volume between MSC-Ns-transplanted and the control group ($p > 0.05$). However, green-fluorescent protein (GFP)-labeled transplanted cells migrated from the injection site into the ischemic boundary area and integrated mainly into the hippocampus and extended neuritis. Most transplanted cells expressed the neuronal markers neurofilament, MAP-2ab, and beta3-tubulin, while very few cells were positive for GFAP. The reason why cognitive function showed significant recovery may partly be due to the integration of MSC-Ns into the hippocampus [56].

These results showed that MSC-Ns are effective in the amelioration of the rat stroke model. The potential of other kinds of stem cells, such as NSCs and umbilical cord blood cells, in stroke has been reported [57, 58]. These reports indicate that only a small fraction of NSC (1–3% of the grafted cells survived and 3–9% expressed NeuN) or human umbilical cord blood cell (1–2% of injected cells survived and 2–3% were positive for NeuN and MAP-2) populations are expected to integrate into the host brain and differentiate into neurons. Our study showed that approximately 30–45% of MSC-Ns survived in the host brain 1 month after the transplantation and a large fraction expressed the neuronal markers. Thus, the specific induction of neuronal cells from MSCs has great potential in cell transplantation therapy for stroke.

Application of MSC-Ns to the Parkinson's disease model

For Parkinson's disease, transplantation of dopaminergic neurons is believed to be effective; however, cells committed to the expression of certain transmitters account for lower ratios in MSC-Ns [40]. For example, the percentage of tyrosine hydroxylase (TH)-positive cells was approximately 4%, and that of other transmitters such as acetylcholine, glutamate, and substance P fell within a range of 1–3%. As glial cell line-derived neurotrophic factor (GDNF) is known to be involved in the generation and development of midbrain dopaminergic neurons [59], it was administered to MSC-Ns and finally resulted in nearly 40% of MSC-Ns becoming TH-positive cells (Fig. 2). Furthermore, other dopaminergic markers, Nurr-1, Lmx1b, En1, and Ptx3, were elevated (Fig. 3b). The production of dopamine by these TH-positive cells was confirmed by high-performance liquid chromatography (HPLC); high-potassium medium was administered to the culture and subjected to HPLC using a reverse-phase column and an electrochemical detector system, showing that these cells released dopamine to the culture medium in response to high-K^+ depolarizing stimuli. These results indicate that functional dopamine-producing neuronal cells could be induced effectively from MSCs [40].

To explore the ability of induced dopaminergic neurons to survive and function in the host brain, both rat and human cells were transplanted separately into the striatum in a rat model of Parkinson's disease. Unilateral administration of 6-hydroxy dopamine (6-OHDA) into the

medial forebrain bundle is known to selectively destroy dopaminergic neurons in the substantia nigra, leading to quantifiable rotational behavior and providing a useful and commonly used model of Parkinson's disease [60]. Apomorphine-induced rotational behavior (mean rotation index = the mean rotation number in post-/pre-grafting) was examined every 2 weeks up to 10 weeks following cell implantation. 1×10^5 cells were grafted into the ipsilateral striatum. The control group received no grafting after 6-OHDA administration, which provoked a rotational bias away from the lesioned side which persisted, whereas rats grafted with TH-MSC-N rat-induced dopaminergic neurons demonstrated substantial recovery from rotation behavior up to 10 weeks ($p < 0.01$). The mean rotation index was 1.3 ± 0.1 in the control group and 0.3 ± 0.1 in induced dopaminergic neuron-transplanted rats. In addition, nonpharmacological behavior tests, the adjusting step test and paw-reaching test, were performed. Four and 6 weeks after grafting, these rats showed significant improvement in both step adjustment and paw-reaching tests ($p < 0.01$). Immunohistochemically, grafted GFP-labeled, induced dopaminergic neurons were found to migrate and extend beyond the injected site, and approximately 30% of cells remained in the striatum 10 weeks after transplantation. TH-positive processes extended to the outside of the implantation zone. The grafted striatum showed migration of GFP-positive transplanted cells that expressed the markers of neurofilament, TH and dopamine transporter (DAT). Among GFP-labeled cells, TH- and DAT-positive cells were approximately 45% and 30%, respectively. In contrast, most of the GFP-labeled cells were negative for GFAP and O4, consistent with the *in vitro* data that none of the induced cells were positive for these glial markers. Grafted animals were followed up to 16 weeks and no tumor formation was observed in the brain [40].

Human induced dopaminergic neurons were similarly transplanted into the striatum of 6-OHDA-lesioned rats. Animals were immunosuppressed with FK 506 daily, and rotational behavior was recorded 4 weeks after cell transplantation. Grafting resulted in significant improvement in rotational behavior [40].

In summary, the additional administration of GDNF to MSC-Ns can efficiently induce TH-positive, dopamine-producing cells, and functional improvement could be achieved when grafted in a rodent model of Parkinson's disease.

Application of MSC-Ms to a muscle-degenerative disease model

As induced multinucleated myotubes in MSC-Ms are already post-mitotic, single cells of MyoD-positive myoblasts and Pax7-positive satellite cells were subjected to clonal culture (clonal MSC-Ms) to exclude non-muscle cells, and were transplanted into muscle-degenerative disease models [41].

Human clonal-MSC-Ms were transplanted into immunosuppressed rats whose gastrocnemius muscles were damaged with cardiotoxin pre-treatment. Cells were labeled with 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. 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, indicating that clonal MSC-Ms are able to incorporate into damaged muscles and contribute to regenerating myofiber formation, regardless of the transplantation method [41].

Clonal MSC-Ms contained Pax7-positive satellite cells which integrated into the satellite cell position after transplantation, namely the plasma membrane and the basal lamina in between [61]. In general, muscle satellite cells are known to contribute to the regeneration of myofiber formation upon muscle damage [1]. To confirm the contribution of transplanted satellite cells as *in vivo* satellite cells to muscle regeneration, the following experiment was performed. Four weeks after the initial transplantation of human clonal MSC-Ms i.v., 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 MSC-Ms to the muscles of patients, those retained as satellite cells should be able to contribute to future muscle regeneration [41].

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. Human clonal MSC-Ms were transplanted into cardiotoxin-pre-treated muscles of mdx-nude mice. Immunohistochemistry revealed the incorporation of transplanted cells into newly formed myofibers which expressed human dystrophin [41].

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

[16–19, 62]. Compared to these muscle stem cell systems, the MSC system offers several important advantages. This 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. Thus functional skeletal muscle cells can be obtained within a reasonable time course on a therapeutic scale. In the case of MSCs derived from inherited muscle dystrophy patients, genetic manipulation is possible after the isolation and expansion of MSCs. At present, there are no effective therapeutic approaches for muscle dystrophy. Hopefully, this MSC differentiation system may contribute substantially to eventual cell-based therapies for muscle disease.

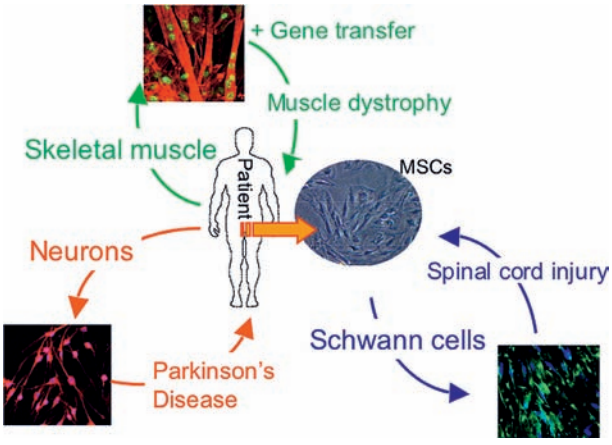

Figure 5. Schematic diagram of an 'auto-cell transplantation system' using MSCs. Neurons, Schwann cells, and skeletal muscle cells induced from patient's MSCs are transplanted back to their owner. Such a self-regenerative system avoids ethical issues and immunorejection.

Conclusions

MSCs provide hopeful possibilities for clinical application, since they can efficiently expand *in vitro* and a therapeutic scale of induced cells are available. In addition, transplantation of MSC-derived cells should pose fewer ethical problems than stem cells, 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, the bone marrow should at least be 'normal and healthy' for transplantation.

Even though transplantation of untreated MSCs is partly supportive in various kinds of degenerative models, probably due to trophic supply, it is desirable to develop a systematic induction system to obtain large amounts of purposeful cells, from the viewpoint of cell-based therapy. Obviously, induced cells must be confirmed to be morphologically and physiologically functional. Moreover, the practical application to human degenerative diseases depends on the ability to control their differentiation with high efficiency and purity into functional cells. As mentioned, 10^7 MSCs can be harvested from 20–100 ml of bone marrow aspirate within 2–3 weeks. If an induction procedure takes the shortest and most perfect course, 10^7 MSCs give rise to nearly 10^7 neurons within 3 weeks and 10^7 skeletal muscle cells within 5 weeks, taking into account the time 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 patient's bone marrow for transplantation therapy within a reasonable time course.

However, 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 [63, 64]. 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, generally adherent MSCs, their properties and plasticity can be studied and defined with more accuracy.

Notch-Hes signaling is known to inhibit neuronal and myogenic differentiation in conventional development [47]. 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 normal development. In our previous report, JAK/STAT inhibitor administration and constitutive active STAT1/3 transfection revealed that downregulation of STATs was tightly associated with NICD-mediated neuronal induction in MSCs, whereas Hes, downstream of Notch, was not involved in the induction event [40]. Skeletal muscle induction was also revealed to be independent of Hes1/5 and the conventional Notch signaling pathway [41]. Thus, our results suggest distinct cellular responses to Notch signals; for example, the repertoire of second messengers and active factors in MSCs may well be different from conventional neural stem cells and myogenic precursor cells, or the susceptibility of MSCs to the Notch signal is probably different from that of known neuronal and myogenic precursor cells. 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 an 'auto-cell transplantation system' using MSCs (Fig. 5). To realize this ideal, it is necessary to develop the regulatory system of differentiating MSCs into cells with a purpose. Our method would be a possible way to regulate MSC differentiation into functional Schwann cells, neurons and skeletal muscle cells which will be applicable to neuro- and muscle-degenerative diseases.

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