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The promises of stem cells: stem cell therapy for movement disorders

Hideki Mochizuki*, Chi-Jing Choong, Toru Yasuda

Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan

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SUMMARY

Despite the multitude of intensive research, the exact pathophysiological mechanisms underlying movement disorders including Parkinson's disease, multiple system atrophy and Huntington's disease remain more or less elusive. Treatments to halt these disease progressions are currently unavailable. With the recent induced pluripotent stem cells breakthrough and accomplishment, stem cell research, as the vast majority of scientists agree, holds great promise for relieving and treating debilitating movement disorders. As stem cells are the precursors of all cells in the human body, an understanding of the molecular mechanisms that govern how they develop and work would provide us many fundamental insights into human biology of health and disease. Moreover, stem-cell-derived neurons may be a renewable source of replacement cells for damaged neurons in movement disorders. While stem cells show potential for regenerative medicine, their use as tools for research and drug testing is thought to have more immediate impact. The use of stem-cell-based drug screening technology could be a big boost in drug discovery for these movement disorders. Particular attention should also be given to the involvement of neural stem cells in adult neurogenesis so as to encourage its development as a therapeutic option.

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1. Introduction

Scientists have known of the existence of stem cells, the unspecialized cells found in all multicellular organisms that can self-renew through self-division and differentiate into diverse specialized cell types, for over a century. Yet it has been only since the late 1990s, when human embryonic stem cells were first cultured in the laboratory, that the field of stem cell research has become the focus of intense scientific interest.

There are essentially three kinds of stem cells: embryonic stem (ES) cells, which are isolated from the inner cell mass of blastocysts; adult stem cells, which are found in various developed tissues such as bone marrow cells; and induced pluripotent stem (iPS) cells, which are artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a "forced" expression of specific genes.

One of the most astounding applications of stem cells is in the treatment and cure of a wide variety of movement disorders including Parkinson's disease (PD), multiple system atrophy (MSA) and Huntington's disease (HD). Several ways of how stem cells are being explored and used in both basic and clinical applications of current movement disorders research include disease modeling, drug toxicity screening/drug discovery, gene therapy and cell replacement therapy.

In most cases, it is difficult to obtain the damaged cells in a disease and to study them in detail. Stem cells, either carrying the disease gene or engineered to carry disease genes, offer an alternative for laboratory studies. Researchers are able to model disease processes *in vitro* and perform more relevant and informative biological assays, thereby better understanding the mechanisms underlying the disease. Stem cells have also been used in the laboratory to screen for new drugs. It has been revealed that very few drugs have been tested on human-diseased cells before human testing. Liver and heart toxicity problems account for about 30% of drugs that fail in early-stage clinical trials, indicating a need for more efficient means of drug toxicity testing before clinical trials. The use of stem cells with specific diseases may correct this situation. Furthermore, given their unique regenerative abilities, stem cells offer the possibility of a renewable source of cell replacement therapies for neurological diseases.

However, stem cell research has been controversial and has raised ethical dilemmas primarily concerning the creation, treatment, and destruction of human embryos inherent to research involving ES cells. The recent discovery of iPS cells, hailed as a potential alternative to ES cells, provides researchers with a unique tool to derive neurons from patient-specific iPS cells for the study of neurological diseases. More importantly, iPS cell research obviates many ethical and resource-related concerns posed by ES cells while prospectively matching their potential for scientific use.

In recent years, the discovery of constitutive ongoing neurogenesis in the adult human brain has challenged the traditional view of a fixed circuitry in functionally normal brains, and has raised high hopes that the adult brain may have the capacity for self-renewal

* Corresponding author. Hideki Mochizuki, MD, PhD, Professor and Chairman, Department of Neurology, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka, 565-0871, Japan.
Tel.: +81 6 6879 5111; fax: +81 6 6879 3579.
E-mail address: hmochizuki@neurol.med.osaka-u.ac.jp (H. Mochizuki).

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after injury, thereby sidestepping the need for transplantation. Primary neural precursor cells reside in specialized zones called "neurogenic niches". A population of neural stem cells (NSCs) preserves enough germinal character to maintain neurogenesis throughout life and, once differentiated, their daughter cells integrate into already existing neuronal networks. Whether adult neurogenesis can be induced under certain circumstances in regions that lack constitutive adult neurogenesis remains controversial, but several studies have reported the isolation of NSCs from different regions of the adult brain, including the substantia nigra pars compacta (SNc). Therefore, there has been considerable interest within the scientific community to gain understanding of the possible correlation between neurogenesis and pathogenesis of movement disorders, which could help the future development of novel therapeutic intervention.

2. Stem cell therapy for PD and MSA

There has been a long history of fetal tissue transplantation for the treatment of patients with advanced PD. Despite the wake of a long series of encouraging open-label studies, initial enthusiasm for cell replacement therapy by grafting fetal neuronal precursor cells into the striatum has vanished after two double-blind placebo-controlled clinical trials showing only moderate symptomatic improvement and the occurrence of severe disabling dyskinesia. These problems should be solved before fetal tissue transplantation can be considered a therapeutic option for PD [1].

Studies have shown ES cell transplanted into the brains of PD rat model differentiated into dopaminergic neurons, restoring partial neural function [2]. PD rodent models subjected to engraftment of dopaminergic neurons derived from human ES cells demonstrated complete behavioral restoration and motor function improvement. Similarly, parkinsonian monkeys receiving transplantation showed excellent DA neuron survival, function and lack of neural overgrowth, indicating potential for the development of cell-based therapies in PD [3].

It was recently shown that reprogramming mouse embryonic fibroblasts with four transcription factors Oct4, Sox2, Klf4, and c-Myc induces pluripotency [4], enabling generation of iPS cells from patients with a variety of diseases [5]. iPSC-derived midbrain dopaminergic neurons from a patient with a triplication in the α-synuclein gene (SNCA) showed accumulation of α-synuclein, inherent overexpression of markers of oxidative stress, and sensitivity to peroxide-induced oxidative stress, precisely recapitulating the cause of disease in the patients [6,7]. Comparably, PARK2 iPSC-derived neurons exhibited mitochondrial dysfunction associated with increased oxidative stress and α-synuclein accumulation, resembling pathogenic changes in patient brains [8]. Neurons derived from mutant PINK1 iPS cells displayed impaired recruitment of lentivirally expressed Parkin to mitochondria, increased mitochondrial copy number and upregulation of PGC-1α, an important regulator of mitochondrial biogenesis, upon mitochondrial depolarization [9]. LRRK2 mutant iPSC-derived DA neurons demonstrated increased susceptibility to oxidative stress, consistent with existing understanding of early PD phenotypes [10]. Such disease-specific iPS cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue formation *in vitro*, thereby facilitating disease investigation and drug development.

Furthermore, generation of iPS cells provides a new avenue for transplantation therapy as it can avoid immunorejection, a major complication in current transplantation medicine. Wernig et al. [11] reported upon transplantation into the fetal mouse brain, iPSC-derived neural precursor cells extensively differentiate into glia and neurons. Functional recovery was observed after transplantation of iPSC-derived midbrain dopamine neurons into the adult brain of Parkinsonian rats. Risk of tumor formation from grafted cells was minimized by the separation of contaminating pluripotent cells and committed neural cells using fluorescence-activated cell sorting. Encouraging data from rodent studies then prompted subsequent assessment in a primate model. Kikuchi et al. [12] observed that human iPSC-derived neural progenitor cells grafted in the brain of a primate PD model survived as dopaminergic neurons for as long as six months, implying the therapeutic potential of human iPS cells. Direct reprogramming of mouse and human fibroblasts into induced neural stem cells (iNSCs) has been proven feasible with a single factor, Sox2. iNSCs express NSC markers and resemble wild-type NSCs in their morphology, self-renewal, ability to form neurospheres and differentiate into several types of mature neurons as well as astrocytes and oligodendrocytes, indicating multipotency. Importantly, implanted iNSCs can survive and integrate in mouse brains without tumorigenic potential. As an additional merit, this method allows shortening of the duration for neuronal cell creation from fibroblasts [13].

Adult stem cells comprise mesenchymal stem cells, hematopoietic stem cells, ectodermal stem cells and so on. Scientific interest in adult stem cells is spotlighted on their ability to divide or self-renew indefinitely, and generate all the cell types of the organ from which they originate, potentially regenerating the entire organ from a few cells. Numerous studies using expanded and/or induced bone marrow-derived mesenchymal stem cells have been reported for animal models and yet only three clinical studies with intracerebral or intravasal application of these cells have been reported for PD and MSA patients. In two open-label studies, subventricular application of both allogenic and autologous bone marrow-derived mesenchymal stem cells showed improvement of motor behavior as reflected by reduction of UPDRS ON and OFF scores in most but not all PD patients [14,15]. In a randomized placebo-controlled trial involving a small number of cognitively intact MSA-C patients, mesenchymal stem cell therapy was safe and was able to delay the progression of neurological deficits with functional improvement in the follow-up period in some of the patients [16].

3. Adult neurogenesis in Parkinson's disease

Increasing evidence points to the presence of adult neural stem cells in many areas of the mammalian brain, mainly in the hippocampus and subventricular zone (SVZ) near the lateral ventricle. It is well known that changes occurring in the SVZ depend upon the pathological condition. Dopamine is an important molecule in neurogenesis. Therefore many investigators now focus on neurogenesis in PD. Höglinger et al. [17] reported reduction in the numbers of proliferating cells in the SVZ of postmortem brains of PD patients, implying that generation of neural precursor cells is impaired in PD as a consequence of dopaminergic denervation. However, controversy regarding neurogenesis in the SVZ in PD models persists. Some groups reported decreased neural precursor proliferation while some reported increased neural precursor proliferation in the SVZ of PD models.

Likewise, whether dopaminergic neurogenesis occurs in the adult substantia nigra (SN) in PD brains or in PD animal models remains a matter of debate. So we evaluated nigral neurogenesis in animal models and PD autopsy brains. We first performed retroviral labeling in a PD rodent model and observed efficient labeling of proliferating cells in SN with retroviral transduction of green fluorescent protein. But many of these labeled cells became microglia and none had differentiated into tyrosine-hydroxylase (TH)-positive neurons. Second, staining for intrinsic markers of neurogenesis showed that there were no proliferating cells in the SN of PD patients but a large number of polysialylated neural cell

adhesion molecule (PSA-NCAM)-positive cells were detected in SN pars reticulata (SNr) of some PD patients. In rat and primate models, dopamine-depleted hemispheres showed more PSA-NCAM staining than the intact side. A small number of TH and PSA-NCAM double positive cells, indicative of newly differentiated dopaminergic neurons, were detected [18]. However, no TH and PSA-NCAM double positive cells in PD patients were detected. Despite not being conclusive enough, these results suggest enhanced neural reconstruction in PD, which may be important in the design of new therapies against the progression of PD.

4. Stem cell therapy for Huntington's disease

Huntington's disease (HD) is characterized by a loss of brain striatal neurons that occurs as a consequence of an expansion of a cytosine adenine guanine (CAG) trinucleotide repeat encoding polyglutamine (polyQ) in the first exon of the Huntingtin gene. Therapeutic strategies are largely based on the amelioration of mutant huntingtin-related metabolic impairment and cellular toxicity. Yet cell replacement may be a potential therapy when cell death has become prominent in later stages of the disease. Numerous preclinical studies reported the efficiency of human fetal striatal tissue in providing functional recovery in various rodent and non-human primate models of striatal neuronal loss. On this basis, several clinical trials have assessed fetal cell transplantation for treatment in HD patients. Delivery of fetal striatal primordium into the caudate putamen of patient's brain was done via surgical stereotactic method. Yet due to heterogeneity in experimental design and small sample size, these clinical trials provided divergent data and reported modest improvements even in the best of cases. Some patients showed symptomatic improvement following the transplant but disease progression ensued with no greater survival. To address this issue, three ongoing randomized controlled clinical trials are reassessing fetal graft efficacy.

The ethical and immunological concerns associated with fetal allografts, along with the practical need to obtain tissue that is precisely staged, accurately dissected and freshly collected, imply that availability of fetal tissues for cell transplantation in the brain is likely to be extremely limited. Thus there is an urgent and active search for alternative sources. Human ES serve as a readily renewable source of potential medium spiny neurons for cell replacement therapy in HD patients. *In vivo* differentiation of striatal progenitor derived from human ES cells into striatal neurons following xenotransplantation into adult rats has first been described by Aubry et al. [19], opening an avenue of human ES cell therapy for HD. However, long-term proliferation of human neural progenitors leads to xenograft overgrowth in the rat brain, hindering its clinical use. HD-specific iPS cells have also been generated and reproduced CAG-repeat-expansion-associated gene expression phenotypes upon differentiation into neural cells, representing a well-characterized resource to elucidate the disease mechanism in HD and providing a human stem cell platform for screening new candidate therapeutics [20]. Also, An et al. [21] reported that iPS cells derived from the HD patient could be corrected by the replacement of the expanded CAG repeat with a normal repeat using homologous recombination, and the correction persists upon differentiation into striatal neurons *in vitro* and *in vivo*. Notably, correction of the HD-iPSCs normalized pathogenic HD signaling pathways and reversed disease phenotypes in neural stem cells. The ability to make patient-specific, genetically corrected iPS cells from HD patients is crucial for the eventual use of these cells in cell replacement therapy.

As mentioned earlier, neurogenesis has recently been observed in the adult human brain, suggesting the possibility of endogenous neural repair. Curtis et al. [22] first reported augmentation of neurogenesis as reflected by increased progenitor cell proliferation in the subependymal layer adjacent to the caudate nucleus, in response to neuronal cell loss in the caudate nucleus in HD. Degree of cell proliferation increased with pathological severity and increasing CAG repeats in the HD gene. Most importantly, proliferating cells were shown to express neuronal markers, indicating the generation of neurons and glial cells in diseased human brain. These results provide evidence for the regenerative potential of the human brain. Further, on the basis that ependymal overexpression of brain-derived neurotrophic factor (BDNF) stimulates neuronal addition to the adult striatum from subependymal progenitor cells while Noggin potentiates this process by suppressing subependymal gliogenesis and increasing progenitor availability, Cho et al. [23] found that BDNF and Noggin induced striatal neuronal regeneration, delayed motor impairment, and extended survival in R6/2 huntingtin transgenic mice, suggesting a new therapeutic strategy for HD.

5. Challenges of stem cell research

Before stem cells can be used to treat a myriad of disorders, many technical obstacles that hinder the clinical use must be overcome. The first major concern is that ES- and iPS-derived grafts have been reported to induce formation of teratomas. The tumor formation depends on the extent to which the cells are selectively enriched and differentiated prior to transplantation. Contamination with undifferentiated multipotent cells permits teratogenesis in the host. There are a number of successful engraftments of human ES cells-derived cells within the brain as treatment for PD and HD without tumor formation. But these studies were conducted in rodents and did not include long assessment periods. This problem may be solved with the establishment of safe stem cells incorporated with an anti-tumorigenic system by virus-mediated suicidal gene introduction [24]. These suicidal genes can serve as cell death switches that halt potentially deadly reactions.

Second, human ES cells express low levels of human leukocyte antigen class I molecules in both undifferentiated and differentiated states and might elicit immune responses. To address this issue, researchers found that short-term immune-dampening treatment enables human embryonic stem cells to avoid rejection after transplantation. Breakthrough of iPS cells also potentially allows generation of patient-specific donor cells that would likely, although not certainly, evade rejection as autograft. However, some researchers opined that iPSC-derived neurons will not be suitable for transplantation until the oncogenes and retroviruses used are replaced with more controlled methods of reprogramming. The problems that remain would likely be overcome through years of intensive research.

Recently a critical issue regarding clinical use of unapproved stem cell treatment in many clinics in some countries has been revealed. Those clinics claimed success in treating patients, including PD patients, but none has published data from controlled clinical trials. PD experts expressed concerns that these treatments might provide anecdotal, poorly controlled and transient improvement in patients and were dubious if the infused cells would survive for more than a few days in patients because so far there are neither scientific nor clinical data to support long-term benefits of hematopoietic or neural stem cell therapies for PD patients. Leading researchers now emphasize the need to strictly regulate stem cell therapy by requiring the organizations using stem cells to register their research and clinical activities, source of stem cells and ethical procedures.

6. Conclusion

In summary, stem cell research has made tremendous progress to date, offering new and promising potentials for the use of

these cells as therapeutic agents. However, it has also been the subject of much debate, and a great deal of research is required to overcome the existing technical hurdles including tumorigenesis and immune response so as to enable development of novel approaches that could be translated into effective and well-tolerated clinical application. Though in its infancy, generation of iPS cells is a breakthrough in stem cell research that, in the long term, may lessen the need to use human ES cells that is always at the crux of ethical concerns.

Conflict of interests

The authors have no conflicts of interest to declare.

References

[1] Greene PE, Fahn S. Status of fetal tissue transplantation for the treatment of advanced Parkinson disease. Neurosurg Focus 2002;13:e3.

[2] Bjorklund LM, Sánchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, et al. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. Proc Natl Acad Sci U S A 2002;99:2344–9.

[3] Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z, et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 2011;480:547–51.

[4] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–72.

[5] Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134:877–86.

[6] Byers B, Cord B, Nguyen HN, Schüle B, Fenno L, Lee PC, et al. SNCA triplication Parkinson's patient's iPSC-derived DA neurons accumulate α-synuclein and are susceptible to oxidative stress. PLoS One 2011;6:e26159.

[7] Devine MJ, Ryten M, Vodicka P, Thomson AJ, Burdon T, Houlden H, et al. Parkinson's disease induced pluripotent stem cells with triplication of the α-synuclein locus. Nat Commun 2011;2:440.

[8] Imaizumi Y, Okada Y, Akamatsu W, Koike M, Kuzumaki N, Hayakawa H, et al. Mitochondrial dysfunction associated with increased oxidative stress and α-synuclein accumulation in PARK2 iPSC-derived neurons and postmortem brain tissue. Mol Brain 2012;5:35.

[9] Seibler P, Graziotto J, Jeong H, Simunovic F, Klein C, Krainc D. Mitochondrial Parkin recruitment is impaired in neurons derived from mutant PINK1 induced pluripotent stem cells. J Neurosci 2011;31:5970–6.

[10] Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, et al. LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. Cell Stem Cell 2011;8:267–80.

[11] Wernig M, Zhao JP, Pruszak J, Hedlund E, Fu D, Soldner F, et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci U S A 2008;105:5856–61.

[12] Kikuchi T, Morizane A, Doi D, Onoe H, Hayashi T, Kawasaki T, et al. Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease. J Parkinsons Dis 2011; 1:395–412.

[13] Ring KL, Tong LM, Balestra ME, Javier R, Andrews-Zwilling Y, Li G, et al. Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell 2012;11:100–9.

[14] Venkataramana NK, Kumar SK, Balaraju S, Radhakrishnan RC, Bansal A, Dixit A, et al. Open-labeled study of unilateral autologous bone-marrow-derived mesenchymal stem cell transplantation in Parkinson's disease. Transl Res 2010;155:62–70.

[15] Venkataramana NK, Pal R, Rao SA, Naik AL, Jan M, Nair R, et al. Bilateral transplantation of allogenic adult human bone marrow-derived mesenchymal stem cells into the subventricular zone of Parkinson's disease: a pilot clinical study. Stem Cells Int 2012;2012:931902.

[16] Lee PH, Kim JW, Bang OY, Ahn YH, Joo IS, Huh K. Autologous mesenchymal stem cell therapy delays the progression of neurological deficits in patients with multiple system atrophy. Clin Pharmacol Ther 2008;83:723–30.

[17] Höglinger GU, Rizk P, Muriel MP, Duyckaerts C, Oertel WH, Caille I, et al. Dopamine depletion impairs precursor cell proliferation in Parkinson disease. Nat Neurosci 2004;7:726–35.

[18] Yoshimi K, Ren YR, Seki T, Yamada M, Ooizumi H, Onodera M, et al. Possibility for neurogenesis in substantia nigra of parkinsonian brain. Ann Neurol 2005; 58:31–40.

[19] Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons *in vitro* and in quinolinic acid-lesioned rats. Proc Natl Acad Sci U S A 2008;105:16707–12.

[20] HD iPSC Consortium. Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. Cell Stem Cell 2012;11:264–78.

[21] An MC, Zhang N, Scott G, Montoro D, Wittkop T, Mooney S, et al. Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. Cell Stem Cell 2012;11:253–63.

[22] Curtis MA, Penney EB, Pearson AG, van Roon-Mom WM, Butterworth NJ, Dragunow M, et al. Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc Natl Acad Sci U S A 2003;100:9023–7.

[23] Cho SR, Benraiss A, Chmielnicki E, Samdani A, Economides A, Goldman SA. Induction of neostriatal neurogenesis slows disease progression in a transgenic murine model of Huntington disease. J Clin Invest 2007;117:2889–902.

[24] Onodera M. Gene and cell therapy for relapsed leukemia after allo-stem cell transplantation. Front Biosci 2008;13:3408–14.