

Motor Neuron Disease and Stem Cell Approach for Its Remediation

Jong Deog KIM^{1*}, Jyoti Bhardwaj², Narendra Chaudhary², and Hyo Jin SEO²

접수: 2013년 5월 4일 / 게재승인: 2013년 10월 21일
© 2013 The Korean Society for Biotechnology and Bioengineering

Abstract: Motor neuron disease (MND) is a fatal neurodegenerative disorder caused by progressive and selective degeneration of motor neurons (MNs). Because of the versatile nature, stem cells have the potential to repair or replace the degenerated cells. In this review, we discussed stem cell based therapies including the use of embryonic stem cells (ESCs), neural stem cells (NSCs), induced pluripotent stem cells (iPSCs) and genetically engineered cells to produce the neurotrophic factors for the treatment of MND. To achieve this goal, the knowledge of specificity of the cell target, homing and special markers are required.

Keywords: Embryonic stem cells, Induced pluripotent stem cells, Motor neuron diseases, Neural stem cells, Superoxide dismutase

INTRODUCTION

Motor neuron disease (MND) is a fatal neurodegenerative disorder caused by progressive and selective degeneration of motor neurons (MNs). The pathological feature of MND is characterized by the degeneration of upper motor neurons (giant cells of Betz and corticospinal tracts) and lower motor neurons (ventral horn, laminae IX of the spinal cord and directly innervate striated muscle of the axial skeleton in both upper and lower

limbs) or both. Upper and lower motor neurons are the final effectors leading to all voluntary movements of the body [1]. MND is used synonymously with amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS) and progressive muscular atrophy (PLS). ALS is the most common motor neuron disease.

The pathogenesis of the disease appears to be multi-factorial, resulting from the interaction of unknown susceptibility gene and environmental factors as well as physiological cellular ageing. Majority of MND cases are sporadic (sporadic ALS, sALS) i.e., only about 5-10% of all cases show familial inherited form of the disease (familial ALS, fALS) [2,3]. The inherited forms of this disease are associated with mutations in the gene encoding Cu/Zn superoxide dismutase (*SOD1*) [4,5]. The mutant *SOD1* protein leads to motor neuron degeneration and accumulation of free radicals by a toxic gain of function which is likely to have multiple elements including oxidative stress, mitochondrial dysfunction, excitotoxicity and protein misfolding [6]. *AV4* gene mutation was most commonly found in North America up to 50% of *SOD1* cases. In such a way different region and countries have different mutation which directly or indirectly affect *SOD1* gene. For instance, *H46R* gene mutation is commonly determined in Japan. *D90A* mutation is associated with the slow progression of ALS found in the people of Scandinavian [1].

Mutations in the *TARDBP* and *FUS* genes account for another 8% of all fALS cases and a small percentage of sporadic amyotrophic lateral sclerosis cases [7]. *TARDBP* encodes the protein TDP-43 which is involved in splicing regulation and mRNA biogenesis. Mislocalisation and accumulations of ubiquitinated TDP-43 has been shown to be the cause in many patient. Mutation or deletion of survival motor neuron (*SMN1*) has been determined in the majority of spinal muscular atrophy (SMA) cases [8]. *TARDBP* mutation was not determined in Finnish patients [9]. Also, about one third of patients have addition in a gene called *C9orf72* and abnormality of this gene develops

¹Research Center on Anti-Obesity and Health Care (RCAOHC), Chonnam National University, San 96-1, Dun-Duk Dong, Yeosu 550-749, Korea
Tel: +82-61-659-7305, Fax: +82-61-659-7305
e-mail: pasteur@jnu.ac.kr

²Department of Biotechnology, Chonnam National University, San 96-1, Dun-Duk Dong, Yeosu 550-749, Korea

MND in some patients whereas some develop frontotemporal dementia [10]. Lesions in the upper and lower motor neuron are also one of the major causes of MND [11].

The causes of MND are the combination of factors and pathogenesis is poorly understood. Several neurotrophic compounds shows neuroprotective and MN promoting effect in animal models but lacks significant effect in clinical trials in MND. Patient with MND dies within three years from the development of symptoms due to respiratory failure [12]. So far riluzole has only shown to alter the course of ALS and prolongs survival by an average of 3-4 months. It effects by inhibiting neurotransmitter glutamate in motor neuron [13]. Two novel ALS potential biomarkers cytoplasmic FMR Interacting Protein 2 (CyFIP2) and Retinoblastoma (Rb) Binding Protein 9 (RbBP9) were recently found in non-neural tissues from ALS patients [14].

Over last two decades, several approaches were used to protect from MND including a placebo trials in animal and human models. Though the therapeutic failures reinforced the idea that MND is multi-factorial disease and single factor treatment may not be useful. Recent advancement and understanding in the field of stem cell technology led to interest in the use of neural stem cells (NSCs), embryonic stem cells (ESCs) or adult stem cells.

STEM CELLS

Stems cells are unspecialized cells capable of renewing themselves and possess ability to differentiate into specialized cells. Two types of stem cells are found in multicellular organisms, embryonic stem cells (ESCs) and adult stem cells. ESCs are pluripotent cells, they can generate every cell types in the body and adult stem cells are multipotent cells that are able to generate those cell types that make up the tissue of origin. For example, neural stem cells can generate the neurons, astrocytes and myelinating cells that form our nervous system [15].

The discovery of reprogramming technologies led the foundation of induced pluripotent stem cells (iPSCs) in 2006 [16] from mouse cells and in 2007 [17] from human cells. iPSCs are similar to natural pluripotent stem cells that can be generated from adult stomach, skin fibroblast and blood cells. Some tissue specific stem cells could be isolated from cord blood, bone marrow and foetal tissue which are being investigated as possible treatments for MND. Among these neural stem cells (NSCs) isolated from foetal tissue and spinal cord are multipotent cells. NSCs are able to differentiate into neurons, astrocytes and oligodendrocytes. In addition to ESCs and iPSCs, mesenchymal stem cells (MSCs) are also multipotent cells and they can be differentiated into osteoblasts, adipocytes, chondrocytes, myocytes and

neuron like cells [18].

STEM CELL BASED APPROACHES TO TREAT MND

ALS is a neurodegenerative disease, for which there is no effective treatments because neurons in the brain are unable to regenerate. Thus, cell based therapies are categorized in two ways, cell replacement and manipulation of the environment around the injured neurons using stem cells. The neural progenitor cells are also investigated which are responsible for generating new neurons in body. Blood stem cells, neural and mesenchymal and other types of stem cells are being tested for their differentiation potential. Among them spinal cord stem cells are more suitable which can produce both motor neurons and glial cells. Instead of replacement of damaged neurons, it may also be possible to use supporting cells such as glial cells. Glial cells are able to repair and to prevent the further damage to the motor neurons. Recent studies have indicated that it is possible to generate motor neurons from direct differentiation of ESCs [19]. Motor neurons derived from mouse ESCs transplanted into rat spinal cord extend axons into periphery. Mouse ES cells are treated to retinoic acid and developmental morphogen sonic hedgehog (Shh) to neutralize and establish a positional identity for ES cells. Transplantation of ES cells derived motor neuron into paralyzed rat resulted in generation of several new motor neurons and led to improved motor function [20]. Several studies have indicated that human ES cells are able to generate dopaminergic neurons. Fibroblast growth factor 8 (FGF8) and Shh induces the human ES cell to generate dopaminergic neurons. Similar results were obtained using monkey ES-derived dopaminergic neurons cultured in a medium supplemented with fibroblast growth factor-20 (FGF20) [21]. The use of ES cell-derived dopaminergic neurons remains limited because the long term survival and phenotypic stability of human ES cell-derived neurons have been problematic. Intravenous infusion of human umbilical cord blood cells in SOD1G93A mice provides neuro-protective effect; they repair the lost and degenerated motor neurons and migrate towards the injured neuronal sites [22,23]. CD133 (+) stem cells from ALS patients, like the stem cells of healthy subjects, are capable of differentiating into pre-neuron cells [24].

NSCs are able to differentiate into neurons, astrocytes and oligodendrocytes which are influenced by cell source, age and passage number. NSCs isolated from embryonic spinal cords generated motor neurons, and improved the phenotype and survival of spinal muscular atrophy mice [25]. NSCs transplantation is beneficial for several neurological disease such as Hun-

tington disease (HD), Parkinson disease (PD), stroke, spinal cord injuries, amyotrophic lateral sclerosis (ALS). Some studies have investigated that genetically engineered NSCs are able to replace damaged or lost cells by releasing glial cell derived neurotrophic factor (GDNF) [26]. Hofstetter et al. showed that neurogenin-2, a transcription factor induce the differentiation of NSCs into oligodendrocytes which improve the recovery of motor function and increased remyelination in the injured area of spinal cord [27]. In recent studies of PD (loss of dopamine neurons) two genetically engineered immortalized mouse NSC line C17.2 and human NSC line HB1.F3 were transduced to carry the *TH* gene and *GTPCH-1* gene for the production of L-DOPA [28]. Neural stem cell therapies are also useful for HD because it results from GABAergic projection neurons in striatum. In HD, loss of GABAergic neurons is caused by mutation in huntingtin gene. Transplantation of NSCs derived striated neuron can replace the damaged GABAergic neurons and form synapses by activation of several transcription factors such as *Dlx-2*, *Pax6* and *Gsh2*. During transplantation of NSCs to replace the damaged striatal neurons, NSCs and genetically modified NSCs producing neuro-trophic factors have been used to protect the neurons against excitotoxicity. Some previous studies have demonstrated that the intrastriatal transplantation of NSCs in rat model of HD promotes the active production of brain-derived neuro-trophic factor (BDNF) which could block the neuronal injury in animal model of HD [29,30].

A recent study showed that the replacement therapy for stroke is more complicated because transplanted NSCs needs to replace a large number of neuronal types and transplantation of human fetal derived NSCs in brain after stroke survived and differentiated into various types of neurons and non neuronal cells which act as supporting cell to induce the differentiation of NSCs into neurons [31]. During transplantation of NSCs to replace the damaged striatal neurons, neurons generated from NSCs and NT2 human teratocarcinoma cell line was also effective in delaying disease progression in mice ALS model [32]. Recent studies demonstrated that the intrathecal transplantation of human NSCs in transgenic SOD1/G93A mouse model of ALS over expressing VEGF induced functional improvement and delayed disease progression. These results indicate that the human NSCs transplantation might be beneficial for ALS patients without significant side effects [33]. In another study, human neural progenitor cells (hNPCs) isolated from postmortem fetal brain tissue, were used to deliver neuro-trophic factors such as GDNF and to replace the glial cell in rat model of ALS [34]. Spinal muscular atrophy (SMA) is an inherited autosomal disease associated with selective loss of motor neurons. NSCs have recently been examined for their potentiality as a treatment of SMA. Intrathecal administration of NSCs in mice

model of SMA induces the growth factors including GDNF, BDNF and neurotrophin. Engrafted animal showed 39% increase in lifespan and 8% of total transplanted cells express choline acetyl transferase [35]. Human NSCs are also used for ex-vivo delivery of protease gene for the treatment of Alzheimer's disease (AD) because it is caused by the increase levels of both soluble and insoluble A β peptides. In multiple sclerosis (MS), oligodendrocytes (OLs) and myelin are destroyed [27]. Most recent study investigated the new F3.Olig2 human NSC line by transduction of F3 with a retroviral vector encoding Olig2 bHLH transcription factor gene which is responsible for the generation of spinal motor neurons and these cells express cell type specific markers for OL [36].

Previous works with the transplantation strategies provided evidences of MN survival or improved motor neuron behavior and extension in lifespan to the production of neuro-trophic factors or other neuro-protective molecules by the grafted cells. As well as effect of growth factors, such as GDNF, IGF-1, CNTF, and VEGF on motor neuron survival and function has been shown in other work in the experimental models of ALS [38].

In 2006, Takahashi and Yamanaka reported the development of induced pluripotent stem cells (iPSCs) from mouse fibroblast by reprogramming adult cells to pluripotency by the expression of *oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. Next, human somatic cells were reported reprogrammed to pluripotent stem cells which could be differentiated into cells of endoderm, mesoderm, or ectoderm [38,39]. Human iPS cells can be generated from patient specific tissue which solved the problems associated with graft rejection, complications of using immunosuppressive drugs and ethical issues with the use of cells from embryonic or fetal origin. Human iPS has its own problems or limitations. The generation, characterization, and differentiation of patient specific iPS cells are a time-consuming and costly procedure. iPS-derived cells has shown therapeutic potential against sickle cell anemia, hemophilia, Parkinson's disease, spinal cord injury, and diabetes in animal model [40-44]. Recently, motor neurons generated from induced pluripotent stem cells (iPSCs) from familial ALS patients with mutations in Tar DNA binding protein-43 (TDP-43) were used for drug screening for ALS disease [45]. Thus, iPSCs hold much hope for future cell based therapies.

SAFETY ISSUES

We have learned from the gene therapy field that human disease treatment must be both safe and effective. Human populations are genetically diverse, thus the derived cells for trans-

plantation should be histocompatible with every individual. Differentiation properties of transplanted pluripotent stem cells should be evaluated for safety issues. Whether the transplanted pluripotent stem cells will form tumors or it will change the differentiation pattern after transplantation. Understanding what happens to transplanted cells in *in-vivo* environment is essential to predict the safety and efficacy of neural stem cell therapies. It is important to develop conditions for growth of neural stem cells in the absence of any pathogenic agents that may come across from growth medium like feeder cells, bovine serum etc. [9].

The Food and Drug Administration (FDA) has made framework for stem cells trials: "All cellular products present many complex issues not encountered with other classes of biologicals. These products can easily support growth of many pathogenic microorganisms and cannot be sterilized. Moreover, they quite likely will be administered to very sensitive sites, such as the central nervous system (CNS). Thus, efforts to minimize risks (e.g., stringent microbiological controls) and to justify these animal risks (e.g., a rationale for human use supported by appropriate animal studies) are of special importance" (Biologics Response Modifiers Advisory Committee Meeting 2000). The questions raised by FDA for stem cell therapies falls into several areas of cellular therapies which still require to be addressed.

THE FUTURE CHALLENGES

Several researches are going on for the development of stem cell-based therapies for neurodegenerative disorders with no effective cure reported to date and still it has to go long way. The results of mouse ES cells transplantation in animal models have provided some evidence of motor neuron regeneration and the treatment of MND. However, the results from human trials are not satisfactory to be clinical. Investigation of disease model, cell dosage, transplant preparation, transplant location, immune response, trophic factors and biomarkers to track transplanted stem cells, cell source and patients are essential factor for the success of stem cell therapies for neurodegenerative disease. For example, neuronal replacement in some patients with Parkinson's disease has been proven to work well, but safer biological and technical advancement have been underestimated. Likewise, more research requires to be conducted into the neuronal cell generation, immunocompatible cell selection, and transplantation of cells into the brains. The chances of rapid tumors development are also high when ESCs are transplanted into adult patients.

Transplantations of fetal brain cells into brains of Parkinson's

disease patients resulted to uncontrollable movements such as writhing, twisting, head jerking, arm-flailing, and constant chewing [46]. Stem cells show variation in their ability to make neurons even the cells from the same individual. There are number of reasons for cells to become dysfunctional like genetics, diseases, injury or aging.

CONCLUSION

Recent discoveries in stem cell based therapies and ongoing clinical trials offers hope to treat MND in future. Degenerated motor neurons are being replaced by the new cells produced by stem cells. Stem cells are already proven to maintain the biological environment and normal physiology by regenerating motor neuron. Although, research in stem cell therapies for MNDs has achieved well in the translation of stem cell therapies from the bench to bedside, but it still lacks approved therapies. To date, actual mechanism of motor neuron remains unclear. There is a clear requirement of more researches to understand the mechanism of motor neuron disease to provide safe and effective treatments. Also, it is essential to understand the surrounding environment of degenerated motor neurons. Recent clinical trials and human stem cell biology offer more distant hope.

REFERENCES

1. Kanning, K. C., A. Kaplan, and C. E. Henderson (2010) Motor neuron diversity in development and disease. *Annu. Rev. Neurosci.* 3: 409-440.
2. Bradley, W. (1996) Overview of motor neuron disease: classification and nomenclature. *Clin. Neurosci.* 3: 323-326.
3. Fiszman, M. L., L. N. Borodinsky, K. C. Ricart, O. P. Sanz, and R. E. Sica (1999) Cu/Zn superoxide dismutase activity at different ages in sporadic amyotrophic lateral sclerosis. *J. Neurol. Sci.* 162: 34-37.
4. Pramatarova, A., D. A. Figlewicz, A. Krizus, F. Y. Han, P. I. Ceballos, A. Nicole, M. Dib, V. Meininger, and R. H. Brown (1995) Identification of new mutations in the Cu/Zn superoxide dismutase gene of patients with familial amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* 56: 592-596.
5. Millicamps, S., F. Salachas, C. Cazeneuve, P. Gordan, B. Bricka, A. Camuzat, L. N. Guillot, O. Russaouen, G. Bruneateau, P. F. Pradat, N. Le-Forestier, V. Danel-Brunaud, N. Guy, R. C. Thauvin, L. Lacomblez, P. Cauratier, D. Hannequin, D. Seilhean, I. Ler-Ber, P. Corcia, W. Camu, A. Brice, G. Rouleau, E. LeGuern, and V. Meininger (2010) SOD1, ANG, VAPB, TARDBP, and FUS mutations in familial amyotrophic lateral sclerosis: Genotype-phenotype correlations. *J. Med. Genet.* 47: 554-560.
6. Ross, C. A., and M. A. Poirier (2004) Protein aggregation and neurodegenerative disease. *Nat. Med.* 10: S10-17.

7. Mackenzie, I. R., R. Rademakers, and M. Neumann (2010) TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. *Lancet. Neurol.* 9: 995-1007.
8. Lefebvre, S., L. Burglen, S. Reboullet, O. Clermont, P. Bulet, L. Viollet, B. Benichou, C. Cruaud, P. Millasseau, M. Zeviani, *et al.* (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80: 155-165
9. Hanna, K. M., L. Tuovinen, S. Penttilä, T. Suominen, B. Udd, and J. Palmio (2012) TARDBP mutations are not a frequent cause of ALS in Finnish patients. *Acta. Myol.* 31: 134-138.
10. Snowden, J. S., S. Rollinson, J. C. Thompson, J. M. Harris, C. L. Stopford, A. M. Richardson, M. Jones, A. Gerhard, Y. S. Davidson, A. Robinson, L. Gibbons, Q. Hu, D. DuPlessis, D. Neary, D. M. Mann and S. M. Pickering-Brown (2012) Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. *Brain* 135: 693-708.
11. Talbot, K. (2002) Motor neurone disease. *Postgrad. Med. J.* 78: 513-519.
12. Siddharthan, C. and S. Andrea (2011) The use of stem cells in Motor Neurone Disease (MND), *Adv. Clin. Neurosci. Rehabil.* 11: 10-11.
13. Oliveira, A. S. B. and R. D. B. Pereira (2009) Amyotrophic lateral sclerosis (ALS): Three letters that change the people's life. For ever. *Arq. Neuropsiquiatr.* 67: 750-782.
14. Henny, N., W. Shane, A. Michal, B. Shlomo, and Weil, Miguel (2012) Two potential biomarkers identified in mesenchymal stem cells and leukocytes of patients with sporadic amyotrophic lateral sclerosis. *Dis. Markers* 32: 211-220.
15. Hima, B. A. and B. Srilatha (2011) Potency of various types of stem cells and their transplantation. *J. Stem Cell Res. Ther.* 1: 115.
16. Takahashi, K. and S. Yamanaka (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 4: 663-676.
17. Takahashi, K., K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, and S. Yamanaka (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861-872.
18. Paspala, S. A., A. B. Balaji, P. Nyamath, K. S. Ahmed, A. A. Khan, M. N. Khaja, M. L. Narsu, Y. P. Devi, T. V. Murthy, and C. M. Habibullah (2009) Neural stem cells & supporting cells-the new therapeutic tools for the treatment of spinal cord injury. *Indian. J. Med. Res.* 30: 379-391.
19. Wichterle, H., I. Lieberam, J. A. Porter, and T. M. Jessell (2002) Directed differentiation of embryonic stem cells into motor neurons. *Cell* 110: 385-397.
20. Deshpande, D. M., Y. S. Kim, T. Martinez, J. Carmen, S. Dike, I. Shats, L. L. Rubin, J. Drummond, C. Krishnan, A. Hoke, N. Maragakis, J. Shefner, J. D. Rothstein, and D. A. Kerr (2006) Recovery from paralysis in adult rats using embryonic stem cells. *Ann. Neurol.* 60: 32-44.
21. Daadi, M. M., B. A. Grueter, R. C. Malenka, D. E. Redmond, and G. K. Steinberg (2012) Dopaminergic neurons from midbrain-specified human embryonic stem cell-derived neural stem cells engrafted in a monkey model of parkinson's disease. *PLoS ONE* 7: e41120.
22. Svitlana, G. D., E. W. Alison, Z. Tanja, S. Saporta, B. J. Eleanor, J. C. Lane, J. E. Hudson, N. Chen, C. D. Davis, and P. R. Sanberg (2003) Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J. Hematother. Stem Cell Res.* 12: 255-270.
23. Garbuzova-Davis, S., M. C. O. Rodrigues, S. Mirtyl, S. Turner, S. Mitha, J. Sodhi, S. Suthakaran, D. J. Eve, C. D. Sanberg, N. N. Kuzmin, and P. R. Sanberg (2012) Multiple Intravenous Administrations of Human Umbilical Cord Blood Cells Benefit in a Mouse Model of ALS. *PLoS ONE* 7: e31254.
24. González-Garza, M. T., H. R. Martínez, E. Caro-Osorio, D. E. Cruz-Vega, M. Hernández-Torre, and J. E. Moreno-Cuevas (2013) Differentiation of CD133+ stem cells from amyotrophic lateral sclerosis patients into preneuron cells. *Stem Cells Transl. Med.* 2: 129-135.
25. Corti, S., F. Locatelli, D. Papadimitriou, C. Donadoni, R. Del Bo, M. Crimi, A. Bordoni, F. Fortunato, S. Strazzer, G. Menozzi, S. Salani, N. Bresolin, and G. P. Comi (2006) Transplanted ALDHhiSS-Clo neural stem cells generate motor neurons and delay disease progression of nmd mice, an animal model of SMARD1. *Hum. Mol. Genet.* 15: 167-187.
26. Einstein, O. and T. Ben-Hur (2008) The changing face of neural stem cell therapy in neurologic diseases. *Arch. Neurol.* 65: 452-456.
27. Hofstetter, C. P., J. P. Card, and L. A. Olson (2005) A spinal cord pathway connecting primary afferents to the segmental sympathetic outflow system. *Exp. Neurol.* 194: 128-138.
28. Ryu, M. Y., M. A. Lee, Y. H. Ahn, K. S. Kim, S. H. Yoon, E. Y. Snyder, K. G. Cho, S. U. Kim (2005) Brain transplantation of genetically modified neural stem cells in parkinsonian rat. *Cell Transplant.* 14: 193-202.
29. Kim, S. U., I. H. Park, T. H. Kim, K. S. Kim, H. B. Choi, S. H. Hong, J. H. Bang, M. A. Lee, I. S. Joo, K. S. Lee, and K. S. Kim (2006) Brain transplantation of human neural stem cells transduced with tyrosine hydroxylase and GTP cyclohydrolase 1 provides functional improvement in animal models of Parkinson disease. *Neuropathology* 26: 129-140.
30. Kim, S. U. and J. de Vellis (2009) Stem cell-based cell therapy in neurological diseases: A review. *J. Neurosci. Res.* 87: 2183-2200.
31. Lindvall, O. and Z. Kokaia (2010) Stem cells in human neurodegenerative disorders-time for clinical translation? *J. Clin. Invest.* 120: 29-40.
32. Gowing, G., and C. N. Svendsen (2011) Stem cell transplantation for motor neuron disease: current approaches and future perspectives. *Neurotherapeutics* 8: 591-606.
33. Benkler, C., D. Offen, E. Melamed, L. Kupersmidt, T. Amit, S. Mandel, M. B. H. Youdim, and O. Weinreb (2010) Recent advances in amyotrophic lateral sclerosis research: perspectives for personalized clinical application. *EPMA J.* 2: 343-361.
34. Suzuki, M., J. McHugh, C. Tork, B. Shelley, S. M. Klein, P. Aebischer, and C. N. Svendsen (2007) GDNF secreting human neural progenitor cells protect dying motor neurons, but not their projection to muscle, in a rat model of familial ALS. *PLoS One.* 2: e689.
35. Corti, S., M. Nizzardo, M. Nardini, C. Donadoni, S. Salani, R. Del Bo, D. Papadimitriou, F. Locatelli, N. Mezzina, F. Gianni, N. Bre-

- solin, G. P. Comi (2009) Motoneuron transplantation rescues the phenotype of SMARD1 (spinal muscular atrophy with respiratory distress type 1). *J. Neurosci.* 29: 11761-11771.
36. Hwang, D. H., B. G. Kim, E. J. Kim, S. I. Lee, I. S. Joo, H. S. Kim, S. Sohn, and S. U. Kim (2009) Transplantation of human neural stem cells transduced with Olig2 transcription factor improves locomotor recovery and enhances myelination in the white matter of rat spinal cord following contusive injury. *BMC Neurosci.* 10: 117-132.
 37. Henriques, A., C. Pitzer, and A. Schneider (2010) Neurotrophic growth factors for the treatment of amyotrophic lateral sclerosis: where do we stand? *Front Neurosci.* 4: 32-45.
 38. Yu, J., M. A. Vodyanik, O. K. Smuga, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, I. I. Slukvin, and J. A. Thomson (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 318: 1917-1920.
 39. Park, I. H., R. Zhao, J. A. West, A. Yabuuchi, H. Huo, T. A. Inca, P. H. Lerou, M. W. Lensch, and G. Q. Daley (2008) Reprogramming of human somatic cells to pluripotency with defined factors. *Nature.* 451: 141-146.
 40. Alipio, Z., W. Liao, E. J. Roemer, M. Waner, L. M. Fink, D. C. Ward, and Y. Ma (2010) Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic beta-like cells. *Proc. Natl. Acad. Sci. USA* 107: 13426-13431.
 41. Hanna, J., M. Wernig, S. Markoulaki, C. W. Sun, A. Meissner, J. P. Cassady, C. Beard, T. Brambrink, L. C. Wu, T. M. Townes, and R. Jaenisch (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318: 1920-1923.
 42. Tsuji, O., K. Miura, Y. Okada, K. Fujiyoshi, M. Mukaino, N. Nagoshi, K. Kitamura, G. Kumagai, M. Nishino, S. Tomisato, H. Higashi, T. Nagai, H. Katoh, K. Kohda, Y. Matsuzaki, M. Yuzaki, E. Ikeda, Y. Toyama, M. Nakamura, S. Yamanaka, and H. Okano (2010) Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. *Proc. Natl. Acad. Sci. USA* 107: 12704-12709.
 43. Wernig, M., J. P. Zhao, J. Pruszak, E. Hedlund, D. Fu, F. Soldner, V. Broccoli, M. Constantine-Paton, O. Isacson, and R. Jaenisch (2008) Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. *Proc. Natl. Acad. Sci. USA* 105(15): 5856-5861.
 44. Xu, D., Z. Alipio, L. M. Fink, D. M. Adcock, J. Yang, D. C. Ward, and Y. Ma (2009) Phenotypic correction of murine hemophilia A using an iPS cell-based therapy. *Proc. Natl. Acad. Sci. USA* 106: 808-813.
 45. Egawa, N., S. Kitaoka, K. Tsukita, M. Naitoh, K. Takahashi, T. Yamamoto, F. Adachi, T. Kondo, K. Okita, I. Asaka, T. Aoi, A. Watanabe, Y. Yamada, A. Morizane, J. Takahashi, T. Ayaki, H. Ito, K. Yoshikawa, S. Yamawaki, S. Suzuki, D. Watanabe, H. Hioki, T. Kaneko, K. Makioka, K. Okamoto, H. Takuma, A. Tamaoka, K. Hasegawa, T. Nonaka, M. Hasegawa, A. Kawata, M. Yoshida, T. Nakahata, R. Takahashi, M.C. Marchetto, F. H. Gage, S. Yamanaka, and H. Inoue (2012) Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci. Transl. Med.* 4: 145-153.
 46. Kelly, H. (2002) Ten problems with embryonic stem cell research. *Acts & Facts.* 31: 2-5.