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INVITED REVIEW

Stem Cell Transplantation: A Promising Therapy for Parkinson's Disease

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Abstract Parkinson's disease is one of the most common neurodegenerative diseases caused by the loss of dopaminergic neurons in the substantia nigra pars compacta. Pharmacological therapies are valuable but suffer from two main drawbacks: side effects and loss of efficacy with disease progression. Surgical treatment is no better than drugs. Transplantation of embryonic mesencephalic tissue has emerged as a therapeutic alternative, but the unstable efficiency and the shortage of embryonic donors limit its clinical application. Recent advances in stem cell research inspire our hope that stem cell transplantation to replace degenerated neurons may be a promising therapy for Parkinson's disease. There are three sources of stem cells currently in testing: embryonic stem cells, neural stem cells, and mesenchymal stem cells. The stem cell transplantation in the animal model of Parkinson's disease proves that it is capable of relieving symptoms and restoring damaged brain function. Future stem cell research should focus not only on ameliorating the symptoms of Parkinson's disease but also on neuroprotection or neurorescue that can favorably modify the natural course and slow the progression of the disease.

Keywords stem cells · Parkinson's disease · dopaminergic neurons · transplantation · regeneration

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Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases caused by the progressive and selective loss of mesencephalic dopaminergic neurons of the nigrostriatal pathway. The characteristic symptoms of PD include tremor, bradykinesia, rigidity, and postural instability. Current therapies center on medical and surgical treatment for controlling these symptoms. Pharmacological therapies such as levodopa, dopamine agonists, and monoamine oxidase-B inhibitors are effective in the early stages of the disease. Levodopa is still the most effective drug, but the side effects of the drug such as fluctuations and dyskinesias influence patients' quality of life (Chapuis et al. 2005; Isacson 2004). Numerous studies have demonstrated that bilateral subthalamic nucleus or globus pallidum internus stimulation improves Parkinsonian symptoms and prolongs the "on" time. This intervention reduces the daily levodopa dose and ameliorates levodopa-related motor complications and dyskinesias. Unfortunately, all of these therapies cannot stop the disease from progression (Anonymous 2001; Krack et al. 2003). Future therapeutic strategies need to focus not only on ameliorating the symptoms of PD but also on protecting or rescuing the degenerated neurons to slow the progression of the disease.

Stem cells are undifferentiated cells without mature tissue-specific characteristics, and they are able to proliferate to reproduce themselves. They are also able to transform into progenitor cells that can differentiate into one or more cell types in response to proper stimuli. The key properties of stem cells, namely, self renewal and multipotentiality, have made such cells very attractive alternative cell sources for neural transplantation. Because PD is mostly caused by the loss of specific type of neurons from midbrain, the stem cell therapy for PD is relatively competitive. The clinical trials of embryonic mesencephalic tissue graft seem to be able to relieve motor symptoms and reduce levodopa uptake (Brederlau et al. 2006; Hagell et al. 1999). Although the outcome of the therapy is uncertain, and it is difficult to get donor tissues, the embryonic mesencephalic tissue transplantation is still an alternative therapy for PD.

The stem cell transplantation in PD is simply to provide dopaminergic neurons to replace the lost functions of degenerated neurons. These cells are typically classified according to their origins. Embryonic stem (ES) cells are found in the inner cell mass of the preimplantation blastocyst, whereas adult or tissue-derived (somatic) stem cells are found in developed tissues of the fetus or in the newborn, juvenile, and adult organism. Accordingly, there are three groups of stem cells commonly used to treat PD: ES cells, neural stem cells (NSCs), and mesenchymal stem cells (MSCs; Fig. 1). ES cells are pluripotent cells that can give rise to all cell lineages. ES cells after differentiating to NSCs and/or neural progenitor cells can be used for transplantation. NSCs and MSCs belong to somatic stem cells that can develop into mature neurons in the adult central nervous system (CNS). NSCs isolated from embryonic or adult brains are restricted to regenerate cell lines of the nervous system and have less propagation potential than ES cells. MSCs in the bone marrow and umbilical cord are capable of differentiating along multiple lineages including neural cells and have significant expansion capacity. This article will review the current status of stem cell research in PD and provide different views of the promising but challenging therapeutic potential for the treatment of this devastating disease.

Embryonic stem cells

ES cells have a unique characteristic to proliferate in an undifferentiated state. Unlike transformed tumor cell lines, ES cells can retain normal karyotypes after extensive passaging in cultures. Another critical characteristic of ES cells is their ability to differentiate into all lineages in vivo and into many cell types in vitro. Therefore, ES cells are

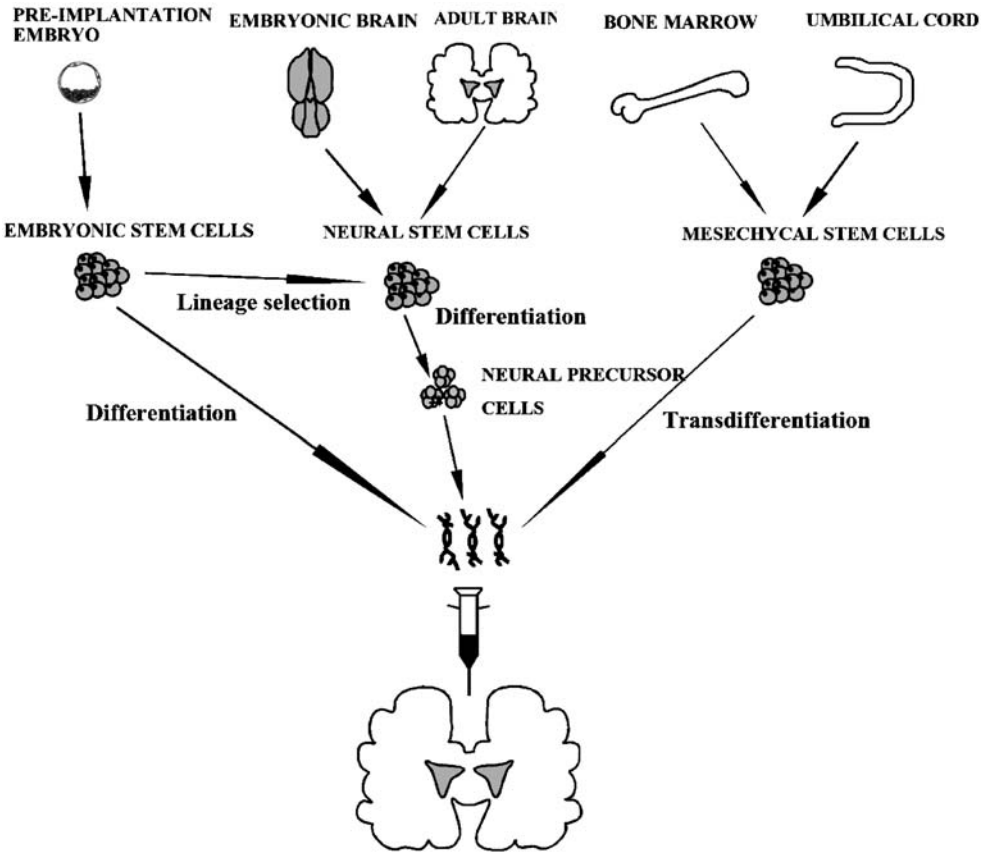

Fig. 1. Alternative sources of stem cells for transplantation in PD.

considered the best candidate of stem cell sources for transplantation. The success of isolation of human ES cells in 1998 dramatically arose the public interest in ES cell therapy (Thomson et al. 1998). To maintain being undifferentiated status, ES cells are cocultured with feeder cells and treated with leukemia inhibitor factor (Smith et al. 1988; Williams et al. 1988).However, direct differentiation of ES cells is far more complicated.

Each of the three major cell types of CNS—neurons, astrocytes, and oligodendrocytes—could be generated and isolated under appropriate conditions from ES cells (Barberi et al. 2003; Okabe et al. 1996). Two aspects of dopaminergic neuron generation in vitro are considered: genetic modification and manipulation of culture condition. Tyrosine hydroxylase (TH) is the rate-limiting enzyme for dopamine synthesis, which indicates the dopaminergic neuron phenotype. Nuclear receptor-related factor (Nurr1)-deficient mice failing to generate TH^+ midbrain dopaminergic neurons suggests that the overexpression of Nurr1 strategy promoted the differentiation of stem cell (Zetterstrom et al. 1997). It was supported that the introduction of Nurr1 facilitated mouse ES cells to differentiate into dopaminergic neurons (Kim 2004). Pitx3 is another factor necessary for the complete maturation and survival of the midbrain dopaminergic neuron population (Nunes et al. 2003). Nurr1 and Pitx3 cooperatively promoted terminal maturation to the midbrain dopaminergic neuron phenotype in murine and human ES cell cultures (Martinat et al. 2006).

The five-stage method is a typical approach for ES cells differentiating into dopaminergic neurons in vitro. Undifferentiated ES cells are expanded and induced to embryonic bodies (EBs). The selected $nestin^+$ cells from EBs are expanded and induced to TH^+ neurons. Compared with the five-stage method, stromal cell-derived inducing activity (SDIA) method is not only faster and easier but also increases the efficiency of mouse ES cell differentiation (Kim 2004). Mouse ES cells cocultured with PA6 stromal cells contain a significantly high proportion (~90%) of TH^+ neurons when treated with the signaling molecules sonic hedgehog (Shh), fibroblast growth factor (FGF)-8, and ascorbic acid. Transplantation experiments show the efficient generation of TH^+ neurons from implanted ES cells in mouse striatum (Kim et al. 2006a). Mouse ES cells differentiated on PA6 cells significantly reduce the amphetamine-induced turning behavior in grafted animals (Baier et al. 2004). Besides PA6, 90% of primate ES cells when cocultured with mouse Sertoli cells turn to TH^+ after 3 weeks induction. These grafted cells can survive for 2 months in murine models (Yue et al. 2006). Human ES cells can also be induced to dopaminergic neurons in vitro and in vivo. Several human ES cell lines are able to differentiate into dopaminergic cells with a feeder-free method similar to the five-stage method and appear dopaminergic traits after transplantation (Iacovitti et al. 2007). Bone morphogenic protein (BMP) promote cell death and inhibits the proliferation of early ventricular zone progenitor cells (Mehler et al. 2000). Noggin, a BMP antagonist, markedly enhances the yield of neuroepithelial progenitors from human ES cells that could give rise to dopaminergic neurons in vitro and in vivo with the SDIA method (Sonntag et al. 2007). Human ES cells exposed to both Shh and FGF-8 with telomerase-immortalized human fetal midbrain astrocytes potentiate dopaminergic neurogenesis differentiating to dopaminergic neurons. The graft of cells yield a significant, substantial, and long-lasting restitution of motor function in Parkinsonian rats (Roy et al. 2006).

Neurospheres are floating heterogeneous spheroid structures that contain neural stem cell progenitors and differentiated cells. These cells are usually embedded in a complex extracellular matrix with a core of differentiating glial fibrillary acidic protein $(GFAP)^+$ and tubulin III^+ cells surrounded by $nestin^+$, epidermal growth factor receptor $(EGFR)^+$, and β 1 $integrin^+$ undifferentiated cells (Campos et al. 2004). Mouse ES cells on PA6 cells could differentiate into neurospheres in the presence of FGF-2 and EGF (Kitajima et al. 2005). FGF-20, a novel member of FGF family, preferentially expresses in the substantia nigra pars compacta and protects dopaminergic neurons (Ohmachi et al. 2000). Synergistic action of FGF-20 and FGF-2 increases the number of dopaminergic neurons in monkey ES cells-derived neurospheres. These transplanted cells function as dopaminergic neurons and attenuate 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurological symptoms in primate models (Takagi et al. 2005).

ES cells are the most useful cell source for transplantation. However, several concerns have been raised of tumor formation. For example, the ES cells diluted into single-cell suspensions for engraftment encounter tumor formation in 44% of animals (Bjorklund et al. 2002). The naïve ES cells doped in cell graft or the late-passage ES cells with genomic alteration may give rise to teratoma or other uncontrolled cell growth. Another concern is the genomic alterations in the late passages of cultured human ES cells, some of which are similar to cancer cells (Maitra et al. 2005). Although most ES cells keep normal karyotypes, periodic monitoring will be required before they are used in clinical application.

The genetical modification of ES cells may reduce the chance of tumor formation. Cripto is a member of the EGF–CFC family, which expresses in multiple cancer cells (Baldassarre et al. 2001; D'Antonio et al. 2002). The anti-CFC domain antibody is able to block Cripto's function and decelerated the growth of tumor cells (Adkins et al. 2003). In vitro differentiation of $Cripto^{-/-}$ ES cells in rat model of

PD result in increasing dopaminergic differentiation and behavioral and anatomical recovery without tumor formation (Parish et al. 2005). Another alternative way to avoid these potential problems is to express a "suicide" gene in ES cells. Human ES cell lines can be genetically engineered to express the herpes simplex virus thymidine kinase (HSV-tk) gene, which can render the ES cells sensitive to ganciclovir and induce destruction of HSV-tk(+) cells in the presence of ganciclovir that are nonlethal to other cell types in vivo (Schuldiner et al. 2003).

Neural stem cells

NSCs exist in the nervous system, with the ability to self renew and to give rise to only cells belonging to all three lineages in the nervous system, namely neurons, oligodendrocytes, and astrocytes. In the embryonic brain, neurons are produced at two stages and two modes of cell division. First, progenitor cells are formed in a narrow zone around the telencephalic ventricle. By symmetric cell division, this ventricular zone grows exponentially. This is followed by asymmetric cell division, in which one precursor cell gives rise to another precursor cell and neuron. Then, the precursor cell migrates from the ventricle to distant positions forming the cortical plate (Roth and Dicke 2005). Recent studies have demonstrated that NSCs exist not only in the developing brain but also in the adult brain (Palmer et al. 2001). However, because of the poor proliferation potential, stem cells in the adult brain cannot retrieve the loss of dopaminergic neurons in PD patients, which requests the transplantation of foreign NSCs. To obtain enough NSCs in vitro for graft, there are two main methods currently in use: (1) as free-floating, clonally derived neurospheres grown in the presence of the mitogens EGF and/or FGF-2; or (2) as adherent immortalized NSC lines typically carrying an oncogene to facilitate continued proliferation, again grown in the presence of FGF-2 and/or EGF.

The NSCs induced to neurospheres differentiate into several phenotypes of neural cells, including dopaminergic neurons. NSCs can integrate in the brain, restore the nigrostriatal pathway, and ameliorate symptoms, and these cells can survive up to 5 months in the host environment after implantation (Armstrong et al. 2002; Harrower et al. 2006). Manipulation of culture condition and genetic engineering can enhance the differentiation of NSCs. It has been reported that FGF-20 can promote the differentiation of NSCs into TH^+ neurons by proximately 80% (Timmer et al. 2006). Nurr1 is a transcription factor that can facilitate both ES cells and NSCs to differentiate into dopaminergic neurons (Kim et al. 2002; Park et al. 2003). Bcl-X_L-overexpressing can increase the capacity of spontaneous dopaminergic differentiation of human NSCs in vitro and in vivo and also enhance the generation of human NSCs (Liste et al. 2004).

In addition, NSCs may rescue neurons by releasing nutrition factors. Glial cell line-derived neurotrophic factor (GDNF) has been reported to enhance the survival of midbrain dopaminergic neurons in vitro and to rescue degenerating neurons in vivo (Love et al. 2005). However, to avoid the serious side effect of GDNF, it must be injected into putamen directly and continuously (Gill et al. 2003; Nutt et al. 2003). NSCs transfected with GDNF or neurturin can survive several months and obviously improved the motor behavior after grafted in murine models of PD (Akerud et al. 2001; Liu et al. 2007; Ostenfeld et al. 2002). Transplantation of human NSCs cloned by v-myc gene transfer exerted neuroprotective effects against dopaminergic depletion in vitro and in vivo by suppressing apoptosis through Bcl-2 upregulation. Parkinsonian behavioral symptoms of 6-hydroxydopamine-lesioned rats are significantly ameliorated compared with controls for trophic factor secretion and neuronal differentiation of human NSCs (Yasuhara et al. 2006). Brain transplantation of human NSCs transduced with TH and GTP cyclohydrolase 1 provides functional improvement in animal models of PD (Kim et al. 2006b).

The neuroprotective role of NSCs in implants may be as important as functional replacement. NSCs seem to migrate in a wider range and integrate better than ES cells, and there is no report about tumorigenesis. However, NSCs are not good at proliferation and survival in vivo as ES cells.

Mesenchymal stem cells

Bone marrow stem cells have great potential as therapeutic agents as they are easily isolated and can be expanded from patients without serious ethical or technical problems. There are hematopoietic and mesenchymal stem cells in the bone marrow. Bone marrow stromal stem cells, namely MSCs, can differentiate into not only skeletal muscle and cardiac muscle cells derived from mesoderm (Ferrari et al. 1998; Orlic et al. 2001), but also lung and liver cells that are usually derived from the ectoderm (Theise et al. 2002, 2000). The term transdifferentiation is originally used by developmental biologists to describe the ability of apparently fully differentiated cells derived from a given tissue to change into cells with characteristics of a different tissue in response to either cell culture or surgical removal of adjacent tissue. Today, it is commonly used to describe the plastic ability of stromal stem cells to differentiate into cell lineages of tissues different from the one in which the somatic stem cell resides and even into cells originating from other germ layers. The MSCs are of interest in this

aspect because transdifferentiation would allow generation of, for example, neural progenitor cells for autologous stem cell therapy.

Numerous studies have shown that rat and human MSCs can differentiate into cells that display neuronal characteristics in vitro (Woodbury et al. 2000). More researchers are interested in neuronal differentiation potential of MSCs in vivo. Several teams have proved in different ways that bone marrow-derived cells are capable of entering mouse CNS and differentiating into neurons through blood circulation (Orlic et al. 2001; Sanchez-Pernaute et al. 2005). Intrastriatal transplantation of mouse MSCs exhibits significant improvement on the rotarod test, and the cells survives more than 4 months (Li et al. 2001). The TH-engineered rat MSCs grafted to Parkinsonian rat decreased the rounds of asymmetric rotation, and the TH gene expression efficiency is about 75%. Therefore, MSCs can be used as delivery vehicles for gene therapy (Lu et al. 2005). Unlike other stem cells, MSCs are capable of migrating to repair diseased cells and tissues (Hellmann et al. 2006).

Once considered as a biological waste product, umbilical cord blood (UCB) has emerged as a viable source of hematopoietic stem cells for transplantation, which contains one tenth of the number of stem cells found in the bone marrow. Human umbilical cord-derived $CD133^+$ hematopoietic stem cells can transdifferentiate into neural cell types of neuron-like cells, astrocytes, and oligodendrocytes by RA treatment (Jang et al. 2004). Human unrestricted somatic stem cells derived from UCB can differentiate into cells with neural features in serum-withdrawal medium, which express transcripts of genes associated with development and/or survival of dopaminergic neurons including En1, En2, Nurr1, Ptx3, Pax2, Wnt1, and Wnt3a (Fallahi-Sichani et al. 2007). Stem cells derived from the human umbilical cord Wharton's Jelly, called umbilical cord matrix stem (UCMS) cells, are another MSCs source. Human UCMS cells were induced to transform into dopaminergic neurons in vitro through stepwise culturing in neuron-conditioned medium, Shh, and FGF-8, and 12.7% of cells are TH^+ (McGuckin et al. 2004).Undifferentiated human UCMS cells are transplanted into the brains of hemiparkinsonian rats without immune-suppressed ameliorated apomorphine-induced rotations in the pilot test (Weiss et al. 2006).

MSCs are plentiful, safe, and ethically acceptable source of stem cells. Autograft of MSCs will be a perfect plan to prevent immunologic rejection. They can migrate into the brain from peripheral circulation and gather in the damaged location, which is quite convenient to graft. A significant advantage of UCB is that most patients who do not have a matched bone marrow donor are likely to have a suitably matched UCB unit. However, the MSCs won't survive and be effective as ES cells. Future improvement in purification and culture may help in counteracting these shortcomings.

Other cell courses

Other stem cell sources may include embryonic germ cells and amniotic fluid-derived stem cells that are considered multipotent cells. Cultured human embryonic germ (hEG) cells can be induced to TH^+ in vitro (Pan et al. 2005), and differentiated hEG cells can replace neurons in the damaged mice brain (Mueller et al. 2005). These data suggest that hEG cells may provide a potential cell source for transplantation. Human amniotic fluid-derived stem cells stably express dopaminergic markers in vitro, including Pitx3 and Nurr1, which are essential for induction and survival of midbrain dopaminergic neurons (McLaughlin et al. 2006). High performance liquid chromatography analysis shows the evidence of dopamine release in the extract of dopaminergic-induced clonal amniotic fluid-derived stem cells (Tsai et al. 2006).

Immune concerns in stem cell therapy

A common concern of stem cell therapy is immunological rejection. Although the brain is recognized as an immunologically privileged site, rejection emerges in allograft experiment. There are numerous immunosuppressive drugs and protocols designed for allografting. Glucocorticoids are the first immunosuppressants used in transplantation. Despite their wide use in clinics, they are the least selective agents and affect multiple leukocyte cell lines, including T and B cells, macrophages, granulocytes, and monocytes. Other immunosuppressive treatments, like cyclosporine, are not very well tolerated by patients and may compromise the effectiveness of the transplanted cells (Barker and Widner 2004). It is quite difficult to find a matched cell source, but ES cells generated by nuclear transfer of recipient's somatic nuclei into oocytes may avoid immune response (Chen et al. 2003). Another method to avoid immune rejection of nonautologous cells is to place them in microcapsules (Black et al. 2006; Blanco-Bose et al. 2006). Although efficacy for immunoisolation of the microcapsules still remains for future

Table 1 Essential properties of stem cells for use in clinical transplantation

Stem cells: properties required for clinical transplantation
Capable of clonal propagation in vitro to ensure homogeneity
Genetic stability at high passage
Integration within the host brain after transplantation
Connectivity within host circuits
Migration and engraftment at sites of damage
Correct differentiation into appropriate neural cell types
Functional benefits
Lack of side effects

in vivo studies, encapsulated ES cells differentiate just as nonencapsulated ES cells do (Dean et al. 2006).

Conclusion

Although stem cell therapy is very promising, there are many scientific, clinical, and ethical issues that need to be resolved before testing in PD. The stem cells for implantation must meet certain criteria as outlined in Table 1. ES cells are the best candidate of cell sources, but the ethical debate on using aborted embryo and the risk of tumor formation obstruct the use of these cells. NSCs and MSCs taken from the patients themselves might be another cell source for that purpose with little concern for immune rejection. The potential modification in gene expression and the unstable genome in long-term passage increase the danger of uncontrolled cell growth. The safety of stem cell transplantation must be confirmed by further studies.

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