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Stem cells and neurodegenerative diseases

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Neurodegenerative diseases are characterized by the neurodegenerative changes or apoptosis of neurons involved in networks, which are important to specific physiological functions. With the development of old-aging society, the incidence of neurodegenerative diseases is on the increase. However, it is difficult to diagnose for most of neur odegenerative diseases. At present, there are too few effective therapies. Advances in stem cell biology have raised the hope and possibility for the therapy of neurodegenerative diseases. Recently, stem cells have been widely attempted to treat neurodegenerative diseases of animal model. Here we review the progress and prospects of various stem cells, including embryonic stem cells, mesenchymal stem cell and neural stem cells and so on, for the treatments of neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic lateral sclerosis/Lou Gehrig's disease.

stem cells, neurodegenerative diseases, therapy

The damage and repair of the central nervous system (CNS) has long been known to be the keystone of neurobiological research. Neurodegenerative change is one of the major reasons of the CNS damage. Neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), Amyotrophic lateral sclerosis/Lou Gehrig's disease (ALS) and Creutzfeldt-Jakob disease (CJD), are characterized by the neurodegenerative changes or apoptosis of neurons involved in networks, important for specific physiological functions. Most of neurodegenerative disorders are typically late-onset and develop slowly. With the development of old-aging society, the incidence of neurodegenerative diseases is on the increase. These disorders affect human health severely. At present, there are too few effective therapies. Recent progress in stem cell biology raise the hope for the development of stem cell therapies in human neurodegenerative disorders**.**

Stem cells are immature cells with prolonged self-renewal capacity and ability to differentiate into multiple cell types or all cells of the body. During the generation and development of human and animal, there are stem cells with self-renewal capacity and differentiation potential in embryonic and adult tissues. Stem cells possess of wide sources. The cell preparations could be standardized and quality-controlled with respect to viability and purity. In an appropriate environment, stem cells are able to be induced to differentiate into multiple cell types or all cells of the body. Stem cell is an ideal model system for the study of embryonic generation, cell differentiation, gene expression and regulation. With stem cell engineering developing, stem cell-based replacement therapy will be a new way for clinical tissue damages, genetic defects and degeneration diseases. At present, stem cell therapy has been used clinically in the treatment of haematological diseases, surgical diseases, nervous system diseases and other clinical disorders.

1 Stem cell and Parkinson's disease

PD is a progressive neurodegenerative disease. The main pathology includes the specific degeneration and

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damage of some neurons from a region of the midbrain involved in the control of movement, including nigrostriatal dopaminergic neurons, noradrenalin neurons, 5-hydroxytryptamine neurons and acetylcholine neurons. PD is characterized by tremors, slowness of movement, rigidity, and balance problems, etc. PD patients with different type neuron loss display different symptoms. Their causes are not confirmed. Although some cases (classified as familial or inherited) have been associated with a family history of the disorder, the investigation shows that inheritance factor is not the main cause. The average age of onset varies between about 55 and 65 years of age and the incidence increases with age to about 1% of those over 65. Because of this, it is generally seen as a disease of the elderly. However, about 5%—10% of cases are diagnosed below the age of 40. At present, the causes of PD are not yet fully understood. There are too few effective therapies.

Investigators attempted to treat PD with tissue transplantation ten years ago. They transplanted human fetal mesencephalic tissue with rich postmitotic dopaminergic neurons into striatum of PD patient. The grafted neurons survived and reinnervated the striatum for as long as 10 years[1,2]. The grafts were able to normalize striatal dopamine release[2] and to reverse the impairment of cortical activation underlying akinesia[3]. Although this therapy strategy can alleviate or treat PD, it is improbable that transplantation of human fetal mesencephalic tissue will become routine treatment for persons with PD because of tissue availability and too much variation in functional outcome and ethical problem. After this, some laboratories tried to transplant fetal midbrain precursors-derived dopaminergic neurons to treat PD. They found that transplanted midbrain precursor-derived dopamine neurons can be integrated into neuronal circuitries in the brain and lead to recovery in a rat model of Parkinson's disease[4–7]. However, they also found that midbrain precursor-derived dopamine neurons could not secrete dopamine persistently, and that dyskinesia, chronic inflammatory and immune responses could develop after transplantation and become troublesome in some grafted patients[8,9].

Stem cells possess wide sources and prolonged self-renewal capacity and multilineage differentiation potential, and can be easily isolated and genetically manipulated. So they are excellent seed cells for replacing and repairing damages or senile tissues. At present, embryonic stem cells have already been used to treat PD in some laboratories. Björklund LM and colleagues reported that transplanting low doses of undifferentiated mouse embryonic stem (ES) cells into the rat striatum resulted in a proliferation of ES cells into fully differentiated dopamine neurons after 14 weeks. Such dopamine neurons could restore cerebral function and behavior in an animal model of PD[10]. Kim and colleagues developed a five-stage method that led to the efficient differentiation of ES cells into tyrosine hydroxylase positive (TH^+) neurons. They transplanted these ES-cell-derived TH^+ cells into the PD rat striatum and found that these cells released dopamine, extended axons into the host striatum, formed functional synaptic connections and modulated spontaneous and pharmacologically induced behaviours, as well as led to recovery in a rodent model of Parkinson's disease[11]. After this, Nishimura and colleagues[12] also transplanted mouse ES-cell-derived TH^+ cells into the PD mice and found a significant reduction in rotational behavior.

Recently, there was great progress in PD treatment by ES cells. Takagi and colleagues[13] produced a highly enriched population of proliferating neural progenitors derived from stromal cell-derived inducing activity (SDIA)-treated monkey ES cells. Furthermore, treatment of these cells with cytokine combination induced the generation of a large population of dopamine neurons from ES cell-derived neural progenitors. Then they transplanted these dopamine neurons into the striatum of a primate model for PD. Their results showed that the transplanted cells functioned as dopamine neurons, attenuating the monkeys' PD symptoms after 10 weeks, and also suggested that consciousness was not disturbed in any animals, and no deterioration was observed postoperatively. In addition, none of the treated animals developed dyskinesia[13]. This trial provided a reliable science basis for a clinical therapy of human PD by ES cells.

Hellmann and colleagues reported that transplanted bone marrow mesenchymal stem cells (MSCs) could survive in the brain of PD animal model. Moreover, these cells could differentiate into neurons and migrate through the corpus callosum to populate the striatum[14]. Weiss and Fu[15,16] transplanted undifferentiated human umbilical cord matrix stem (UCMS) cells into the brains of PD rats respectively and found that UCMS cells could survive for at least 4 months, the rotations were ameliorated and these transplanted cells did not produce brain tumors and a frank host immune rejection re-

sponse[15,16]. The recent report suggested that MSCs transplanted at sites of nerve injury could promote functional recovery by producing trophic factors that induce survival and regeneration of host neurons[17]. Another study indicated that transplanting of stem cells from human bone marrow into the brain of immunodeficient mice markedly increased the proliferation of endogenous neural stem cells[18]. These results provided test evidence to explain recent observations in which MSCs or related stem/progenitor cells were found to produce improvements in disease models even though a limited number of the cells engrafted.

In the study of Barker, primary porcine fetal neural tissue or cortically derived neural stem cells were transplanted into 6-hydroxy dopamine lesioned rat striatum and the results demonstrated that cortically derived neural stem cells (NSCs) survived up to 5 months in contrast to the poor survival of primary porcine xenografts. Moreover, histological analysis demonstrated good graft integration with fibers extending into the surrounding host tissue including white matter with synapse formation. This demonstrates that such xenografted cells may be able to recreate the damaged circuitry in PD[19]. In the study of Sladek Jr[20], human neural stem cells (hNSCs) were implanted into the caudate nucleus and the substantia nigra of the 1-methyl-4-phenyl-1,2,3,6-tetra-hydropyridine(MPTP)-treated monkeys. 4 and 7 months respectively after hNSCs implants, the size and number of TH^+ cells in the brain were assessed. The results showed that in the presence of hNSCs, the number and size of caudate TH^+ cells returned to non-MPTP-treated control levels[20]. This indicated that hNSCs may be beneficial to maintaining a normal striatal environment. Redmond and colleagues reported that undifferentiated human neural stem cells (hNSCs) implanted into MPTP-treated Parkinsonian primates were able to survive, migrate, and had a functional impact by behavioral improvement in this dopamine-deficit model. A small number of hNSC progeny differentiated into TH^+ and/or dopamine positive cells and most of them differentiated into astrocytes to regulate microenvironment around the neurocytes and to deal with the injury[21].

All these investigations suggest that ES cells, MSCs and NSCs are capable of becoming ideal seed cells for therapy of PD. Moreover, it has been proved that hNSCs can attenuate primates' (the monkey) PD symptoms. This prominent progress provides a theoretical basis for PD therapy by using stem cells.

2 Stem cells and Alzheimer's disease (AD)

AD is a progressive, degenerative and fatal brain disease. Some studies suggest that brains from AD patients show several distinct neuropathological features, including degeneration of cholinergic neurons of the Meynert nucleus, 50 percent loss of neurons containing choline acetyltransferase, selective loss of cholinergic neurons of the hippocampus and reduction of acetylcholinesterase and choline acetyltransferase of cerebral cortex[22,23]. Alzheimer's destroys brain cells, causing problems with memory, thinking, attention, language, understanding, behavior severe enough to affect work and daily life. Not only older people but also younger people of age 30—50 may also get AD. However, most of patients aged 65 and older can be diagnosed. The early form of AD is usually found in younger people. These patients account for 10 percent of the total and are thought generally inherited cases. In 2025, there will be 22 million AD patients in the world. The incidence of AD is 7 percent in Beijing. It is similar to that of occident. At present, no effective treatment has been proven to stop AD. Acetylcholinesterase inhibitors and neuron protecting factors are currently available to alleviate AD symptom.

With the development of stem cell biology, stem cell replacement therapy becomes a new way. Martinez-Serrano and colleagues[24] attempted to transplant forebrain cholinergic neurons into the host striatum and Meynert nucleus, and found that the cells survived well in the host brain for long periods of time, and induced a hypertrophic response of cholinergic neurons. Sinden and colleagues[25] reported that transplanting of NSCs with rich choline alleviated AD symptoms of rat. In the study of Qu et al.[26], the human undifferentiated NSCs were injected into the brain of 6-month-old and 24-month-old rats respectively. Their results demonstrated that human neural stem cells improved cognitive function of aged brain. Wu and colleagues[27] proved that human fetal brain-derived neural stem cells grafted in adult rat brain generated cholinergic neurons in specific region.

Wang and colleagues[28] transplanted ES cells-derived neurospheres into mouse model frontal cortex of Meynert nucleus lesion. They found that transplanted neurospheres survived, migrated and differentiated into many choline acetyltransferase-positive neurons and a

few serotonin-positive neurons. The working memory error decreased significantly in the mice grafted with neurospheres. In contrast, ES cells developed into teratomas in all of the control mice and expressed no neurons, and the working memory deteriorated remarkably[28].

Pathologyof AD is characterized by the deposits of amyloid β-protein (Aβ), an amino acid peptide fragment derived from sequential proteolytic cleavage of the amyloid precursor protein (APP) by beta- and gamma-secretases. The study indicates that APP signal pathway is a regulation system associated with the differentiation and migration of NSCs[29]. APP expression increases in the growth brain and injured brain[30,31]. Overexpression of APP results in the differentiation of NSCs to glia. These NSCs-derived glia secrete trophic factors to support injuried cells and to promote the neuron migration and the differentiation of NSCs[32]. Sugaya and colleagues[33] found that glial differentiation took place in stem cells transplanted into APP transgenic mice. RNA interference of APP or reduction of APP levels in the brain significantly reduced glial differentiation of stem cells. In another study, human umbilical cord blood mononuclear cells containing rich MSCs were transplanted into APP transgenic mice brain and ameliorated AD[34]. Recent study demonstrated that the alpha-secre-tase-cleavedfragment of the amyloid precursor protein (sAPPalpha), a potent neurotrophic factor, potentiated the nerve growth factor (NGF)/retinoic acid (RA) induced transdifferentiation of bone marrow-derived adult progenitor cells (MAPCs) into a cholinergic-like neuronal phenotype. These findings suggest the combined use of sAPPalpha and MAPCs offers a new and potentially powerful therapeutic strategy for AD treatment[35].

The above progress suggests both NSCs and bone marrow-derived stem cells can ameliorate AD in different degrees and show excellent potential for AD treatment. However, till now, most of investigations are limited in the animal model. There are still many problems before the clinical treatment of human AD by stem cells, such as how one determines dosing of the cells, what brain sites should be targeted for transplantation, and how about the long-term effect is.

3 Stem cell and Huntington's disease

HD is a fatal hereditary and neurodegenerative disease. It is inherited as an autosomal dominant condition. The presence of an expanded stretch of CAG repeats seems to correlate well with the presence of HD. The DNA sequence, CAG (cytosine-adenine-guanine), is part of huntingtin gene sequence. The presence of CAG repeats results in the loss of specific subpopulations of neuronal cells (GABAergic neurons), primarily in the striatum of the basal ganglia and, to a lesser extent, in other extrapyramidal and cortical areas. HD usually develops in middle age. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult. Typical clinical features include rapid, uncontrolled movements of the limbs and trunk, cognitive impairment and emotional disturbances such as depression and irritability. There is no treatment to halt the progression, which leads to death after ten to twenty-five years. Experts estimate that nearly 30000 have HD and 150000 are in danger in the United States. At this time, there is no way to stop or reverse the course of HD. Some drugs can only alleviate the symptom.

In the 1990s, fetal striatum was transplantated to treat human HD[36−38]. These studies firstly demonstrate that human fetal striatum is able to survive and develop in the host brain, and support the theory that cell transplantation can replace damaged neurons and reconstitute the neural circuits. The experiments indicated that transplantation of rat striatal neurons into the lesioned striatum of nonhuman primates replaced damaged neurons and reconstructed the neural circuits[39,40]. A recent report indicated that fetal neural tissue transplantated into the brain of HD patient differentiated into neurons and survived in human neostriatum for more than 6 years. Despite prolonged survival, these grafts had poor integration with host striatum that was likely responsible for the lack of clear clinical improvement in these patients[41].

With the further development of stem cell biology, it has been demonstrated that NSCs, MSCs and ES cells can differentiate into neurons and other neural cells in specific environment. Recently, some laboratories attempted to treat HD by transplanting stem cells and made some progress. Lescaudron and colleagues[42] transplanted autologous bone marrow stem cells in the damaged striatum of the rat HD model and found that the transplant significantly reduced working memory deficits. However, most of the transplanted cells appeared quite primitive[42]. In the study of Kordower and colleagues[43], HD rats were transplanted with stem cells derived from human fetal cortex that were either pre-

treated in culture media with the differentiating cytokine or allowed to grow in culture media alone. They found that rats transplanted with cytokine-treated neurospheres had a greater striatal volume compared with those receiving transplant of untreated neurospheres. Grafted cells migrated to the globus pallidus and substantia nigra pars reticulate and differentiated into neurons and astrocytes. The symptoms of HD rats were alleviated[43]. Roh and colleagues[44] injected immortalized NSCs into the ventricle or via a tail vein following intrastriatal lesioning in rats. The results showed that transplanted NSCs migrated into the striatum either from ventricle or from the systemic circulation, and that tissue damage or tumor formation was not observed[44]. Kim and colleagues[45] also transplanted NSCs intravenously into rat brain. Their result indicated that intravenously injected human NSCs could migrate into the striatal lesion and differentiate into neurons and glias, and decrease the following striatal atrophy, and induce long-term functional improvement in the rat model[45]. Modo and colleagues[46] also reported that transplanted NSCs alleviated the symptoms and prevented the development of brain injury in HD rat. Modo and colleagues[47] injected neural progenitor cells-derived from the subventricular zone of adult rats in quinolinic acid (QA) lesion rat model striatum. Eight weeks after the transplantation, approximately 12% of transplanted cells survived and migrated extensively throughout the lesioned striatum, and differentiated into either astrocytes or mature neurons. Rats transplanted with adult neural progenitor cells also demonstrated a significant reduction in motor function impairment. Johann and colleagues[48] further demonstrated that the method of graft preparation of NSCs for transplantation, as well as the timing of the transplantation procedure strongly affected the survival of the donor cells. The best survival was found with the combination of early transplantation (i.e., 2 d after QA-lesion) with the use of neurospheres instead of dissociated cell suspension.

Recently, there is prominent progress in treatment of HD by NSCs. Bachoud-Lévi and colleagues[49] transplanted human fetal NSCs into the brains of five patients with HD. The results showed that three out of five patients with HD produced motor and cognitive improvements 2 years after intracerebral fetal neural graft. Clinical improvement plateaued after 2 years and then faded off variably 4－6 years after surgery. This study is a breakthrough and attempt of stem cell therapy for HD from the animal model to human. Although it has made an elementary success, there are still some problems to be resolved, such as how can stem cells produce a permanent cure for the HD, how to select suited patient, how to avoid the side effect, how dose stem cell therapy combine with neurotrophic support and neuroprotective treatment.

4 Stem cell and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS), sometimes called Lou Gehrig's disease, is a rapidly progressive and invariably neurological disease, which is characterized by the gradual degeneration and death of motor neurons in cerebral cortex and cholinergic neurons in ventral horn of spinal cord. Motor neurons located in the brain and spinal cord are responsible for controlling voluntary muscles. The degeneration or death of these motor neurons leads to the muscle atrophy and functional loss. The cause of ALS is not known. Mutations in the gene that produces the superoxide dismutase 1(SOD1) enzyme are associated with some cases of familial ALS. SOD1 gene mutation leads to cholinergic motor neuron degeneration and death, and causes random damage to proteins within cells[50]. The ALS symptoms include muscle weakness, paralysis, slurred and nasal speech, difficulty chewing or swallowing and breathe weakness. The disease usually does not impair a person's intelligence, memory and sense. The progress of ALS is rapid. Most people with ALS usually die within 2 to 5 years from the onset of symptoms. ALS most commonly strikes people between 40 and 70 years of age. As many as 120000 people have ALS in the world. No cure has yet been found for ALS.

Most of ALS cases are diagnosed in the late stage. Neuroprotective treatment can postpone the progress of disease but not prevent the ALS. Combining neuroprotective treatment with cell replacement therapy may be a better way for ALS. The replacement and regeneration of cholinergic motor neuron in a specific region of spinal cord is very important for the treatment of ALS[51]. The study indicated that the expression of Hb9 could induce mouse ES cells to differentiate into cholinergic motor neuron. These ES cell-derived motor neurons were able to populate the embryonic spinal cord, extend axons, and form synapses with target muscles[52]. Recent studies demonstrated human NSCs transplanted into the

injured spinal cord of marmosets could survive and differentiate into neurons, astrocytes, and oligodendrocytes, and promote functional recovery in marmosets. These NSCs-derived motor neurons replace injured motor neurons of ALS[53].

Lastly, Mazzini and colleagues[54] applied stem cells to clinical ALS patients. MSCs were isolated and expanded *ex vivo*, and suspended in autologous cerebrospinal fluid, and then transplanted into seven ALS patients. No patient manifested major adverse events such as respiratory failure or death. Mild adverse events were intercostal pain irradiation and leg sensory dysesthesia, both reversible after 6 weeks. The spinal cord volume was not modified. Abnormal cell proliferation was not observed. The linear decline of the forced vital capacity was significantly slowed down in four patients 36 months after MSCs transplantation. These results demonstrate that transplantation of MSCs into the spinal cord of ALS patients is safe, with no significant acute or late toxicity. This clinical result provides the theoretical basis and important experience for ALS treatment.

5 Perspectives

At present, great progress and breakthrough have been achieved in stem cell-based therapies for neurodegenerative disorders. However, the development of stem cell-based therapies is still at an early stage. Many basic issues remain to be resolved. Although ES cells seem to have an unrestricted potential to differentiate towards neuroectodermal phenotypes, the differentiation *in vitro* has enormous random. ES cells can give rise to teratomas and immune rejection response after transplantation. The risk for teratoma from ES cells as well as the consequences of introducing new genes in stem cell-derived neurons should be carefully evaluated. The isolation process of NSCs is standardized. They can differentiate to all of the major neural cells type *in vivo* and *in vitro*. When transplanted into the brain they are able to survive, migrate and integrate in a functionally active way. However, the source of human NSCs is absent. Stem cells isolated from adult brain as neurospheres generate fewer neurons than that isolated from embryonic or fetal brain, both in transplantation cases as well as in differentiating conditions *in vitro*. MSCs have broad sources. They can differentiate into neurons *in vitro*. However, the number of these transdifferentiated cells is extremely low in the host brain after transplantation. In addition, the stability and validity of investigation results in the animal model need to be further verified. Adverse effects and security of stem cell therapy must be evaluated seriously. Better criteria for selecting the patients suitable for cell therapy have to be defined. Before surgery, the transplantation procedure should be customized with respect to the dose and location of grafted cells so that the repair will be as complete as possible. Success of stem cell-based therapies for neurodegenerative disorders will depend on further investigation in stem cell biology and further understanding to pathogenesis of neurodegenerative disorders.

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