Stem Cell Therapy for Neurodegenerative Diseases: Strategies for Regeneration against Degeneration

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ABSTRACT

Neurodegeneration is a general term for the progressive loss of structure and/or function of neurons, gives rise to dysfunction or death of neurons. Neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), spinal cord injury (SCI) and brain ischemia (BI) occur as a result of neurodegenerative processes leading to different degrees of paralysis and loss of sensation and cognition in the patients. Unfortunately, no successful cure for neurodegenerative disorders has been developed so far, and most of the currently available pharmacological therapies are mainly palliative. In recent years, stem cells have provided a great opportunity to develop potentially powerful innovative strategies to cure neurodegenerative diseases. Stem cells transplantation is capable of restoring injured neuronal tissue by replacement of the damaged cells via using directly differentiated cells or by protecting of existing healthy neurons and glial cells from further damage, or by repairing through providing a conductive environment in favour of regeneration. Here we have brought together some of these examples, discuss possible therapeutic means using different types of stem cells, mainly adult stem cells (ASCs), to treat neurodegenerative diseases.

Keywords: Neurological disorders, Stem cell therapy, Genetically modification, Paracrine effects, Differentiation, Combinatorial treatment, Cell cartridge

Neurodegenerative diseases are divided into acute cases, like spinal cord injury (SCI) and brain ischemia (BI), in which different types of both neurons and glial cells restricted to the stroke site are lost over a short period of time (1, 2), and chronic cases such as Alzheimer disease (AD), Parkinson...
disease (PD), Huntington’s disease (HD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), that dysfunction or death of neurons and/or glial cells happen over the years. In chronic state, degeneration process could be widespread/general (i.e. affect many types of neurons in AD) or selective involving just one specific type of cell (e.g. dopaminergic neurons in PD and motor neurons in ALS) (3-5). However, currently there are no available treatments, and drugs are entirely palliative for these diseases, finally leading to progressive loss of sensation, cognition, and motor neurons and gradual paralysis of the patient (4). Therefore, stem cells have been proposed as a promising treatment for neurodegenerative diseases (1).

Most of the studies in stem cell-based therapy for neurodegenerative diseases have been conducted preclinically in animal models which have shown stem cells could differentiation into neuronal and glial cells, promote functional recovery of nervous tissue, affect endogenous cells, decrease motor impairments by trophic support and also prevent detrimental events associated with neurodegenerative disorders (6-9). In addition, there are some evidence from clinical trials indicates the same results as preclinical studies, although they are not consistent and convincing at this time (7, 10-13). Moreover, there are many planned and ongoing clinical studies investigating different aspects of stem cell therapies for neurodegenerative disorders (please refer to https://clinicaltrials.gov), which is growing every day.

Many different sources of stem cells have been examined to determine which one is the most efficacious for stem cell therapy of neurodegenerative diseases (14-16). In addition to embryonic stem cells (ESCs), stem cells derived from adults tissues/organs are of keen interest because they are readily available sources of stem cells and easily expanded in vitro without ethical problems and no tumor formation report so far (17, 18). The key benefit of adult stem cells (ASCs) is that they can be harvested from various sources frequently and possibility to use in autologous therapy. This omits the risks, ethical and religious problems and immunorejection issues that allogeneic ESCs have. However, their limited differentiation potential restricts universal use of ASCs (17, 19). In recent years, extensive efforts have been carried out by investigators to successfully generate neurons and glial cells from different types of stem cells, and to exploit other beneficial aspects of stem cells to treat neurological diseases. We review here previously published experiments, animal studies and clinical trials involving stem cells based treatment for neurodegenerative diseases and discuss the potential future perspective for stem cell therapy of neurological diseases in the clinical setting.

Degenerative disorders of the central nervous system (CNS)

Central nervous system (CNS) consists of brain and spinal cord, as well as their coverings which has special features such as diverse and complex cytology and topography, axoplasmic transport, neurotransmitters, myelin, three classes of intermediate filaments (i.e. vimentin, glial fibrillary acidic protein (GFAP), and neurofilaments), separate population of interstitial cells, glia cells, cerebrospinal fluid, blood brain barrier, absent lymphatic vessels and lymph nodes, and etc. Some conditions such as programmed aging (20), disruption of extracellular matrix (ECM) (21), deterioration by oxidative stress (22), and insufficient protein degradation and subsequently accumulation of misfolded proteins (23) could give rise to synaptic loss and neural or glial cells damage in CNS. For instance, aging through loss of brain parenchyma, shrinkage of large neurons, cellular gliosis, intraneuronal (intraglial) aggregation of proteins, viruses or lysosomal substrates affects CNS to develop neurodegenerative conditions (20).

Generally, neurodegenerative diseases such as AD, PD, HD, AML and MS have unknown etiology and associated with progressive dysfunction/death of neurons or their systems due to biochemical, structural and functional changes selectively or widespread which result in dementia (memory and cognitive impairment) and/or severe motor impairment (1, 4, 20). However, understanding of disease pathology in each case would be certainly required to develop stem cell based strategic plan/s. Hence, we aimed to mention pathology of each specific disease. For instance, AD is a widespread chronic case caused by neuronal loss throughout the brain; involve the basal forebrain cholinergic system, hippocampus, amygdala and several cortical areas.
(grey matter) with pathological characteristics of cortical atrophy, β-amylloid senile plaques (extraneuronal), and neurofibrillary tangles (intraneuronal) accumulation. One of possible mechanisms of synaptic and neural toxicity in Alzheimer’s disease has been shown, in part, is due to β-amylloid and Tau proteins hyperphosphorylation and aggregation (4). AD is the most prevalent form of dementia. This intractable degenerative disease was first explained by German neuropathology’s and psychiatrist Alois Alzheimer in 1906 and was entitled AD after him (24).

Other neurodegenerative disease, PD, is a selective chronic disease caused by the progressive death of a specific population of the cells, i.e., dopaminergic (DA) neurons in the substantia nigra and reduced DA stimulation in the striatum. PD is the second most prevalent form of dementia that like AD, researchers haven’t yet found the cure of PD. People with PD use therapies that increase their dopamine levels (10). The most current effective drug for treatment of PD is levodopa (Sinemet), because it directly converted into dopamine in the brain (25).

Moreover, AML is an adult-onset neurodegenerative disorder caused by degeneration and loss of motor neurons in the cerebral cortex, brainstem and spinal cord, leading to fatal paralysis. HD is another incurable disorder which caused by expansions of polyglutamate in the huntingtin protein. HD characterized by neuronal dysfunction and degeneration contribute to the progressive physiological, cognitive, sensational and motor impairments. MS is a CNS autoimmune disease contributed to degradation of myelin that sheath affects axonal signals transport of neurons, eventually leading to progression of the disease range from fairly benign to extremely debilitating. Unlike AD, PD, HD and ALS, MS predominately affects usually young adults, and also females affected nearly twice as often as males. Again, purely symptomatic treatments are currently available for MS patients.

In addition to chronic neurodegenerative processes, acute disorders of the CNS as a result of ischemic or trauma still are a big challenge for medicine (26). Focal tissue loss caused by cerebral artery occlusion in ischemic stroke of brain leads to the death of oligodendrocytes, and astrocytes, in addition to multiple types of neurons (5). Similar situation exist in spinal cord injury (SCI) due to traumatic damage to the spinal cord which commonly caused by vehicle accidents, sports injuries, and traumatic injury in the workplace (2). Loss of oligodendrocyte and axonal demyelination are major secondary damages after injury of SCI contribute to pathological processes (27).

How regeneration works

There is homeostasis in the human body which means that the tissues/organs are able to regulate internal conditions against external changing conditions, usually by feedback controls, and stabilize whole body functioning and health (28, 29). One part of homeostasis is the periodic or constant generation of new cells to repair or replace damaged/dying cells which is called regeneration. Adult (tissue) stem cells (ASCs) normally remain quiescent unless they received activation signals to divide through a process called asymmetric cell divisions. Through this process they can maintain their populations and also differentiate into the desired cell types by creation of a progenitor (more committed cells) for tissues regeneration (29, 30).

ASCs have located in throughout the body. These stem cells reside in a particular microenvironment of tissues/organs called the “niche” which fosters the growth, proliferation and differentiation of resident stem cells. Damages to tissues, signals they receive, and changes in the stem cell niche can activate them to take part in tissue/organ regeneration (28-30).

The CNS has a limited regeneration potential which is a main challenge to develop new effective therapeutic strategies to induce its functional repair. Already, various types of stem cells have been proposed as a viable therapeutic option for degenerative diseases as they possess high proliferation capacity and able to differentiate into multiple lineages (30-32). Alternatively, through paracrine mechanisms, stem cells are also capable of influencing their microenvironment, maybe by sharing soluble secretory factors, and exosome (vesicle) containing proteins, coding RNAs and even non-coding RNAs including miRNAs and IncRNAs (29, 33, 34) (Figure 1).
Figure 1. Mechanism of regeneration for neurodegenerative diseases by stem cells. Stem cells by asymmetric cell division proliferate, maintain cell populations, create progenitor cells, help to heal, and play a critical role in regeneration, more importantly, by secreting paracrine factors influence on neighboring cells.

There are four wide categories of stem cells considering their origin: embryonic, fetal, induced pluripotent stem (iPS) cells and ASCs (4, 30). Among ASCs, hematopoietic stem cells (HSCs) frequently found in the bone marrow, as well as in umbilical cord blood and placental tissue. They produce all the blood cell types including myeloid (e.g. macrophages, erythrocytes, monocytes, eosinophil, neutrophils, basophils, dendritic cells, and platelets) and lymphoid (i.e. B-cells, T-cells, and NK-cells) (35, 36). As it is obvious, HSCs could not be differentiated into neurons and glial cells, rather it normally used to completely replace abnormal
immune system of a patient in usually autoimmune-based disease like MS (37). Another type of ASCs is neural stem cells (NSCs) located in (1) subventricular zone lining the lateral ventricles, and (2) subgranular zone, part of the hippocampus (38). NSCs can easily differentiate into neurons, oligodendrocytes and astrocytes (39, 40), however, they are hard to obtain, and also their ex vivo expansion and maintenance are hard (38, 40).

Rather than HSCs and NSCs, MSCs is considered as a more attractive type of ASCs possesses ten clinically interesting properties as shown in Figure 2. MSCs found in many adult organs but currently from bone marrow, adipose tissue, and cord blood are easiest to isolate (33). MSCs could be differentiated into cartilage cells, muscle cells, fat cells, bone cells, ligaments, tendons, and connective tissue cells. Although MSCs has mesodermal origin, interestingly they might differentiate into endodermal cell linages such as hepatocytes and ectodermal origins including neurons and glial cells (31), but with low efficacy and under special condition medium (3, 41).

Figure 2: Ten properties of mesenchymal stem cells (MSCs) that make them more attractive for clinic. These properties represent intriguing aspect of MSCs, introducing the possibility that these cells might be used as effective therapy in neurodegenerative diseases.

**Neural replacement purpose**

Clinically, stem cell therapy of neurodegenerative diseases must give rise to long-lasting amendment in sensory, cognitive and motor neurons, disease symptoms, or undo disease stem cell-based therapies by replacement strategies; stem cell-based therapies by replacement strategies; generating neurons and glial cells successfully from stem cells (7, 42).

Studies have indicated that it is possible to generate all neural cell types as well as glial cells in culture from stem cells of various sources, including ESCs (43-49), iPSCs (50, 51), NSCs (52-56), and even from the MSCs (3, 40, 57). For example, for stem cell therapy of PD, dopaminergic (DA) neurons with the properties of substantia nigra neurons have been generated from human ESCs by several studies (58-60). More recently, Hwang et al. have shown that ESC-derived neural progenitor cells (NPCs) cultured in media
supplemented with sonic hedgehog (SHH) and retinoic acid (RA) could be efficiently differentiated into DA neurons and following transplantation in SCI rat model, dramatically attenuate the chronic neuropathic pain (61). Additionally, there are several great review article about clinical trials have examined transplantation of human fetal DA neurons or NSCs; suggesting that cell replacement strategy can produce long-lasting improvement in PD patients (62, 63).

In another study, ESC-derived NPCs genetically modified by brain-derived neurotrophic factor (BDNF) to promote differentiation into the GABAergic neurons which are suitable to treatment of PD and SCI (64). Recently, allograft of ESC-derived NSCs/NPCs into the cerebrospinal fluid of nonhuman primate model of SCI led to improved motor function, but whether these neurons could integrate into existing circuitries has not been yet determined (65). Importantly, Pan et al. by studying axonogenesis of ES-derived motor neurons have shown that aberrant axon morphology was still present after engraftment of GFP-positive neurons into the SCI, suggesting that even a mature neural environment may fail to provide a proper niche to guide normal axon formation. These findings emphasis necessity for examining the functionality and morphogenesis of neurons before the clinical trials using ESCs or ASCs (66). First-in-human (FIH) trials have received approval in the United States in January 2009 to initiate a clinical trial assessing a human ESCs-derived oligodendrocyte, named GRNOPC1, by the Geron Corporation, for treatment of severe SCI (67). Chapman et al. have evaluated the ethical issues raised by the Geron FIH trial and then recommended ways to improve future proposed trials with novel stem cell therapies (67).

As mentioned above some examples, for replacement therapy, most of studies have used ESCs, or NSCs; however, several attempts have been done to induce MSCs towards neural and glial cells aim to use them as a neural replacement strategy (57, 68-71). For instance, to solve the problems of low differentiation rate of MSCs, Dezawa et al. efficiently induce differentiation of both adult and human BM-MSCs into neuronal cells using gene transfection with Notch intracellular domain (NICD) and subsequent treatment with trophic factor such as bFGF, forskolin, and ciliary neurotrophic factor (CNTF). Interestingly, further pretreatment of the induced neuronal cells with GDNF generated more specialized population, i.e. tyrosinehydroxylase (TH) cells and DA neurons. Following intrastriatal transplantation of these GDNF-treated cells, behavioural improvements appeared in animal model of PD (57).

In other study, it has been revealed that rat BM-MSCs induced by treatment of bFGF and neurotrophin 3 (NT-3) can transdifferentiate into neural-like cells in culture (68). Additionally, it has been further demonstrated human BM-MSCs under conditions of cocultured with olfactory ensheathing cells (OECs) and daily supplement of bFGF could be differentiation into neural-like cells (71).

**Paracrine effects and trophic supports**

Whereas neuronal and glial cells replacement in neurodegenerative disease seems to be a long-way goal, using stem cells to prevent CNS cells from dying is a more realistic and short-term approach to reach clinic. This perspective is supported by the fact that most of clinical trials (107 out of 190) focused on using MSCs injection into the CNS to treat neurodegenerative diseases, traumatic spinal cord injury and brain ischemia, mainly by beneficial paracrine effects of these cells (Table 1).

Unlike studies using other stem cells, MSCs have been transplanted without prior in vitro differentiation in most studies aimed to use its beneficial paracrine effects. As a proof of the concept, Vercelli et al. demonstrated that human MSCs are a good candidate for ALS cell therapy because they can migrate and survive after transplantation in the lumbar spinal cord; prevent microglial activation and astrogliosis, decrease motor neuron cell death through paracrine actions, thus ameliorate the motor performance in an experimental ALS model (72).

**Table 1.** Clinical trials that have been conducted using stem cells to treat various neurodegenerative diseases. The number clinical trials targeting treatment of these diseases by stem cells have been presented in this table. Up to now, more than 190 clinical trials worldwide (completed, planned, and ongoing) have applied different types of stem cells, mainly MSCs, as treatment for a neurodegenerative disease including Alzheimer’s disease (AD), Huntington’s disease (HD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), spinal cord injury (SCI) and brain ischemia (BI). The data collected from https://clinicaltrials.gov on 1th, January, 2016.
However, more recently, Mazzini et al. presented the results of a long-term follow-up of 19 ALS patients using autologous MSCs transplanted into the dorsal spinal cord which showed no obvious clinical benefits (73). Recently, Salem et al. have explored the possible therapeutic potential of single intravenous (IV) injection of BM-MSCs in treatment of AD experimental model compared with 2 conventional therapies of AD; cerebrolysin and rivastigmine administered daily. They observed significant improvement BM-MSCs after 4 months against AD rather than the reference drugs, suggesting that paracrine properties have important therapeutic roles. In addition, histopathological examination showed that BM-MSCs could remove beta-amyloid plaques from hippocampus (74). Moreover, it has been revealed that BM-MSCs were able to migrate on the brain and remove beta-amyloid senile plaques from the hippocampus and reduce beta-amyloid depositions by the activation of endogenous microglia in an AD mouse model (74, 75). Similarly, in additional studies, it has been shown that hMSCs reduce deposition of beta-amyloid, improve synaptic transmission and memory deficits (76, 77).

Recently, the potential efficacy of adipose tissue-derived hMSCs in treatment of HD has been also confirmed. They observed that even xenotransplantation of human AT-MSCs could counteracted neurodegeneration caused by HD and ameliorate some behavioral impairments (78). In a clinical study, followed-up to 5 years, intravenous autologous MSCs transplantation showed safe for ischemic stroke patients. Interestingly, clinical improvements had been associated with serum levels of stromal cell-derived factor-1 (SDF-1) and the degree of involvement of the subventricular region of the lateral ventricle, showing that recovery after stroke is depend on the specific characteristics of the patients (79).

Many other studies have shown that MSCs primarily through paracrine actions can promote endogenous neurogenesis (80-82), decrease apoptosis of bystander cells (81, 83), reduce levels of free radicals especially in ischemic condition (84), encourage neurorestoration (85, 86), modulate inflammation (87-89) and etc. Also, NSCs has been demonstrated that act through neurotrophic factors and promote axonal growth in spinal cord injury (90-92). For instance, a study has been shown that hESC-derived NPCs transplanted into the cerebral ventricles of an MS mouse model exert therapeutic benefits by immunosuppressive neuroprotective mechanism. This animal experiment may serve as a first step forward to further developments of hESC for stem cell therapy in MS (93).

Additionally, many experimental studies have shown the beneficial neuroprotective effect of HSCs-releasing factors such as erythropoietin (EPO),
Granulocyte colony-stimulating factor (G-CSF), stem cell factor (SCF), VEGF, and SDF-1-alpha (94, 95). Interestingly, HSCs have innate tropism towards the site of injury (96), could be exploited this features for delivering neurotrophic and protective factors.

**Genetically modification of stem cells to get better outcomes**

The efficacy of both differentiation and paracrine approach could be improved by genetically modifying the stem cells for carrying new genes to better differentiate towards neuronal lineages or to secrete a specific therapeutic molecules in addition to their innate trophic support (97, 98). Stem cells naturally produce various neurotrophic factors, such as BDNF, nerve growth factor (NGF), cerebral dopamine neurotrophic factor (CDNF) or GDNF, and facilitate neuronal differentiation and maintenance of endogenous stem cells of CNS (83, 85, 99-101). For instance, transplantation of genetically engineered MSCs overexpressing BDNF showed stronger therapeutic benefits following transplantation in cerebral ischemia animal models rather than MSCs alone (102-104). The strategies to exploit therapeutic effects of stem cells have been shown in Figure 3.

Alternatively, stem cells could be genetically engineered to have high migratory ability following transplantation to delivery of factors that can modify the course of the disease by their innate trophic support (105, 106).

Furthermore, as understanding of the pathology of the specific disease is key factor to develop stem cell based treatments for neurodegenerative diseases, many studies directed to genetically modified stem cells to express disease-relevant genes (107, 108). Therefore, creating model system have advantages of investigating basic issues of neural development and cell replacement, gene therapy, disease-specific cellular pathways and testing new therapeutic approaches; would accelerate way to reach clinical (107, 109-111).

**Stem cells in combination with other drug or treatment**

There is still no cure for neurodegenerative diseases since the precise mechanisms of these diseases are largely unknown. However, advantages of stem cells due to the restorative and trophic abilities are far beyond drugs (108, 112).

To develop successful and more effective stem cell-based therapy for neurodegenerative diseases, several studies have suggested that it would likely require that the stem cells based therapy complement with other drugs or treatments, such as antioxidants and/or therapeutic molecules (108, 113, 114). For instance, it has been demonstrated that antioxidants such as vitamin E thorough reducing ROS levels, and protecting against peroxidation of lipids in the brain could reduce the risk of AD (113).

Many of neurodegenerative diseases are characterized by the accumulation of disease-specific misfolded proteins in the CNS (23, 115, 116). Therefore, for instance, limited proteolysis and clearance of β-amyloid plaques by activation of Cathepsin B could be offered a complement therapeutic strategy with stem cells to get better results for AD (117).

**Stem cells as a cartridge**

It has been suggested that the prolonged and controlled delivery of GDNF, one of the neurotrophic factors for dopamine and motor neurons recovery, into the brain could be used to long-term and more effective therapy of neurodegenerative disorders like PD (118). However, as GDNF is a large peptide, it cannot efficiently enter the brain from blood and cerebrospinal fluid (CSF) (119, 120). Therefore, it needs to be directly delivered to tissue. Possible solutions might be direct infusion into brain tissue with pumps (118, 121), delivery by injection of virus producing GDNF (122), delivery by using encapsulated cells secreting GDNF (123, 124) and delivery using stem cells secreting GDNF (125-128).
Alternatively, the novel cartridge based bioreactors have been developed in supporting liver cells with good viability and functional performance which can be an effective design for delivering trophic factors. Recently, Niu et al. have developed a machine that controls the flow of blood through the cartridge which contains billions of liver cells used as a bioartificial liver (129). The significance of these devices is that they supply an environment to maintain cells in a way that allows performing key cellular and tissue functions. In a study, expanded human BM-MSCs have been used as a cartridge system to work like a bioartificial liver (130). Therefore, this innovative approach could be exploited for neurodegenerative diseases either. However, the main drawback of this technique is impossibility to maintain cell viability effectively for the long period of time.

**Conclusion**

The use of stem cells, although in its early stages, appears likely to contribute to future clinical treatments of neurodegenerative disease through replacement of dysfunctional or dying neurons as well as neuroprotective and neurorestorative approaches. Stem cells from a variety of sources have shown effective in improving motor function after
neurodegenerative diseases in animal experiments, but still need further investigation in clinical trials. Regardless of differentiation potential among different stem cells, MSCs due to having great paracrine properties are of keen interest to use in clinical settings. Furthermore, combinatorial administration of other drugs/therapeutic molecules with stem cells is proposed as a desirable approach especially to complement the gaps yet existing by the single therapeutic application of stem cells neurodegenerative disorders.

Transplantation of stem cells or stem cell-derived motor neurons or glial cells in neurodegenerative diseases in a clinical setting to replaces lost neurons, and integrates into existing neural circuitry neuronal by replacement strategy seems to be currently unrealistic and long-distance goal. Rather, using stem cells for the delivery of trophic factors to prevent disease progression seems to be a more realistic and short-term achievable goal for clinic. However, factors that control the differentiation, survival, and maturation of stem cells in the context of degenerative diseases must be more thoroughly understood before stem cell therapy that could be transferred to clinic.

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