Stem Cells Transplantation and Huntington’s Disease

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Huntington’s disease (HD) is a progressive and devastating neurodegenerative disorder that results in movement abnormalities, cognitive impairments, dementia, and affective disturbances. As no proven medical therapy for this genetic disease is currently available, symptoms mitigation is the primary treatment for HD. Stem cells can play an important role in cell therapy therapeutic strategies to replace dysfunctional or dying cells in HD. Here, we present a brief overview of the current state of stem cells therapy and of the results obtained in animal models of HD, and discuss neuroprotective approaches that utilize stem cells-derived paracrine factors.

Keywords: Stem cells, Animal model, Huntington’s disease

Pathogenesis of Huntington’s disease

Huntington’s disease (HD) is a progressive and heritable neurodegenerative disorder, which is characterized by movement abnormalities, cognitive impairments, dementia, and affective disturbances (1). HD was first described by George Huntington in 1872 in The Medical and Surgical Reporter. It is caused by an expansion of trinucleotide CAG within exon 1 of the huntingtin gene on the short arm of human chromosome 4 (2). The huntingtin gene was discovered in 1993, and encodes a 350 kDa ubiquitously expressed protein called huntingtin (Htt) (2). At the gene locus implicated in normal individuals, there are commonly 17 to 20 repeats of the CAG triplet at the 5’ end (2, 3). However, HD patients have abnormally expanded CAG repeats, and numbers range from 36. This expansion results in the transcription of mutant huntingtin (mHtt). Furthermore, CAG repeat length is inversely correlated with age at disease onset (3), and mHtt has been linked with altered protein-protein interactions, abnormal protein aggregations, transcriptional dysregulation, excitotoxicity, and mitochondrial dysfunction, which in particular, induce the deaths of medium spiny neurons in the striatum and lead to a loss of normal protein function (4-8). Normal Htt is expressed ubiquitously and is essentially required for embryonic development (9).

mHtt leads to the progressive loss of medium-sized spiny neurons in striatum, which represent the most important population of degenerated neurons in HD (10, 11). However, the mechanism of neuronal cell death induced by mHtt has not been fully established. Neuronal dysfunction and degeneration lead to chorea, dystonia, psychiatric impairment cognitive, and emotional disturbances that are characteristics of HD (12). As no proven medical therapy for HD is currently available, HD is treated by addressing its symptoms. Furthermore, although cell transplantation has been developed for the treatment of HD over the last decade, its clinical merits and efficacy have remained elusive.

Experimental animal models for stem cell transplantation researches in HD

The ideal animal model of HD should replicate both the genotype and phenotypes of human HD. HD animal models that show rapid progression, early disease onset,
abnormal behavior, and neuropathological features are useful for rapid evaluating therapeutic hypothesis. However, more precise replications of the disease in man require slowly progressive disease models. Rodent HD models, usually rats and mice, are widely used as experimental models to investigate cell therapy efficacy and the effects of other therapeutic agents. Furthermore, recently, a transgenic nonhuman primate model of HD was developed by Yang et al. (10). Because there are many rodent models of HD, here, we present a brief overview of two rat models induced using chemical reagents and three transgenic mouse models.

**Rat models**

Rat models of HD have been established using the mitochondrial toxins, including 3-nitropropionic acid (3NP) and malonate, or excitotoxic glutamate analogs, such as quinolinic acid (QA), ibotenic acid (IA), and kainic acid (KA) (13-17). Animals with striatal lesions induced by QA mimic many of the neuropathological features of HD. QA has been shown to induce glutamate excitotoxicity and to cause selective neuronal loss in the striatum, which resembles the histopathologic characteristics of HD patients (17). Thus, cell transplantation in QA rat models can be used to both observe the differentiation of grafted cells into medium-sized spiny neurons and to examine neuroprotective effects. One research group tried the systemic transplantation of neural stem cells in the QA rat model of HD (18, 19). HD rats receiving human neural stem cells showed functional recovery (18, 19).

Impaired energy metabolism also causes excitotoxicity mediated by voltage-dependent N-methyl-D-aspartate (NMDA), and 3NP inhibits succinate dehydrogenase and activates neuronal death (13, 14). In terms of HD pathophysiology, mitochondrial abnormalities are considered significant, and two types of factors are known to affect striatal degeneration in 3NP models. One causes cell death and involves c-Jun N-terminal kinase and Ca\(^{2+}\)-activated protease calpains whereas the other involves glutamate, dopamine, and adenosine which affect the striatal degeneration induced with 3NP (20, 21). For these reasons, HD models produced using 3NP can be efficient at the investigation of neuroprotection and molecular modifications related with toxicity from mitochondrial dysfunction. There is a report that transplantation of human NSC released a large amount of brain-derived neurotrophic factor (BDNF), which protects neuronal damage induced by 3NP, and prevented degeneration of striatal neurons in a 3NP rat model of HD (22).

**Transgenic Mouse models**

Transgenic mouse models continue to play a decisive role in the study of HD because of the physiological, neurological, and genetic similarities between these models and human HD (23-25). R6/2 mice have a small N-terminal fragment of huntingtin containing 150 CAG repeats (24), and have been used predominantly for preclinical drugs trials because they allow survival results to be obtained in about three months. Such studies have also been used to study neuroprotection by using motor performance improvements to assess functions impaired in HD, such test include, the rotarod test and body weight findings. In terms of neuropathological studies of R6/2 mice, cellular striatal atrophy and aggregates/inclusions in the nucleus are detected (26, 27). Recently, we showed that the growth factors secreted from stem cells could alleviates neuronal damage in R6/2 mice (28).

The YAC128 mouse model of HD contains the entire human HD gene containing 128 CAG repeats (29). The representative behavioral characteristics of YAC128 mice are hyperactivity, motor abnormalities, and hypokinesis (23). These behavioral symptoms result from brain atrophy, which can be explained by pathological phenomena, such as neurodegenerations in the cortical and striatal regions (23). Furthermore, YAC128 mice show very little variability, which adds to their attractiveness as an experimental model (23). In addition, the age-dependent brain neurodegeneration exhibited by these mice makes them a suitable practical model for investigating therapeutic methods.

Hdh Q111 mice, a knock-in mice, possess the murine HD gene containing 111 CAG repeats (30). Knock-in Hdh mouse provide a more precise transgenic model of HD than R6/2 or YAC128 mice. Hdh Q111 mice show little neuronal cell death in the striatum and do not develop a gait abnormality until 2 years old, whereas Hdh Q150 mice show gait abnormalities at 7 months. Knock-in mice with 72–80 CAG repeats have mHtt aggregates in neuropils and show aggressive behavior (25, 31).

Because of its complicated disease pathways, no HD therapy has been clearly defined as yet, and thus, many animal models of HD have been established. In order to identify the most efficient curative treatments for HD, it is important that an appropriate animal model be selected to evaluate novel therapeutic methods.

**Cell therapy**

Cell transplantation strategies have been most actively
pursued in the context of HD therapy, and can be classified into two broad categories, namely, the replacement or neuroprotection of dead or dying cells: (I) using fetal tissues or cells; (II) using stem cells.

The ameliorating effect of grafted fetal tissue on neuronal dysfunction in a QA animal model of HD was reported by Nakao et al. (32). Furthermore, Dunnett et al. found that fetal striatal tissue can integrate and survive within the degenerated striatum in a HD mouse model (23, 32). Several clinical trials on fetal tissue transplantation have been performed in HD patients since 1990, and although full recovery has not been reported in any HD patient, these studies indicated that fetal tissue transplantation could provide cellular and behavioral improvements (33). However, these effects did not persist long term, and in most transplanted patients clinical improvements declined after reaching a plateau. In particular, although grafted regions were found to be protected from neurodegeneration, surrounding brain regions continued to degenerate. Furthermore, the impact of transplantation on disease stage remains to be elucidated. Human striatal degeneration includes not only cortical and subcortical components but also neocortical and other areas of the brain as the disease progresses (34). In other words, neural tissue transplantation only into striatum is not enough to ameliorate pathology. However, the migration ability and paracrine effects of stem cells can affect whole brain. The use of fetal tissues from spontaneously aborted fetuses or elective abortions raise profound ethical questions, and thus, stem cell transplantation provides an alternative strategy for HD.

**Stem cells as a source of cell therapy**

It is difficult to justify basing developments on fetal cells because it is difficult to secure supplies of embryonic striatal cells or tissues. On the other hand, stem cells are an ideal for cell transplantations purposes in HD because they are relatively easy to obtain, and somatic stem cells, in particular, offer a means of eliminating immune rejection problems (35, 36). Furthermore, stem cells can self renew continuously, produce progeny, and differentiate into many cell types. Several types of stem cells, such as, neural stem cells (NSCs), mesenchymal stem cells (MSCs) and adipose-derived stem cells (ASCs) have been isolated from specific adult human tissues. In addition, Takahashi and Yamanaka group recently induced pluripotent/ESC-like cells from somatic cell, named induced pluripotent stem cells (iPS cells) (37).

**Embryonic stem cells (ESCs)**

ESCs are pluripotent stem cells that have ability to make all body organs. Unlike adult stem cells, ESCs are derived from inner cells mass of blastocysts (38, 39). Furthermore, the differentiation of mouse ESCs into neuronal progenitor cells, that can differentiate into neurons, astrocytes and oligodendrocytes, has been developed (40). In relation to HD, transplanted ESCs are able to differentiate into neurons in striatum lesions of a QA animal model of HD and show to migrate around cortical regions (41). Furthermore, it has been reported that human ESC-derived neural precursors show behavioral recovery in a QA animal model of HD (41). Although human ESCs have potential as a source of cell therapy for HD, further study is needed to ensure that the transplantation of human ESCs introduces the possibilities of adverse effects, such as tumor formation and immune rejection. In addition, there are ethical and practical issues associated with use of human embryos.

**Neural stem cells (NSCs)**

NSCs can be found in embryonic and adult neural tissues, induced from ESCs and can differentiate into neurons, oligodendrocytes, and astrocytes (40, 42, 43). In the adult brain, NSCs are located in the subventricular zone (SVZ) of the lateral ventricle and subgranular zone of the hippocampus and migrate into the dentate gyrus (DG) or olfactory bulb (44-46). NSCs can be isolated from adult mammalian brain with the use of the neurosphere assay (47). In this assay, NSCs in vitro in the presence of growth factors, such as basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF), undergo proliferation, generating round multicellular spheroids, so-called neurospheres (47, 48). The differentiation of NSCs into specific cells, such as neurons or glia, involves the consideration of many factors (49). In the case of HD, modification of neurogenesis and transplantation of neural progenitors is attractive trial for disease. Rodent and primate models of HD transplanted with murine or human NSCs have shown potential effects of cell therapy (41, 50-52). NSCs transplanted into rodent HD models have been found to improve motor function, alleviate aggregation formation, and extend life span (41, 50-52). Although the majority of preclinical trials have used intrastrital injections, some authors have claimed that systemically administered NSCs are also effective in several neurological disorders (43). Intravenous administration of human NSCs induces functional recovery, migrates to the striatum, and attenuates striatal atrophy in a rodent lesion model of HD (41, 53).
NSCs are an ideal raw material for the treatment of neurological diseases, but they present a substantial ethical limitation, because they can only be obtained from aborted fetuses for cell therapy.

**Mesenchymal stem cells (MSCs)**

MSCs can be defined as non-hematopoietic, multipotent cells that originate from adult stromal structures in bone marrow (54). In vivo, MSCs are known to give rise to osteocytes, hematocytes, adipocytes, and chondrocytes, and to have the potential to trans-differentiate to non-mesenchymal cell types, like neurons (55, 56). Furthermore, MSCs have ability to differentiate into a neuronal lineage, which make them a powerful therapeutic transplantation option for the treatment of neurodegenerative diseases like HD (54-56). Moreover, MSCs isolated from rats and human bone marrow have been induced to differentiate into dopaminergic neurons using several factors, such as bFGF, GDNF, forskolin, and ciliary neurotrophic factor, and a gene transfection method (57).

Despite these results, we cannot be sure that MSCs implanted in vivo differentiate and function properly (58). Lescaudron et al. tried transplantation of autologous adult bone marrow MSCs in a QA rat model of HD (59), and although an improvement in behavioral functions was observed for HD rats implanted with bone marrow stem cells, only a small number of transplanted cells in the host were found to express the neural phenotype (59). Nevertheless, the findings obtained suggest that the growth factors released by implanted cells improved host cell survival and protection for neurons susceptible of death.

**Adipose-derived stem cells (ASCs)**

ASCs can be easily isolated from materials obtained during liposuction, and also have the ability to differentiate into several lineages, such as adipocytes, bone, cartilage, skeletal muscle, endothelium, hematopoietic cells, and neuronal cells (60-62). In addition, hASCs secrete multiple anti-apoptotic growth factors, including granulocyte monocyte colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), bFGF, and insulin-like growth factor-1 (IGF-1) (63, 64). Accordingly, human ASCs offer a clinically feasible source of stem cells for cell-based therapy. Recently, we showed that the intrastriatal transplantation of human ASCs reduced lesion volumes in a QA rat model of HD, improved rotarod performance and limb clasping, and increased survival in R6/2 mice, and that these improvements were associated with reductions in the loss of striatal neurons and mHtt aggregates (28).

**Induced pluripotent stem cells (iPS cells)**

Despite of great therapeutic potential of ESCs in HD, they have two limitations, namely, the ethical issue regarding the use of human embryos mentioned above, and allogenic immune rejection. Thus, attempts have been made to use pluripotent/ESC-like cells reprogramming the somatic cell nucleus obtained from adult somatic cells. In order to transfer somatic cell nuclear, the nucleus of an adult somatic cell is inserted into an unfertilized oocyte whose nucleus are removed (65). This method results in the production of pluripotent cells, but unfortunately it does not overcome too the need for a human embryo or oocytes (66, 67).

The Takahashi and Yamanaka group recently found that the over expression of four transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) induced the development of ESC-like cells from embryonic and adult mouse fibroblasts (37). These cells referred to as induced pluripotent stem cells (iPS cells) and have ability to differentiate into various cell types. Human iPS cells are also possibly obtainable from HD patients (68), and may be a near ideal therapeutic source for HD. However, despite iPS cells are the potential source of cell therapy, there are some problems, such as animal derived culture conditions and viral manipulation required to form iPS cells, to be resolved necessarily before clinical trials are attempted.

Overall, stem cells have much potential for use as a source of cell therapy for HD, however stem cells used for autologous transplantation, such as MSCs, ASCs and iPS cells, themselves also carry the mHtt gene. Thus, more study is needed to ensure the survival and functional effects of stem cells derived from HD patients.

**Conclusion**

Currently, no cure is available for HD and the objective of treatment is to minimize symptoms, and prevent complications. Although stem cell transplantation has been examined in various animal models of HD, the efficacy of this process is limited. In the past, cell therapy strategies in HD have targeted the replacement and protection of cellular depletion during the disease course, and thus, preventing disease progression. The current objectives of stem cell therapy in HD are: (I) to promote endogenous neurogenesis and improve self-repair in the brain; (II) to replace damaged or dying neurons; and (III) to protect neurons from disease progression using the factors released by stem cells. Researchers need to consider the multiple of mechanisms induced by stem cell trans-
plntation, for example, the effects of growth factors after ASC transplantation in HD mice (28). Although still in its infancy, factor-based or direct replacement stem cell therapy appears to be effective. Considerably more experimentation and clinical trials are required before a stem cell based therapy is available for HD.

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Potential Conflict of Interest

The authors have no conflicting financial interest.

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