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# Pluripotent Stem Cells to Model and Treat Huntington's Disease

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## Abstract

Stem cell therapies hold considerable promise for the treatment of neurodegenerative diseases. Pluripotent stem cells (PSCs) have been of particular clinical interest because of their ability to generate neuronal cells and to be used in animal models of neurodegenerative disease as well as for testing new drugs. Several PSCs isolated from humans and animals that carry the genotype of Huntington's disease (HD) have been used in aforementioned studies. HD-PSCs obtained can produce *in vitro* neural progenitor cells (NPCs). These NPCs applied in HD models show several advantages: they engraft into the brain in animal models and differentiate into neuronal cells, thus promoting behavioral recovery and motor impairment. Although progress has been made using PSCs, additional tests should be done to overcome several limitations as, for example, tumorigenicity, before their clinical application. We focus this chapter on current knowledge regarding HD-PSC lines and their helpfulness as an *in vitro* model for basic research. Next, we discuss the advances of disease-free PSCs in preclinical HD models aiming to their potential application in patients. Additionally, we discuss their potential use as a test system for anti-HD drug screening by the pharmaceutical industry, especially considering HD patients' welfare.

**Keywords:** Huntington's disease, neural progenitor cells, pluripotent stem cells, stem cell transplantation

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## 1. Introduction

HD is an autosomal dominant neurodegenerative genetic disease caused by an expansion of polyglutamine (CAG) repeats in the huntingtin (HTT) protein. Clinically, HD patients present cognitive decline, motor dysfunction and psychological problems. The age of onset for these

symptoms is directly associated with the number of repeats. Pathological threshold is reached when patients present more than 36 repeats [1, 2]. Conventional therapies have no effect on HD [3–6]. Stem cells, which have amazing potential to develop into many different cell types in the body during early life, may offer new therapeutic approaches for treating HD disease [7–9]. Fetal neural grafts, neural progenitor cells (NPCs) and mesenchymal stem cells (MSCs) have already been used in several preclinical and even in preliminary clinical trials [10–14]. Other options of stem cells to be used in HD studies are embryonic stem cells (ESCs) and, especially, induced pluripotent stem cells (iPSCs), which have recently been developed in the field of human stem cells [15–19].

In devastating HD, the loss of neurons and the incapacity to mobilize inherent regenerative mechanisms to recover from progressive damage underlies the pathology and prognosis [1, 2]. Stem-cell-based therapies hold promise for the future treatment of these symptoms and to study the progress of disease. The establishment of *in vitro* cellular HD models for testing new drugs is under development and is of great importance. Furthermore, *in vitro* HD cell models help to better understand HD at the molecular and cellular levels and to identify new HD biomarkers [20–22]. Recently, NPCs have been derived from HD-iPSC [23]. The present chapter discusses PSCs use as a model study HD, and to carry out drug screening and study stem cell-based therapy in animal models of HD.

## 2. HD clinical aspects

HD has been reported in almost all countries and occurs in all races, equally affecting both genders. The diagnosis of HD depends on a detailed clinical evaluation and positive family history, which may be confirmed through the use of molecular genetic techniques. The average age of onset varies between 35 and 45 years, although it may manifest at any age. In about 10% of cases, the onset of symptoms occurs before 20 years of age, when patients are said to have “juvenile HD” and, in 25% of cases, the onset arises after 50 years of age—the so-called “late HD” [24]. The median survival time in HD ranges from 14 to 17 years, while it may be as long as 40 years [25]. The most frequent complaint in HD patients is a lack of “coordination” and occasional involuntary tremors in several body segments, which can usually be attributed to the presence of chorea [26]. Other early motor abnormalities include interrupted saccadic eye movements or hypometric balconies, motor impersistence of tongue protrusion and difficulty performing rapid alternating movements. Patients are described as being excessively irritable, impulsive, unstable or aggressive. The most common early symptom is mental depression. Symptoms of emotional nature or personality changes, preceding or concurrent with the onset of tic movements, are reported in half of the patients with HD [27]. A striking feature in a large number of HD patients is the intense weight loss. Cause of this weight loss is unclear. Premature aging is another obvious feature of HD [26].

## 3. HD genetic and pathophysiological aspects

HD is a hereditary autosomal dominant condition. The Htt gene is located on chromosome 4p16.32 and the genetic alteration is an increase in the number of repetitions of three nucleotides

(C, A and G) in the coding region of the first exon of the HD gene [1]. The CAG “triplet” is normally repeated about 20 times in humans, but an estimated doubling in the number of repeats (40 CAG repeats or more) results in the development of HD. Intermediate numbers of repeats, between 27 and 35, are not associated with the HD phenotype. Expansions above 36 are most frequent in the paternal lineage, due to the instability of the number of CAG repeats during spermatogenesis. The presence of 36–39 repeats is related with reduced penetrance, whereby HD may develop or not, and is considered uncertain [1]. The majority of adult onset cases have 40–50 CAG repeats, whereas expansions of 50 or more repeats generally causes the juvenile form of the disease [28, 29]. Additionally, the greater the number of CAG repeats in the Htt gene, the earlier the disease will manifest [30]. In HD, the number of CAG repeats explains many of the genetic features of this disorder, including its progression and severity.

CAG is a codon that codes for glutamine, and the mutation leads to an abnormally expanded polyglutamine tract in huntingtin [1]. In HD, the expanded polyglutamine is cleaved, resulting in an N-terminal fragment containing the polyglutamine expansion [31]. Huntingtin (HTT) is a protein of approximately 300 kDa, which is located in the cytoplasm of all somatic cells, except for neurons, where it is found in both the cytoplasm and nucleus. Normal gene function and how this mutation produces HD is still unknown, however, huntingtin is essential for life. These aggregated proteins accumulate in excess in neuron axons or dendrites and may block neurotransmitter action, impairing the normal neuronal function and leading to onset of behavioral deficits [32]. During disease progression, HTT protein aggregates accumulated in neuron cells cause cell death. Striatum and cerebral cortex are the structures that have most prominent neuronal loss. In fact, the most striking anatomopathological feature of HD is the degeneration of the basal ganglia, especially the caudate nucleus and putamen, with progressive and intense atrophy and gliosis [30].

PSCs are an important tool for HD *in vitro* studies, since these cells can model the disease, informing, for instance, the outcome of different CAG repeat numbers in HD neurons, including the possible interactions of the mutant HTT with different proteins. Conversely, PSCs can also be used *in vitro* to study the effect of different number of CAG repeats in PSCs development and ability to differentiate.

#### 4. Mouse pluripotent embryonic stem cells: variability and heterogeneity

Pluripotency is a transient property of stem cells during early embryogenesis. It refers to an unrestricted developmental potential of the cells to give rise to all three embryonic germ layers: endoderm, ectoderm and mesoderm, and to contribute to the formation of all tissues of the developing organism. After isolation from early embryos at morulae or at blastocyst stage, PSCs are able to retain pluripotency during long-term *in vitro* cultivation [33, 34]. In mice, there are two different PSC types isolated from early embryo, which are naive and primed ESCs. Naive cells are extracted from the inner cell mass (ICM) of preimplanted embryos at day 4.5 [33, 35], while primed cells are obtained from the epiblast of postimplantation embryos around day 7 [36, 37]. Naive ESCs and primed epiblast stem cells (EpiSCs) differ in the expression levels of pluripotent key markers, such as the POU-family transcription factor Oct-4, the homeodomain DNA-binding protein Nanog and the Sox-family transcription factor Sox2 [38]. Naive female

ESCs have both X chromosomes active; in contrast, female-derived EpiSCs have only one of the X chromosomes activated. When injected into immunocompromised or syngeneic mice, both naive and primed EpiSCs are able to produce teratomas which contain the derivatives of all three germ layers [36]. Reintroduction of naive and primed EpiSCs into the mouse blastocyst leads to the formation of chimeras (animals composed by donor and recipient cells) with a high percentage of donor cell contribution, thus demonstrating their ability to participate efficiently in normal development. Only naive ESCs are able to generate germline-competent chimeras, which are able to pass on their donor cells genotype to the next generations [39–42].

## 5. Human pluripotent embryonic stem cells: variability and heterogeneity

In humans, so far only primed ESCs are known. They are isolated from the ICM of preimplantation human blastocysts [33]. They express several key markers of pluripotency, such as OCT4, NANOG and SOX2 [33, 43–45], and are able to generate teratomas *in vivo* [33, 46–48]. Due to ethical considerations, live chimeras cannot be obtained from hESCs. Nevertheless, efficient hESC integration into the postimplantation mouse epiblast has been shown, although, at a later stage, these cells were rapidly eliminated during embryo development probably because of the difference in cell cycle timing between the two species [49].

The production of human ESCs involves destruction of human embryos, which is of ethical concern. An alternative, the generation of iPSCs by adult somatic cell reprogramming, has been proposed. These cells are initially obtained *in vitro* using a defined cocktail of transcription factors (Oct3/4, Sox2, c-Myc and Klf), called reprogramming factors, which are able to restore pluripotency when introduced into terminally differentiated cells or into adult cells [17, 19, 20, 50]. These human iPSCs are able to produce teratomas that contain the derivatives of all three germ layers. Recent studies showed that X chromosome reactivation, an important event in cell reprogramming, occurs in hiPSCs [51, 52]. Additionally, hiPSCs are able to integrate into different anatomic sites in mouse embryos at E10.5 [52]. Both these studies suggest that, hypothetically, hiPSCs might form chimeras, thus showing the characteristic of primed PSCs.

Given the pluripotent ability of hESCs and hiPSCs, both cell types are of great interest to generate HD *in vitro* models that can be used in basic research characterizing juvenile and adult HD molecular and cellular mechanisms as well as in the pharmaceutical industry, to screen new drugs. The capacity of hiPSCs to differentiate into neural cells and produce functional neurons [53–55] has potentially great impact given the possibility of the use of these cells in cell therapy and tissue regeneration. However, due to the potential risk that these cells can derive teratomas, hiPSC application in patients is still under the investigation.

## 6. Isolation of pluripotent hESCs from HD embryos

Primary cell cultures from adult tissues can be obtained from HD patients. However, this is not always possible, can pose a risk for patients and there is a limited variety of tissues that can be used for cell isolation. Therefore, frequently HD cells are isolated post mortem from



tissue samples. Thus, isolation of hESCs from HD embryos was a cutting-edge discovery in HD cellular models. These hESCs with genetic disease inheritance that have unlimited proliferating and self-renewing potential are unique sources to reproduce heredity of diseases *in vitro* [56, 57]. However, only a few studies reported isolation of these cells so far [57–61].

The first derivation of hESCs from HD embryos occurred in 2005 [57]. Since then, other HD lines have been obtained from donated embryos that mainly contain 37–51 CAG repeats. These cells express the Htt gene, and mutated Htt mRNA and protein levels, and thus have the potential to model HD pathology at the cellular level. The HD-hESCs isolated so far (**Table 1**) can be considered primed hESCs according to the existing classification [43, 62]. They express core pluripotency markers and present a normal karyotype [58–61]. Only one study demonstrated that HD-hESCs are able to form teratomas [59]. *In vitro*, HD-hESCs are able to differentiate into neurons and astrocytes [58–61] through neurosphere formation by the cells positive for the neuroectodermal marker Pax6 [60]. Another study showed that HD-hESCs differentiate preferentially into astroglial cells [58]. Glial cells comprise 90% of the brains cells and provide support neuroprotective for neurons. In healthy brain, astroglial cells protect against excitotoxicity by removing excess of glutamate from the extracellular space [63]. However, in the HD brain, mutant HTT accumulates in glial nuclei and decreases the expression of glutamate transporters in neurons and atrogliial cells (**Table 1**). This is an important outcome for further HD studies that investigate the effect of mutant HTT on astroglial cells and the potential therapeutic potential of these cells in HD.

HD is considered as a disease of the striatum, characterized by vulnerability to degeneration and death of the medium spiny neuron (MSN) [64]. Thus, the ability of HD-hESCs to differentiate into gamma-aminobutyric acid GABAergic MSNs, which are susceptible to neurodegeneration in HD, has also been tested. MSNs receive a massive combination of dopa-

Ref	Source	CAG repeats	Number of lineages	Pluripotent markers expressed	Teratoma formation	Formation of Htt aggregates	Neuronal differentiation
[59]	HD embryo	40–48	4	OCT4, SSEA3, SSEA4, TRA-1-60, and TRA-1-81	Positive	N/A	Neurons
[60]	HD embryo	37 and 51	2	SSEA-3, SSEA-4, Oct-4, TRA-2-39, TRA-1-60 and TRA-1-80	N/A	absent	Neurons and astrocytes
[61]	HD embryo	37 and 51	3	TRA-1-60,D9	N/A	N/A	Neurons and GABAergic neurons
[58]	HD embryo	47	1	POU5F1, SSEA3 and 4, TRA1-61 and 1-80CD9	N/A	N/A	Neurons and astrocytes

N/A, non available.  
Chromosomal abnormalities were absent in all derived cell lines.

**Table 1.** Human ESC lines derived from HD patients.

minergic and glutamatergic inputs, which result in preferential vulnerability of these cells to the toxicity of polyQ-HTT [65]. However, only one report has shown that HD-hESCs are able to differentiate into GABAergic MSNs [61].

As discussed before, repeat size of CAG is a major determinant of the severity and pathology in HD. The longer the repeats, the more severe the symptoms [66]. After differentiation, neuronal precursor populations derived from HD-hESCs do not present any alteration in the incidence of CAG repeats [58, 60]. These findings indicate that the presence of Htt mutation does not prevent HD-hESCs from differentiating into neural cells *in vitro* [57, 59, 60], implying that HD-hESCs can be used as an *in vitro* model of HD. This model has the potential to increase the understanding of the mechanisms of neurodegeneration and can be used for efficient screening for new anti-HD drugs, selecting only the most efficient for further testing in human clinical trials.

## 7. Isolation of induced pluripotent stem cells from HD patients

HD-iPSCs that carry different number of huntingtin gene repeats (from 39 CAGs to 180 CAGs) have been isolated [23, 67–72] (**Table 2**). To produce HD-iPSCs, the most common original cell type isolated from HD patients is fibroblasts. Fibroblasts from HD patients show HD-related phenotypes, such as alterations in proteasome activity and altered Htt gene expression [23, 67–73]. The majority of HD-iPSCs have been generated by retroviral infection that promotes the expression of four transcription factors: Oct-4, Sox2, c-Myc and Klf4 [23, 67–69, 71, 72] (**Table 2**). After retroviral infection, HD-reprogrammed fibroblasts gain hESCs-like morphology, start to express markers of pluripotent cells, such as OCT4, NANOG, SSEA4 and alkaline phosphatase (AP) [23, 67, 68, 71, 72]; TRA-1-60 [67, 71, 72, 74], SSEA3 [67, 72]; and TRA-1-81, REX1, GDF3 and hTERT [67]. At present, the pluripotency of human HD-iPSCs is less studied when compared with that of human iPSCs derived from healthy donors. Only few studies perform the teratoma formation assay, which is essential for the characterization of the pluripotency of any reprogrammed cell. This assay is a reliable method to verify the *in vivo* differentiation potential of HD-hiPSC [71, 74, 75]. An important aspect of HD-iPSC technology is a unique possibility to study the mechanism of HD patient-specific neuronal differentiation, since HD-iPSCs are able to form neurospheres that express neuronal progenitor markers [67, 71, 72, 74]. These neurospheres are able to produce neurons, including GABAergic MSN, and glial cells [23, 68, 71, 72, 74]. Overall, these studies show that the Htt mutation and the number of CAG repeats seem not to affect neural cells fate *in vitro*, although HD *in vivo* is associated with changes in neural function and survival.

In order to use autologous HD-iPSCs therapeutically, it is critical to develop reprogramming methods that can provide a correction of the expanded Htt allele in iPSCs in these cells upon their expansion *in vitro*. A gene targeting technique has been used to achieve a correction of the expanded Htt allele in HD-iPSCs, replacing the expanded CAG repeat with 21 repeats (within the normal, non-pathological range, which varies from 6 to 34) using homologous combination [72]. The resulting cells maintain the pluripotent characteristics and can differentiate into MSNs *in vitro* and *in vivo*. This study demonstrated that non-pathological iPSCs potentially can be produced from diseased patients for stem cell replacement therapy [72].

Ref	Source	CAG repeats	Inducing method	Correct allele CAG repeat	Number of lineages	Pluripotent markers expressed	Teratoma formation	Formation of mHtt aggregates	Neuronal differentiation	Chromosomal abnormalities
[72]	HF	72	Retroviral iPSC (OCT3/4, SOX2, KLF4, C-MYC)	21 CAG	8	NANOG, SOX2, OCT4, N/A SSEA4, and TRA-1-60	N/A	Absent	GABAergic neurons	Absent
[67]	HF	72	Retroviral iPSC (OCT3/4, SOX2, KLF4, C-MYC)	N/A	N/A	TRA-1-81, TRA-1-60, N/A OCT4, NANOG, SSEA3, and SSEA4 REX1, GDF3, and hTERT	N/A	N/A	N/A	Absent
[74]	HF	60 and 180	Retroviral iPSC (OCT4, SOX2, C-MYC, KLF-4) and LIN 28	N/A	14	OCT4, SSEA4, or TRA-1-60, PAX6	N/A	Absent	GABAergic, neurons and astrocytes	Absent
[23]	HF	72	Not informed	N/A	N/A	OCT4, NANOG, SOX2 and SSEA4	N/A	N/A	GABAergic neurons	N/A
[68]	HF	39 to 44	Retroviral and lentiviral iPSC (OCT4, KLF-4, SOX2, C-MYC)	N/A	5	OCT4, SOX2	Positive	N/A	GABAergic neurons	Positive
[71]	HF	50 and 109	Retroviral iPSC (OCT3/4, SOX2, KLF4, C-MYC)	N/A	N/A	NANOG, OCT3/4, SSEA4 and TRA 1-60	Positive	N/A	Astrocytes neurons	N/A
[75]	MF	72	Retroviral iPSC (OCT4, SOX2, KLF-4)	N/A	N/A	OCT4, SSEA4, TRA1 -60, RIPS3 alkaline phosphatase	Positive	Positive	Astrocytes neurons	N/A
N/A, non available; HP, human fibroblasts; MF, monkey fibroblasts.										

**Table 2.** Human iPSC lines derived from HD patients.



## 8. HD animal models

Models of HD recapitulate disease pathogenesis and predict response to experimental treatments. In general, there are two ways of generating animal models of HD: use of toxic/chemical pharmacological agents or of genetically modified animals [76, 77]. The majority of studies *in vivo* with NPCs from PSCs used quinolinic acid (QA)-HD models [69, 78–81].

QA can be found endogenously, where it binds and activates the *N*-methyl-D-aspartate receptor, a glutamate receptor and ion channel protein found in nerve cells. At high concentrations, QA is neurotoxic by over-exciting the same receptors, eventually leading to neuronal cell death [76]. This toxin mimics several aspects of human HD, such as extensive degeneration in striatum, death of dopamine-expressing GABAergic neurons [82, 83], weight loss [84] and motor and cognitive deficit [85]. However, motor deficit is discrete, the main motor alterations including tremor, seizures, eventual paralysis and recumbence [84]. Another aspect of the QA-HD lesions are symptoms that mimic deficits seen in early stages of HD (but not later). The lesions produce hyperactivity in animal models, but the hypoactivity that occurs later in the disease is not modeled by any dose of the toxin [86].

With the discovery of the *Htt* mutation in 1993, it became possible to create animal models with a similar genetic background as that found in humans with HD [1]. Hayden and colleagues used a yeast artificial chromosome these YAC vector system to express the entire human *Htt* gene under control of the human *Htt* promoter [87] YAC mouse strains contain either 72 or 128 CAG repeats. The resulting YAC mice present more hallmarks of human HD than toxic models, with a decrease in the number of GABAergic neurons in the striatum, decrease in body weight and pronounced motor deficit (ataxia, gait abnormalities, hind limb claspings) and increased nuclear *Htt* staining. Interestingly, only the YAC 128 CAG shows positive staining for inclusion bodies—a feature found in human HD—at 18 months [88].

Rodent and non-rodent studies *in vitro* and *in vivo* show the potential of these HD models, but there are limitations as to how these models may benefit patients. It is important to choose appropriate animal models according to the question under investigation. Chemical toxicity models, such as QA, are reliable to reproduce neuronal regeneration when associated with massive cells loss; however, they are not appropriate for assessment of later stages of the disease (similar to chronic). Whereas genetic animal models have similar HD symptoms as patients in later stages of the disease, thus allowing investigators to study HD progression.

## 9. Isolation of pluripotent stem cells from HD transgenic animals

Several PSCs have been established as *in vitro* models of HD. Somatic cells, such as fibroblasts and NPCs isolated from HD-transgenic animals (monkeys and rodents), have been reprogrammed using the Oct4, Sox2 and Klf4 transcription factors, producing HD-iPSCs. These cells preserve both the HD-related genotype and phenotype: they express mutant HTT protein and show formation of intracellular HTT protein aggregates [75, 89, 90]. In addition, PSCs have been generated using cell fusion as a tool for reprogramming: transgenic HD monkey

skin fibroblasts and wild-type non-transgenic monkey oocytes were fused and the pluripotent hybrid cells selected after fusion were found to express mutant *Htt* and to have HTT protein intracellular inclusions after the induction of *in vitro* neural differentiation [91]. These studies teach us that HD pluripotent cells can recapitulate the genotype and cellular phenotype of HD-patient cells, which is crucial for the production of cell systems that closely resemble HD. These models can then be used for the screening of anti-HD drugs in pluripotent cells and neurons derived from these cells.

## 10. Transplantation of hESCs and rodent ESC-derived NPCs in HD animal models

Studies have shown the therapeutic potential of hESC-derived NPCs in HD chemical rodent models (**Table 3**). NPCs have been transplanted directly into the striatum at between  $10^4$  and  $10^6$  cells per animal [79–81]. These cells were able to survive and graft into the striatum in a QA-induced HD animal model [79]. After transplantation, the cells were shown to differentiate into GABAergic MSN [80] and astrocytes [79–81]. However, the stage of NPC maturation reflects on their specification. Thus, rosette-forming NPCs are not able to differentiate *in vivo* into MSN, while striatal progenitor cells effectively generate striatal neurons [80]. The main problem in using PSCs in the clinic is the need to control neural cell proliferation, avoiding xenograft overgrowth, which may compromise postgrafting safety. Although published data suggest the beneficial action of NPCs in striatal injury regeneration, the role of hESC-derived NPCs in this process needs to be better elucidated.

Only one study so far has demonstrated an efficient recovery of motor deficit after hESC-derived NPCs transplantation in the QA rat model [81]. The animals treated with NPCs exhibited a significant behavioral improvement in the apomorphine-induced rotation test as compared to sham 3 weeks' posttransplant. None of the studies investigated long-term motor functional recovery following NPC transplantation or the possible mechanisms of therapeutic action of these cells besides differentiation [79–81]. There is no doubt that more ample and rigorous studies using chemical and transgenic animals must be performed to demonstrate the efficiency and stability of hESC-derived NPCs to promote neural tissue restoration and functional recovery of motor deficit in HD animal models.

NPCs derived from rodent ESCs have similar beneficial effects as human NPCs when transplanted into the chemical rodent model. They are able to differentiate into neurons and the animals that receive rodent NPC transplantation show rotation behavior improvement as compared with untreated animals [13, 77, 92].

## 11. Transplantation of hiPSCs-derived NPCs in HD animal models

The beneficial effect of hiPSC, as well as hiPSC-derived NPCs has also been tested in HD animal models aiming to the future clinical application of these cells [69, 70, 78]

Immunosuppression	Immunorejection symptom	Decreased striatal atrophy	Number of cells	Graft survival	Neuronal differentiation <i>in vivo</i>	Behavior improvements	Aggregate formation <i>in vivo</i>	Aberrant cell differentiation <i>in vivo</i>	Time course (weeks)
Cyclosporine A	N/A	N/A	1×10 <sup>5</sup>	Yes	Nestin ,MAP2, DARPP-32, Gaba	Rotation activity	Present	N/A	12 and 33
Cyclosporine A	N/A	N/A	1×10 <sup>5</sup>	Yes	Nestin ,MAP2, DARPP-32	Learning and motor activity	Absent	N/A	12
Absent	Microglia activation	reduced lesion	1×10 <sup>6</sup>	Yes	NeuN ,Darpp32,GFAP,Iba-1	Memory learning	N/A	Absent	4–6
Cyclosporine A	N/A	reduced lesion	N/A	Yes	Pax-6, NeuN, MAP2,GFAP	N/A	N/A	Present	4–8
Absent	Microglia activation	N/A	1×10 <sup>4</sup>	Yes	MAP2, NeuN, DARPP32, GFAP, MAP2, Pax6, NCAM	N/A	N/A	Present	4–6 and 13–21
Absent	N/A	N/A	1×10 <sup>4</sup>	Yes	Nestin,TuJ1,GAD6	Learning and motor activity	N/A	Absent	3
N/A, non available.									
Neurotrophic action was not evaluated in any of the studies.									

**Table 3.** HD treatment by stem cell transplantation in animal models.

(Table 3). Using the ipsilateral ventricular route, these cells were transplanted into both chemical [69, 78] and transgenic HD rodent models [70]. Similar to ESCs, the hiPSCs differentiate *in vivo* into neurons, including GABAergic specification neurons [69, 70, 78], and astrocytes [78]. Such transplantation caused a modest reduction in striatal neuronal atrophy, a hallmark of HD disease that starts long before the onset of motor symptoms [19, 78]. NPCs derived from iPSCs are of particular interest to be used in HD, since patients are dominated by chorea (involuntary movements) and cognitive disability that should improve by the presence of healthy neurons [20]. The ability of these transplanted cells to reverse HD symptoms in animal models was assessed using several motor and memory tests, such as the using rotarod performance test, the staircase test, the stepping test and the Morris water maze spatial memory task. They showed that experimental animals receiving iPSC-derived NPCs showed short- and medium-term functional motor improvements in different Skills, exhibiting a significantly better performance than sham group animals [69, 70, 78]. However, the long-term (<12 months) stability of such behavioral improvements still needs to be demonstrated.

## 12. Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) protein expression is found in the brain and the spinal cord [93, 94]. This protein promotes the survival of nerve cells (neurons) by playing a role in the growth, maturation (differentiation) and maintenance of these cells. In the brain, BDNF is active at the connections between nerve cells (synapses) where cell-to-cell communication occurs [93, 95]. The BDNF protein helps regulate synaptic plasticity, which is important in learning and memory, and is found to be expressed in regions of the brain that control eating, drinking and body weight [96–100].

The deficits in BDNF signaling contribute to the pathogenesis of several major diseases and disorders such as HD and depression [30, 101, 102]. The decrease in BDNF expression that is observed in HD impairs dopaminergic neuronal function [77], which may be associated with HD motor disturbances. In transgenic HD models, the level of BDNF in cortical tissues can be reduced to 45% of that of controls [103]. Such reduction of BDNF levels is attributed to a mutation in *Htt* which prevents BDNF transcription [104]. Additionally, BDNF transport from the cortex to striatum is decreased in HD transgenic models [105, 106].

The significant role of BDNF in neuronal HD cells is also evident *in vitro*. After removing BDNF from the cell culture medium, neurons derived from HD-iPSCs (109 and 180 CAG repeats) have a robust increase in 3/7 caspase activity and die [107]. This and many other studies indicate that BDNF is a critical factor in the pathology of HD and is a putative candidate for HD treatment [108–110]. However, it is difficult to find an ideal dose for each patient because of the variability in neurodegenerative disease manifestation between individuals. Overdoses of BDNF may induce tumor formation in the brain; on the other hand, low BDNF doses may not provide an efficient treatment.

Mouse ESCs have been genetically manipulated by use of knock-in technology and clones overexpressing BDNF-GFP have been generated. These cells differentiated into neural cells

*in vitro* and gave rise to an increased number of neurons as compared to control unmodified ESCs. BDNF-GFP-expressing ESC-derived neurons have a more complex dendritic morphology and differentiate into GABAergic cells more efficiently than control cells. These BDNF-GFP-expressing ESC-derived neurons show similar electrophysiological properties as cortical neurons and release BDNF in an activity-dependent manner [111].

BDNF-secreting iPSCs that were produced using a virus-free reprogramming method can differentiate into neural cells that overexpress BDNF. In this study, mice which were exposed to a stressor regimen and received BDNF-secreting iPSC-derived neural progenitors via intracerebroventricular transplantation reversed the impact of stressor challenge by subventricular zone adult neurogenesis [112].

Both of these studies demonstrate that PSCs may be used to investigate the effects of BDNF in cell transplantation in various neuropathological conditions. Indeed, neurons derived from HD-iPSCs may provide a model to study the role of BDNF secretion in HD, as well as may help to understand whether the number of repeats and the level of mutant Htt protein expression affect the production of BDNF. Furthermore, these cells can be used as a model to develop different pharmacological, genetic and cellular strategies of BDNF delivery into patients, providing potential new treatments for this orphan disease.

### **13. Limitations on neuronal cells derived from pluripotent stem cells in the treatment of HD**

A major concern regarding cell treatment in HD is the propensity of grafted PSCs or their derived cells to form tumors [33]. Two studies showed that after transplantation of neuron progenitors derived from hESCs and iPSCs into HD animal brains, there occurred the formation of teratoma-like cell masses [79, 80]. These studies teach us that PSCs-derived NPCs can be contaminated with residual PSCs, which maintain their pluripotency and may contribute to tumor formation *in vivo*. Coincidentally, in both studies, neural progenitor stem cells expressed paired box 6 (Pax6). Pax6 is a marker of immature NSCs, which play a role in the development of human neuroectodermal tissues; this transcription factor also has an important regulatory function in cancer cell proliferation and tumor progression [113, 114].

Another disadvantage of PSC transplantation is the stimulation of the host immune system, which could lead to rejection of the cell graft [115]. The majority of *in vivo* studies which transplant human PSCs into HD animal models used immunosuppressive drugs [69, 70, 79], making these studies hard to interpret, since these drugs may relieve HD symptoms [116].

The studies conducted with the absence of immunosuppressive protocols show microglia activation in host tissues after transplantation with hESC-derived striatal and NPCs [78, 80]. Neuroinflammation, characterized by activation of microglia and astrocytes, occurs acutely after traumatic injury, and is a main factor contributing to secondary injury in the central nervous system (CNS). Thus, microglia activation can be considered an important parameter to measure the anti-inflammatory process in stem cell therapies [117]. Alternatively, a study claims that microglia activation may be indicative for an immune response, which suggests donor cell rejection [118].



The use of HD-iPSCs as therapeutic tools has significantly increased over the last years. However, autologous HD-iPSCs are not a good choice for stem cell-based therapy since they carry the HD mutation, which compromises such therapy [69, 119]. Thus, transplantation of HD72-iPSC-derived neural precursors, where HD72 is the number of CAG repeats, into a QA rat model showed that a long time after transplantation (33 weeks), grafted cells showed the formation of huntingtin aggregates. Furthermore, in spite of initial improvement in HD, the disease returned after 33 weeks [69]. Later, the formation of aggregates was evaluated using the same cells (HD72-iPSC-derived neural precursors) a short time after transplantation, and no evidence of aggregates was found in the mouse transgenic model. Recently, Jeon et al. [119] performed more studies and confirmed that the mutant HTT protein derived from NPCs generated from iPSC-HD is able to proliferate *in vivo* in fetal host tissue. They associated this effect to the activity of exosomes, since it has been demonstrated *in vivo* and *in vitro* that exosomes can transport mutant Htt.

Previously, it had been considered that the mutant HTT protein causes cellular dysfunction in a cell-autonomous manner that results in aggregation, inclusion body formation and cell death [120]. However, more recent publications suggested that the pathology does not occur purely at the cellular level. Observation of aggregates of mutant HTT within fetal striatal allografts in patients with HD provides strong evidence for the existence of a non-cell-autonomous mechanism of action, which accounts for the HTT protein to spread via pathological cell-cell communication [119, 121].

All studies demonstrate that HD-iPSC transplantation is a very powerful model which should be more intensively explored. More research is still needed to assess the ability of HD-iPSCs with varying number of CAG repeats to form huntingtin protein aggregates as well as to evaluate the disease pathology after short- and long-term cell transplantation.

## 14. Final considerations

A small number of studies have focused on isolation of HD-PSCs and their use in preclinical studies and have already shown that these cells are an appropriate *in vitro* model for studying molecular and cellular aspects of HD. Interestingly, most HD-ESCs derived so far have 40–50 CAG repeats in *Htt*, a number of repeats usually associated with adult onset of HD (Table 2). In contrast, the majority of HD-iPSCs established to date present a variable number of CAG repeats, all ≤50 (Table 3), which is associated with juvenile-onset HD (prior to age 20). Furthermore, although, a subgroup of 5% of juvenile-onset HD patients have a CAG repeat number greater than 60, none of these have derived HD-iPSCs [28].

NPCs derived from ESCs and iPSCs at different stages of maturation (rosette-forming NPCs and striatal progenitor cells) have mainly been used in transplantation studies in chemical and transgenic HD animal models. However, these studies must be interpreted with care due to the limited number of animals used.

Another consideration worth mentioning regards injection route in transplantation assays. Several studies transplant NPCs via parenchymal brain injection. Although these NPCs demonstrate the ability to engraft into brain, to reduce striatum lesion and to differentiate into GABAergic neurons, such intracerebral injection route is strongly invasive, and it is

not advisable to be used in humans. Thus, other routes, for example, the intravenous route, should be explored in NPC transplantation [11, 122–124].

Additionally, though behavior improvements have been achieved after transplantation of NPCs derived from hESC and hiPSC, these improvements were observed during short- and middle-term periods (until 12 weeks), whereas long-term studies are lacking and would be more useful in reproducing sort after effects for human treatment [69, 70, 72, 81].

Some safety aspects regarding future transplantation of PSC-derived NPCs into humans need to be reevaluated. Recent studies suggest that huntingtin aggregates formed in one cell can be transmitted to neighboring cells [125]. Since PSC-derived NPCs show robust engraftment into the injury site and differentiation to neurons, the ability of huntingtin aggregates formed in the neuronal cells of HD animals to pass into donor-derived neurons should be investigated thoroughly before clinical trials are started.

The majority of studies attribute clinical benefits of PSC-derived NPCs in HD animal models mainly to robust cell graft and tissue regeneration [69, 70, 72, 79, 81]. However, previous studies that used fetal NSCs and MSCs derived from adult tissues attribute the clinical improvements observed after cell transplantation to the paracrine action and neurotrophic support provided by these cells (reviewed in [12]. In these contexts, strategies that provide neuroprotective effect for HD neurons are essential for future clinical intervention in HD. Also, recent studies carried out with NPCs show that these cells are sensitive to BDNF withdrawal *in vitro*, thus NPCs could be an appropriate model to carry out NPC-BDNF dose-response assays.

Preclinical studies which used PSC-derived NPCs in HD animal models do not present enough information to support safety and efficiency of these cells for use in humans. It should be also considered that they presented many limitations in their use in rodent models, thus justifying the delay in clinical studies with PSC-derived NPCs until better data are collected.

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