Chapter 5

Pluripotent Stem Cells to Model and Treat Huntington's Disease

Cristiane Valverde Wenceslau, Irina Kerkis, Celine Pompeia and Monica Santoro Haddad

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66589

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

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

http://dx.doi.org/10.5772/66589

(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

http://dx.doi.org/10.5772/66589

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 atroglial 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, 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, 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.

http://dx.doi.org/10.5772/66589

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 clasping) 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 *in vivo*	Behavior improvements	Aggregate formation *in vivo*	Aberrant cell differentiation *in vivo*	Time course (weeks)
Cyclosporine A	N/A	N/A	1×10^5	Yes	Nestin ,MAP2, DARPP-32, Gaba	Rotation activity	Present	N/A	12 and 33
Cyclosporine A	N/A	N/A	1×10^5	Yes	Nestin ,MAP2, DARPP-32	Learning and motor activity	Absent	N/A	12
Absent	Microglia activation	reduced lesion	1×10^6	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	1×10^4	Yes	MAP2, NeuN, DARPP32, GFAP, MAP2, Pax6, NCAM	N/A	N/A	Present	4–6 and 13–21
Absent	N/A	N/A	1×10^4	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 expects 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.

Author details

Cristiane Valverde Wenceslau[1,2]*, Irina Kerkis[2]*, Celine Pompeia[2] and Monica Santoro Haddad[3]

*Address all correspondence to: irina.kerkis@butantan.gov.br and wenceslauvet@yahoo.com.br

1 CellAvita Ltd, Brazil, Campinas, SP, Brazil

2 Laboratory of Genetics, Butantan Institute, São Paulo, SP, Brazil

3 Hospital of the Faculty of Medicine, University of São Paulo, São Paulo, SP, Brazil

References

[1] The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993;72(6):971–83.

[2] Myers RH. Huntington's disease genetics. NeuroRx 2004;1(2):255–62.

[3] Maucksch C, Vazey EM, Gordon RJ, Connor B. Stem cell-based therapy for Huntington's disease. J Cell Biochem 2013;114(4):754–63.

[4] Petersen A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, et al. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. Hum Mol Genet 2001;10(12):1243–54.

[5] Dayalu P, Albin RL. Huntington disease: pathogenesis and treatment. Neurol Clin 2015;33(1):101–14.

[6] Novak MJ, Tabrizi SJ. Huntington's disease. BMJ 2010;340:c3109.

[7] Kim M, Lee ST, Chu K, Kim SU. Stem cell-based cell therapy for Huntington disease: a review. Neuropathology 2008;28(1):1–9.

[8] Golas MM, Sander B. Use of human stem cells in Huntington disease modeling and translational research. Exp Neurol 2016;278:76–90.

[9] Choi KA, Hwang I, Park HS, Oh SI, Kang S, Hong S. Stem cell therapy and cellular engineering for treatment of neuronal dysfunction in Huntington's disease. Biotechnol J 2014;9(7):882–94.

[10] Palfi S, Conde F, Riche D, Brouillet E, Dautry C, Mittoux V, et al. Fetal striatal allografts reverse cognitive deficits in a primate model of Huntington disease. Nat Med 1998;4(8):963–6.

[11] Lee ST, Chu K, Park JE, Lee K, Kang L, Kim SU, et al. Intravenous administration of human neural stem cells induces functional recovery in Huntington's disease rat model. Neurosci Res 2005;52(3):243–9.

[12] Kerkis I, Kerkis A, Lizier NF, Wenceslau CV. Dental stem cells: risk and responsibilities. In: Bhattacharya N, Stubblefield FG, editors. Regenerative Medicine. Using Non-Fetal Sources of Stem Cells. London: Springer; 2015, pp. 171–5.

[13] Armstrong RJ, Watts C, Svendsen CN, Dunnett SB, Rosser AE. Survival, neuronal differentiation, and fiber outgrowth of propagated human neural precursor grafts in an animal model of Huntington's disease. Cell Transplant 2000;9(1):55–64.

[14] Gaura V, Bachoud-Levi AC, Ribeiro MJ, Nguyen JP, Frouin V, Baudic S, et al. Striatal neural grafting improves cortical metabolism in Huntington's disease patients. Brain 2004;127(Pt 1):65–72.

[15] Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007;448(7151):318–24.

[16] Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318(5858):1917–20.

[17] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126(4):663–76.

[18] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861–72.

[19] Montserrat N, Nivet E, Sancho-Martinez I, Hishida T, Kumar S, Miquel L, et al. Reprogramming of human fibroblasts to pluripotency with lineage specifiers. Cell Stem Cell 2013;13(3):341–50.

[20] Ross CA, Akimov SS. Human-induced pluripotent stem cells: potential for neurodegenerative diseases. Hum Mol Genet 2014;23(R1):R17–26.

[21] Mason SL, Barker RA. Emerging drug therapies in Huntington's disease. Expert Opin Emerg Drugs 2009;14(2):273–97.

[22] Chen S-Y, Lin J-S, Lin H-C, Shan Y-S, Cheng Y-J, Yang B-C. Dependence of fibroblast infiltration in tumor stroma on type IV collagen-initiated integrin signal through induction of platelet-derived growth factor. Biochim Biophys Acta, Mol Cell Res 2015;1853(5):929–39.

[23] Zhang N, An MC, Montoro D, Ellerby LM. Characterization of human Huntington's disease cell model from induced pluripotent stem cells. PLoS Curr 2010;2:Rrn1193.

[24] Bruyn GW, Went LN. Huntington's chorea. In: Vinken PJ, Bruyn GW, Klawans HL, editors. Handbook of Clinical Neurology: Extrapyramidal Disorders. Amsterdam: Elsevier Science; 1986, pp. 267–313.

[25] Haddad MS, Cummings JL. Huntington's disease. Psychiatr Clin North Am 1997;20(4): 791–807.

[26] Hayden MR. Huntington's Chorea. London: Springer; 1981.

[27] Folstein SE. The psychopathology of Huntington's disease. Res Publ Assoc Res Nerv Ment Dis 1991;69:181–91.

[28] Ross CA. When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. Neuron 1995;15(3):493–6.

[29] Quarrell OW, Rigby AS, Barron L, Crow Y, Dalton A, Dennis N, et al. Reduced penetrance alleles for Huntington's disease: a multi-centre direct observational study. J Med Genet 2007;44:(3):e68.

[30] Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiol Rev 2010;90(3):905–81.

[31] Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, et al. Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. Mol Cell 2002;10(2):259–69.

[32] DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, et al. Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 1997;277(5334):1990–3.

[33] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282(5391):1145–7.

[34] Wu J, Yamauchi T, Izpisua Belmonte JC. An overview of mammalian pluripotency. Development 2016;143(10):1644–8.

[35] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292(5819):154–6.

[36] Tesar BM, Goldstein DR. Toll-like receptors and their role in transplantation. Front Biosci 2007;12:4221–38.

[37] Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, et al. Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature 2007;448(7150):191–5.

[38] Pera MF. In search of naivety. Cell Stem Cell 2014;15(5):543–5.

[39] Gardner RL. Mouse chimeras obtained by the injection of cells into the blastocyst. Nature 1968;220(5167):596–7.

[40] Bradley A, Evans M, Kaufman MH, Robertson E. Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. Nature 1984;309(5965):255–6.

[41] Nagy A, Gocza E, Diaz EM, Prideaux VR, Ivanyi E, Markkula M, et al. Embryonic stem cells alone are able to support fetal development in the mouse. Development 1990;110(3):815–21.

[42] Hu M, Wei H, Zhang J, Bai Y, Gao F, Li L, et al. Efficient production of chimeric mice from embryonic stem cells injected into 4- to 8-cell and blastocyst embryos. J Anim Sci Biotechnol 2013;4(1):12.

[43] Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, et al. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. Proc Natl Acad Sci U S A 2010;107(20):9222–7.

[44] Adewumi O, Aflatoonian B, Ahrlund-Richter L, Amit M, Andrews PW, Beighton G, et al. Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. Nat Biotechnol 2007;25(7):803–16.

[45] Guo G, von Meyenn F, Santos F, Chen Y, Reik W, Bertone P, et al. Naive Pluripotent stem cells derived directly from isolated cells of the human inner cell mass. Stem Cell Rep 2016;6(4):437–46.

[46] Blum B, Benvenisty N. The tumorigenicity of human embryonic stem cells. Adv Cancer Res 2008;100:133–58.

[47] Prokhorova TA, Harkness LM, Frandsen U, Ditzel N, Schroder HD, Burns JS, et al. Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. Stem Cells Dev 2009;18(1):47–54.

[48] Plaia TW, Josephson R, Liu Y, Zeng X, Ording C, Toumadje A, et al. Characterization of a new NIH-registered variant human embryonic stem cell line, BG01V: a tool for human embryonic stem cell research. Stem Cells 2006;24(3):531–46.

[49] Mascetti VL, Pedersen RA. Human-mouse chimerism validates human stem cell pluripotency. Cell Stem Cell 2016;18(1):67–72.

[50] Kerkis I, Wenceslau CV, Pompeia C. Induced pluripotent stem cells derived of dental pulp stem cells: a new tool for cellular therapy. In: Sahin F, Dogan A, Demirci S, editors. Dental Pulp Stem Cells: Stem Cell Biology and Regenerative Medicine. Switzerland: Human Press; 2016, pp. 125–42

[51] Wang J, Singh M, Sun C, Besser D, Prigione A, Ivics Z, et al. Isolation and cultivation of naive-like human pluripotent stem cells based on HERVH expression. Nat Protoc 2016;11(2):327–46.

[52] Gafni O, Weinberger L, Mansour AA, Manor YS, Chomsky E, Ben-Yosef D, et al. Derivation of novel human ground state naive pluripotent stem cells. Nature 2013;504(7479):282–6.

[53] Brustle O, Jones KN, Learish RD, Karram K, Choudhary K, Wiestler OD, et al. Embryonic stem cell-derived glial precursors: a source of myelinating transplants. Science 1999; 285(5428):754–6.

[54] Renoncourt Y, Carroll P, Filippi P, Arce V, Alonso S. Neurons derived in vitro from ES cells express homeoproteins characteristic of motoneurons and interneurons. Mech Dev 1998;79(1-2):185–97.

[55] Bain G, Kitchens D, Yao M, Huettner JE, Gottlieb DI. Embryonic stem cells express neuronal properties in vitro. Dev Biol 1995;168(2):342–57.

[56] Doss MX, Koehler CI, Gissel C, Hescheler J, Sachinidis A. Embryonic stem cells: a promising tool for cell replacement therapy. J Cell Mol Med 2004;8(4):465–73.

[57] Verlinsky Y, Strelchenko N, Kukharenko V, Rechitsky S, Verlinsky O, Galat V, et al. Human embryonic stem cell lines with genetic disorders. Reprod Biomed Online 2005;10(1):105–10.

[58] Stephenson E. Derivation of human embryonic stem cells to study early development and genetic disease. Department of Biochemical Engineering. PhD. London: University College London; 2009, p. 394.

[59] Bradley CK, Scott HA, Chami O, Peura TT, Dumevska B, Schmidt U, et al. Derivation of Huntington's disease-affected human embryonic stem cell lines. Stem Cells Dev 2011;20(3):495–502.

[60] Niclis J, Trounson AO, Dottori M, Ellisdon A, Bottomley SP, Verlinsky Y, et al. Human embryonic stem cell models of Huntington disease. Reprod Biomed Online 2009;19(1):106–13.

[61] Niclis JC, Pinar A, Haynes JM, Alsanie W, Jenny R, Dottori M, et al. Characterization of forebrain neurons derived from late-onset Huntington's disease human embryonic stem cell lines. Front Cell Neurosci 2013;7:37.

[62] Yu J, Thomson JA. Pluripotent stem cell lines. Genes Dev 2008;22(15):1987–97.

[63] Maragakis NJ, Rothstein JD. Glutamate transporters in neurologic disease. Arch Neurol 2001;58(3):365–70.

[64] Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr. Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 1985;44(6):559–77.

[65] Ouimet CC, Langley-Gullion KC, Greengard P. Quantitative immunocytochemistry of DARPP-32-expressing neurons in the rat caudatoputamen. Brain Res 1998;808(1):8–12.

[66] Zoghbi HY, Orr HT. Glutamine repeats and neurodegeneration. Annu Rev Neurosci 2000;23:217–47.

[67] Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, et al. Disease-specific induced pluripotent stem cells. Cell 2008;134(5):877–86.

[68] Camnasio S, Delli Carri A, Lombardo A, Grad I, Mariotti C, Castucci A, et al. The first reported generation of several induced pluripotent stem cell lines from homozygous and heterozygous Huntington's disease patients demonstrates mutation related enhanced lysosomal activity. Neurobiol Dis 2012;46(1):41–51.

[69] Jeon I, Lee N, Li JY, Park IH, Park KS, Moon J, et al. Neuronal properties, in vivo effects, and pathology of a Huntington's disease patient-derived induced pluripotent stem cells. Stem Cells 2012;30(9):2054–62.

[70] Jeon I, Choi C, Lee N, Im W, Kim M, Oh SH, et al. In vivo roles of a patient-derived induced pluripotent stem cell line (HD72-iPSC) in the YAC128 model of Huntington's disease. Int J Stem Cells 2014;7(1):43–7.

[71] Juopperi TA, Kim WR, Chiang CH, Yu H, Margolis RL, Ross CA, et al. Astrocytes generated from patient induced pluripotent stem cells recapitulate features of Huntington's disease patient cells. Mol Brain 2012;5:17.

[72] An MC, Zhang N, Scott G, Montoro D, Wittkop T, Mooney S, et al. Genetic correction of Huntington's disease phenotypes in induced pluripotent stem cells. Cell Stem Cell 2012;11(2):253–63.

[73] Valenza M, Rigamonti D, Goffredo D, Zuccato C, Fenu S, Jamot L, et al. Dysfunction of the cholesterol biosynthetic pathway in Huntington's disease. J Neurosci 2005;25(43):9932–9.

[74] Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. Cell Stem Cell 2012;11(2):264–78.

[75] Chan AW, Cheng PH, Neumann A, Yang JJ. Reprogramming Huntington monkey skin cells into pluripotent stem cells. Cell Reprogram 2010;12(5):509–17.

[76] Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. FEBS J 2012;279(8):1356–65.

[77] Pineda JR, Canals JM, Bosch M, Adell A, Mengod G, Artigas F, et al. Brain-derived neurotrophic factor modulates dopaminergic deficits in a transgenic mouse model of Huntington's disease. J Neurochem 2005;93(5):1057–68.

[78] Mu S, Wang J, Zhou G, Peng W, He Z, Zhao Z, et al. Transplantation of induced pluripotent stem cells improves functional recovery in Huntington's disease rat model. PLoS One 2014;9(7):e101185.

[79] Vazey EM, Dottori M, Jamshidi P, Tomas D, Pera MF, Horne M, et al. Comparison of transplant efficiency between spontaneously derived and noggin-primed human embryonic stem cell neural precursors in the quinolinic acid rat model of Huntington's disease. Cell Transplant 2010;19(8):1055–62.

[80] Aubry L, Bugi A, Lefort N, Rousseau F, Peschanski M, Perrier AL. Striatal progenitors derived from human ES cells mature into DARPP32 neurons in vitro and in quinolinic acid-lesioned rats. Proc Natl Acad Sci U S A 2008;105(43):16707–12.

[81] Song J, Lee ST, Kang W, Park JE, Chu K, Lee SE, et al. Human embryonic stem cell-derived neural precursor transplants attenuate apomorphine-induced rotational behavior in rats with unilateral quinolinic acid lesions. Neurosci Lett 2007;423(1):58–61.

[82] Ribeiro CA, Grando V, Dutra Filho CS, Wannmacher CM, Wajner M. Evidence that quinolinic acid severely impairs energy metabolism through activation of NMDA receptors in striatum from developing rats. J Neurochem 2006;99(6):1531–42.

[83] McLin JP, Thompson LM, Steward O. Differential susceptibility to striatal neurodegeneration induced by quinolinic acid and kainate in inbred, outbred and hybrid mouse strains. Eur J Neurosci 2006;24(11):3134–40.

[84] Ramaswamy S, McBride JL, Kordower JH. Animal models of Huntington's disease. Ilar J 2007;48(4):356–73.

[85] Shear DA, Dong J, Haik-Creguer KL, Bazzett TJ, Albin RL, Dunbar GL. Chronic administration of quinolinic acid in the rat striatum causes spatial learning deficits in a radial arm water maze task. Exp Neurol 1998;150(2):305–11.

[86] Vazey EM, Chen K, Hughes SM, Connor B. Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease. Exp Neurol 2006;199(2):384–96.

[87] Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, Singaraja R, et al. A YAC mouse model for Huntington's disease with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal neurodegeneration. Neuron 1999;23(1):181–92.

[88] Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci 2005;25(16):4169–80.

[89] Castiglioni V, Onorati M, Rochon C, Cattaneo E. Induced pluripotent stem cell lines from Huntington's disease mice undergo neuronal differentiation while showing alterations in the lysosomal pathway. Neurobiol Dis 2012;46(1):30–40.

[90] Dong G, Ferguson JM, Duling AJ, Nicholas RG, Zhang D, Rezvani K, et al. Modeling pathogenesis of Huntington's disease with inducible neuroprogenitor cells. Cell Mol Neurobiol 2011;31(5):737–47.

[91] Laowtammathron C, Cheng E, Cheng PH, Snyder BR, Yang SH, Johnson Z, et al. Monkey hybrid stem cells develop cellular features of Huntington's disease. BMC Cell Biol 2010;11:12.

[92] Johann V, Schiefer J, Sass C, Mey J, Brook G, Kruttgen A, et al. Time of transplantation and cell preparation determine neural stem cell survival in a mouse model of Huntington's disease. Exp Brain Res 2007;177(4):458–70.

[93] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Fact 2004;22(3): 123–31.

[94] Fang H, Chartier J, Sodja C, Desbois A, Ribecco-Lutkiewicz M, Walker PR, et al. Transcriptional activation of the human brain-derived neurotrophic factor gene promoter III by dopamine signaling in NT2/N neurons. J Biol Chem 2003;278(29):26401–9.

[95] Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. Annu Rev Neurosci 2001;24:677–736.

[96] Bothwell M. Functional interactions of neurotrophins and neurotrophin receptors. Annu Rev Neurosci 1995;18:223–53.

[97] Pencea V, Bingaman KD, Wiegand SJ, Luskin MB. Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. J Neurosci 2001;21(17):6706–17.

[98] Lee J, Seroogy KB, Mattson MP. Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. J Neurochem 2002;80(3):539–47.

[99] Rosas-Vargas H, Martinez-Ezquerro JD, Bienvenu T. Brain-derived neurotrophic factor, food intake regulation, and obesity. Arch Med Res 2011;42(6):482–94.

[100] Kramar EA, Chen LY, Lauterborn JC, Simmons DA, Gall CM, Lynch G. BDNF upregulation rescues synaptic plasticity in middle-aged ovariectomized rats. Neurobiol Aging 2012;33(4):708–19.

[101] Zuccato C, Cattaneo E. Brain-derived neurotrophic factor in neurodegenerative diseases. Nat Rev Neurol 2009;5(6):311–22.

[102] Mughal MR, Baharani A, Chigurupati S, Son TG, Chen E, Yang P, et al. Electroconvulsive shock ameliorates disease processes and extends survival in huntingtin mutant mice. Hum Mol Genet 2011;20(4):659–69.

[103] Zuccato C, Ciammola A, Rigamonti D, Leavitt BR, Goffredo D, Conti L, et al. Loss of huntingtin-mediated BDNF gene transcription in Huntington's disease. Science 2001;293(5529):493–8.

[104] Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nat Genet 2003;35(1):76–83.

[105] Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, et al. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain 2008;131(Pt 4):1057–68.

[106] Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell 2004;118(1):127–38.

[107] Mattis VB, Tom C, Akimov S, Saeedian J, Ostergaard ME, Southwell AL, et al. HD iPSC-derived neural progenitors accumulate in culture and are susceptible to BDNF withdrawal due to glutamate toxicity. Hum Mol Genet 2015;24(11):3257–71.

[108] Martire A, Pepponi R, Domenici MR, Ferrante A, Chiodi V, Popoli P. BDNF prevents NMDA-induced toxicity in models of Huntington's disease: the effects are genotype specific and adenosine A2A receptor is involved. J Neurochem 2013;125(2):225–35.

[109] Canals JM, Pineda JR, Torres-Peraza JF, Bosch M, Martin-Ibanez R, Munoz MT, et al. Brain-derived neurotrophic factor regulates the onset and severity of motor dysfunction associated with enkephalinergic neuronal degeneration in Huntington's disease. J Neurosci 2004;24(35):7727–39.

[110] Zuccato C, Liber D, Ramos C, Tarditi A, Rigamonti D, Tartari M, et al. Progressive loss of BDNF in a mouse model of Huntington's disease and rescue by BDNF delivery. Pharmacol Res 2005;52(2):133–9.

[111] Leschik J, Eckenstaler R, Nieweg K, Lichtenecker P, Brigadski T, Gottmann K, et al. Embryonic stem cells stably expressing BDNF-GFP exhibit a BDNF-release-dependent enhancement of neuronal differentiation. J Cell Sci 2013;126(Pt 21):5062–73.

[112] Liu G, Rustom N, Litteljohn D, Bobyn J, Rudyk C, Anisman H, et al. Use of induced pluripotent stem cell derived neurons engineered to express BDNF for modulation of stressor related pathology. Front Cell Neurosci 2014;8:316.

[113] Zong X, Yang H, Yu Y, Zou D, Ling Z, He X, et al. Possible role of Pax-6 in promoting breast cancer cell proliferation and tumorigenesis. BMB Rep 2011;44(9):595–600.

[114] Xia X, Yin W, Zhang X, Yu X, Wang C, Xu S, et al. PAX6 overexpression is associated with the poor prognosis of invasive ductal breast cancer. Oncol Lett 2015;10(3):1501–6.

[115] Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature 2011;474(7350):212–5.

[116] Aso E, Ferrer I. It may be possible to delay the onset of neurodegenerative diseases with an immunosuppressive drug (rapamycin). Expert Opin Biol Ther 2013;13(9):1215–9.

[117] Lozano D, Gonzales-Portillo GS, Acosta S, de la Pena I, Tajiri N, Kaneko Y, et al. Neuroinflammatory responses to traumatic brain injury: etiology, clinical consequences, and therapeutic opportunities. Neuropsychiatr Dis Treat 2015;11:97–106.

[118] Singhal S, Lawrence JM, Bhatia B, Ellis JS, Kwan AS, Macneil A, et al. Chondroitin sulfate proteoglycans and microglia prevent migration and integration of grafted Muller stem cells into degenerating retina. Stem Cells 2008;26(4):1074–82.

[119] Jeon I, Cicchetti F, Cisbani G, Lee S, Li E, Bae J, et al. Human-to-mouse prion-like propagation of mutant huntingtin protein. Acta Neuropathol 2016; 577–92.

[120] Cisbani G, Cicchetti F. An in vitro perspective on the molecular mechanisms underlying mutant huntingtin protein toxicity. Cell Death Dis 2012;3.

[121] Cicchetti F, Lacroix S, Cisbani G, Vallieres N, Saint-Pierre M, St-Amour I, et al. Mutant huntingtin is present in neuronal grafts in Huntington disease patients. Ann Neurol 2014;76(1):31–42.

[122] Kwon SK, Kim SU, Song JJ, Cho CG, Park SW. Selective delivery of a therapeutic gene for treatment of head and neck squamous cell carcinoma using human neural stem cells. Clin Exp Otorhinolaryngol 2013;6(3):176–83.

[123] Donega M, Giusto E, Cossetti C, Schaeffer J, Pluchino S. Systemic injection of neural stem/progenitor cells in mice with chronic EAE. J Vis Exp 2014;86: 51154.

[124] Doeppner TR, Kaltwasser B, Bahr M, Hermann DM. Effects of neural progenitor cells on post-stroke neurological impairment-a detailed and comprehensive analysis of behavioral tests. Front Cell Neurosci 2014;8:338.

[125] Costanzo M, Abounit S, Marzo L, Danckaert A, Chamoun Z, Roux P, et al. Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes. J Cell Sci 2013;126(Pt 16):3678–85.

IntechOpen