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Stem Cell-Based Therapy for Huntington's Disease

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ABSTRACT

Huntington's disease (HD) is a late-onset neurodegenerative disease characterized by a progressive loss of medium spiny neurons in the basal ganglia. The development of stem cell-based therapies for HD aims to replace lost neurons and/or to prevent cell death. This review will discuss pre-clinical studies which have utilized stem or progenitor cells for transplantation therapy using HD animal models. In several studies, neural stem and progenitor cells used as allotransplants and xenografts have been shown to be capable of surviving transplantation and differentiating into mature GABAergic neurons, resulting in behavioral improvements. Beneficial effects have also been reported for transplantation of stem cells derived from non-neural tissue, for example, mesenchymal- and adipose-derived stem cells, which have mainly been attributed to their secretion of growth and neurotrophic factors. Finally, we review studies using stem cells genetically engineered to over-express defined neurotrophic factors. While these studies prove the potential of stem cells for transplantation therapy in HD, it also becomes clear that technical and ethical issues regarding the availability of stem cells must be solved before human trials can be conducted. J. Cell. Biochem. 114: 754–763, 2013.

KEY WORDS: STEM CELLS; HUNTINGTON'S DISEASE; TRANSPLANTATION; RODENT HD MODEL

Huntington's disease (HD) is a neurodegenerative disorder caused by a genetic mutation in the huntingtin (*htt*) or IT15 (interesting transcript 15) gene. The disease is inherited in an autosomal-dominant manner and shows a prevalence of about 1 in 10,000 individuals [Bates et al., 2002]. HD is characterized by neuronal cell loss mainly in the caudate nucleus, putamen and the cerebral cortex. In later stages of the disease other brain areas, such as the hippocampus and the hypothalamus, are affected [Vonsattel et al., 1985; Kassubek et al., 2004]. Degeneration of mostly medium-sized projection spiny neurons results in motor disturbances together with cognitive and psychiatric dysfunctions. Typically HD patients do not show any symptoms until onset at a median age between 35 and 42 years. However, early and late manifesting HD forms with first symptoms between 2 and 92 years have been described but occur less commonly [Myers et al., 1985; van Dijk et al., 1986]. In early stages of HD, patients develop mild impairment of motor functions, subtle personality changes, hyperactivity, and cognitive dysfunctions. With further progression affected individuals show severe clinical symptoms, for example, depression, anxiety and dementia as well as distinct chorea, lack of coordination, and/or bradykinesia. Whereas all patients exhibit similar physical symptoms, the progression and intensity of cognitive and psychiatric symptoms can vary significantly between individual patients. HD progresses constantly until death occurs within approximately 15–20 years from the onset.

The neuropathology of HD manifests in the progressive degeneration of the basal ganglia and becomes most prominent in the neostriatum, commonly referred to as the striatum, which includes the caudate nucleus and putamen. Postmortem examinations reveal striatal atrophy in 95% of HD brains with a mean volumetric decrease of 58% [Lange et al., 1976; Vonsattel and DiFiglia, 1998]. The cells predominantly affected are the GABAergic medium spiny projection neurons in the basal ganglia. Other parts in the brain have also been found to be affected in HD. De la Monte et al. [1988] found a volumetric loss of up to 29% in the cerebral cortex, 28% in the thalamus, and 29–34% in the telencephalic white matter in HD patients. Thu et al. [2010] compared clinical findings and postmortem analysis of HD patients and demonstrated significant correlations between symptomology and the extent of cell loss in the cortex. Further, total brain volume has been found to

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be reduced by 19% in HD patients compared to healthy control brains [Halliday et al., 1998].

HD is one of a family of diseases of the central nervous system caused by expanded CAG trinucleotide repeats. Expanded CAG triplet repeats are translated into polyglutamine (polyQ) mutant proteins in HD and other disorders like Dentatorubro-pallidoluysian atrophy (DRPLA) and the spinocerebrallar ataxias (SCAs). For HD, expanded CAG repeats have been localized to 4p16.3 locus within the coding region of the *htt* gene (IT15 gene) on the short arm of chromosome 4 [MacDonald et al., 1993]. Numbers of 36 and more CAG trinucleotide repeats have been found in HD [Walker, 2007]. In healthy individuals the *htt* gene contains typically between 8 and up to 35 CAG triplet repeats, although CAG repeats exceeding 28 show instability in replication which can increase risk of developing HD in future generations. However, an overlap between the normal and disease size ranges of 36–39 CAG repeats has been found [Rubinsztein et al., 1996]. The lengths of repeats in HD patients usually indicate the time course and progression of the disease [Brandt et al., 1996]. Patients showing 47 and more CAG repeats have been found to develop HD symptoms earlier with neurologic and cognitive functioning declining significantly faster compared to those with shorter mutations up to 46 repeats, although no corresponding difference in ultimate severity of neurologic or cognitive impairment was observed.

The translated wild-type huntingtin (htt) protein comprises more than 3,100 amino acids and has a molecular weight of ~348 kDa. The polyQ tract, which is expanded in HD is located in the NH_2-terminal of the htt protein. Although cell degeneration is mainly observed in brain tissue, mutant htt protein is ubiquitiously expressed in neuronal and non-neuronal cell types in HD [Vonsattel and DiFiglia, 1998]. To date, no structural homology has been found to other known proteins. Htt protein is localized in the nucleus and in the cytoplasmic matrix as well as in the soluble cytoplasmic compartment, around the membranes of vesicles, dendrites, and nerve terminals [DiFiglia et al., 1995; Sharp et al., 1995]. Expression in almost all tissues and the heterogeneous intracellular distribution has made it difficult to identify the functions of htt protein. Even though conditional and permanent htt-knockout animal models have been created, the physiological roles and functions of the htt protein remain largely unclear [Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995; Dragatsis et al., 2000; Leavitt et al., 2001]. Intranuclear aggregation of the mutated htt protein is found in neurons of the caudate nucleus, putamen, and the cortex in human HD patients [DiFiglia et al., 1997]. Affected neurons appear more frequently in juvenile patients than in adult patients. Furthermore, the presence of intranuclear inclusions in symptomatic HD patients and their absence in a presymptomatic adult indicates a threshold phenomenon regarding mutant htt protein aggregation, which may explain the late onset of the disease.

It is hypothesized that HD either results from a dominant-negative effect of mutant htt leading to a loss-of-function of the wild-type protein, or from a toxic gain-of-function of mutant htt possibly through an interaction with a protein binding partner expressed in vulnerable neurons. To further examine the normal function of htt protein and how mutated htt protein is involved in pathology of HD many studies have been focused on the identification of htt protein–protein interactions. A variety of htt-interacting proteins with different functions have been identified to date. These htt-interacting proteins are involved either in gene transcription (p53 or specificity protein 1), intracellular trafficking and endocytosis (HAP1 and HIP1), signaling (Calmodulin and CIP-4), or metabolism (GAPDH and HIP2) [Li and Li, 2004]. It is suggested that htt protein might play a role in the arrangement of multi-protein signaling complexes by modulating binding properties of associated factors. Interestingly, several proteins bind to the NH_2-terminal region of htt protein. Possible alterations in binding properties due to expanded CAG triplets to other proteins have to be determined to further characterize the pathological mechanisms in HD. Furthermore, htt protein–protein interactions occur in different cell compartments, which underline the current understanding of htt as a multifunction-protein. Zuccato et al. [2001] found that wild-type htt protein increases brain-derived neurotrophic factor (BDNF) expression in CNS cells, whereas the mutated htt protein leads to down-regulation of BDNF, resulting in insufficient neurotrophic support and neuronal cell death; these findings support the theory that HD is the result of a loss-of-function. However, until the pathological mechanism of the mutant htt protein is clarified both, a physiological loss-of-function and a toxic gain-of-function may be considered.

Little progress has been made in the development of medical treatment for HD in recent years. Currently, there is no therapy available to delay the onset of symptoms or the progression of HD. Only symptomatic treatment to improve the quality of life for HD patients is currently available. Tetrabenazine, a human vesicular monoamine transporter 2 (VMAT2)-inhibitor was approved by the US FDA for the treatment of chorea associated with HD in 2008 as the first specific treatment for HD in the US [Hayden et al., 2009]. The VMAT2 is responsible for the transport of monoamines, for example, dopamine from the cytoplasm into synaptic vesicles. However, the exact mechanism underlying the anti-chorea effect of tetrabenazine is not clear. Antidepressants, such as selective serotonin uptake inhibitors, anticonvulsants as well as neuroleptic drugs are further commonly used to treat particular symptoms in HD. In contrast, the potential use of stem cell-based therapy approaches are aimed at providing causal treatment for HD. Preclinical studies utilizing stem cell-derived transplantation therapy will be discussed in this review.

ANIMAL MODELS OF HUNTINGTON'S DISEASE

To investigate the pathology and develop strategies for the treatment of HD, various animal models have been generated. Rodents are the most widely used group of HD animal models, however, non-human primate and non-mammalian HD models, for example, *Caenorhabditis elegans* or *Drosophila melanogaster* have also been generated [Lee et al., 2004; Brignull et al., 2006; Yang et al., 2008]. HD animal models can be described in two broad classes: excitotoxic lesion models which have mainly been used to investigate molecular therapeutics and cell therapy approaches; and genetically modified models, such as transgenic models and knock-in models (Table I).

TABLE I. Summary of Rodent Models of Huntington's Disease

Model (species)	Genetic insertion/exitotoxin	Neuropathology	Phenotype	Refs.
QA (rat, mouse)	Intra-striatal injection of quinolinic acid (QA)	Loss of GABAergic medium spiny projection neurons	Hyperkinesia; rotational asymmetry; impairment of spontaneous exploratory forelimb use	Beal et al. [1991], McBride et al. [2004], Vazey et al. [2006]
3-NP (rat, mouse)	Systemic administration of 3-nitro-propionic acid (3-NP)	Loss of GABAergic spiny projection neurons and aspiny interneurons	Hyperactivity or hypoactivity (3-NP dosage dependent); decreased motor performance	Beal et al. [1993], Borlongan et al. [1997], Blum et al. [2001], Ryu et al. [2004]
R6/1 (CBAxC57BL mouse)	Human exon 1–115 CAG repeats	Neuronal inclusions; loss of striatal neurons	Decreased anxiety; reduced activity, depression-like symptoms	Mangiarini et al. [1996], Morton et al. [2000], Naver et al. [2003], Pang et al. [2009], Dowie et al. [2010]
R6/2 (CBAxC57BL mouse)	Human exon 1–144 CAG repeats	Intra-/extranuclear inclusions in hippocampus/striatum; loss of striatal neurons	Cognitive deficits; aggressive behavior; motor function impairment; loss of weight	Mangiarini et al. [1996], Morton et al. [2000], Hickey et al. [2005], Stack et al. [2005]
YAC128 (FVB/N mouse)	Several copies of entire mutant human gene ~128 CAG repeat	Selective degeneration of striatum/cortex	Hyperactivity (initially); motor dysfunctions; learning and memory deficits; hypokinesis	Slow et al. [2003], Van Raamsdonk et al. [2005]
TgHD rat (Sprague–Dawley)	Rat cDNA fragment 51 human-derived CAG repeats/endogenous rat htt promoter	Inclusions in basal ganglia/cortex/hippocampus; striatal atrophy; enlarged lateral ventricles;	Motor function impairment; involuntary head and neck movements; weight loss	von Horsten et al. [2003], Nguyen et al. [2006]
HdhQ94, HdhQ111 (129Sv/C57BL6 mouse)	Chimeric mouse/human exon 1 94, 140, 111 CAG repeats	Neuronal intranuclear inclusions; *no* loss of striatal neurons	Hyperactive and hypoactive phases of motor dysfunction	Wheeler et al. [2002], Levine et al. [1999], Menalled et al. [2002]
Hdh(CAG)150 (C57BL6/129P2)	Insertion of ~150 CAG repeats into exon 1 of the murine gene	Neuronal nuclear inclusion aggregates; increased striatal gliosis	Motor task deficit, gait abnormalities	Lin et al. [2001]

Intrastriatal injection of glutamic acid analog excitotoxins, particularly quinolinic acid (QA) and kainic acid (KA) induces neuronal cell loss and therefore simulates one aspect of HD pathology. It has been shown that QA causes more specific striatal atrophy than KA, with selective degeneration of the GABAergic medium spiny projection neurons while selectively sparing somatostatin/neuropeptide Y striatal interneurons and fibers of passage [Beal et al., 1991]. Further, due to its capability to mimic early stages of HD with symptoms such as hyperactivity and motor function impairment, QA has emerged as the most important excitotoxin in HD research. QA is a metabolite of the amino acid tryptophan of the kynurenine pathway and functions as an *N*-methyl-D-aspartate (NMDA) receptor agonist. Schwarcz et al. [1988] observed increased amounts of the enzyme 3-hydroxyanthranilic acid oxygenase, which mediates the conversion of 3-hydroxyanthranilic acid into QA, in the human postmortem HD brain. Interestingly, striatal injection of QA in mice results in increased htt protein levels in some remaining neurons [Tatter et al., 1995]. Indeed, over-expression of normal htt protein protects NMDA receptor-mediated apoptotic neurodegeneration after QA administration [Leavitt et al., 2006]. These findings strongly indicate the junction between QA and htt as the crucial protein in HD.

Treatment with 3-nitropropionic acid (3-NP) is also widely used to model HD in rodents as well as in non-human primates [Brouillet et al., 1995; Blum et al., 2001; Yang et al., 2005]. The neurotoxin 3-NP irreversibly inhibits the enzyme succinate dehydrogenase in mitochondria leading to predominantly striatal cell death [Beal et al., 1993]. Both GABAergic spiny projection neurons and aspiny interneurons are equally affected. Due to its capability to cross the blood–brain barrier, 3-NP can be administered systemically. Indeed, systemic application of 3-NP induces selective neurodegeneration in the lateral striatum. Administration of 3-NP can mimic different stages of HD dependent on the frequency of given doses. Intraperitoneal injections of two doses of 3-NP leads to hyperkinetic symptoms in rats, occurring in early- and mid-stages of HD [Borlongan et al., 1997]. In contrast, four or more injections result in hypoactivity, which is observed in late-stages of HD.

Since HD was identified as a monogenetic disease caused by a mutation in the IT15 gene in 1993, several genetically engineered animal models showing a similar pathogenetic pattern to human HD patients have been generated. Rodents have been used predominantly as models to investigate basic molecular mechanisms of HD and potential therapeutic options. Two different categories of rodent HD models have been created: transgenic models and knock-in models. Whereas the mutant *htt* gene is randomly inserted and driven by different promoters in transgenic models (ectopic expression), the mutated *htt* gene is driven by the endogenous promoter in an appropriate genomic context in knock-in models. Mice have almost exclusively been used in genetic models of HD with only one rat transgenic HD model available to date [Heng et al., 2008].

The first transgenic mouse HD models R6/1 and R6/2 were generated by insertion of the exon 1 fragment at the 5′-end of the human *htt* gene comprising approximately 115 and 144 CAG repeats, respectively [Mangiarini et al., 1996]. The R6/2 represents the most widely used transgenic mouse model for HD. The mutant

truncated human *htt* gene is driven by the human *htt* promoter and is expressed in all cell types of the R6/2 model. Due to the relatively long CAG repeat fragment, the R6/2 model shows early-onset and progressive development of various symptoms. Cognitive deficits can be observed as early as 3.5 weeks of age followed by development of aggressive behavior, motor function impairment, and loss of weight. These symptoms are accompanied by a neuroanatomic phenotype showing decreased numbers of striatal neurons and brain volume [Hickey et al., 2005; Stack et al., 2005]. At 12 weeks of age, a reduction of 26% in the number of neurons and a reduced striatal volume by 41% can be observed. However, this cannot entirely explain a 44% reduction of total brain volume, which indicates an additional non-specific loss of cells in the R6/2 brain. A cellular feature of this model is the presence of neuronal intranuclear and extranuclear inclusions in cells of the hippocampus and the striatum. Progressive formation has been observed regarding the number, the size and the distribution of mutant htt inclusions [Morton et al., 2000]. Death occurs in the R6/2 model at an average age of 11–13 weeks.

The R6/1 mouse model is used less frequently compared to R6/2 and therefore information and understanding is limited. Behavioral and histological phenotypes of the R6/1 model are milder, which can be attributed to the shorter CAG repeat sequence [Naver et al., 2003]. Development of the disease follows a slow progression with a lower level of anxiety seen at 15 weeks and reduced activity at 23 weeks of age compared to wild-type mice. However, these symptoms seem not to be consistent and distinct in the R6/1 strain. Recent studies show altered depression-related behavior, which correspond with reduced expression of specific serotonin receptors as well as decreased cannabinoid 1, dopamine 1 and dopamine 2 receptor ligand binding in the striatum [Pang et al., 2009; Dowie et al., 2010].

The YAC128 transgenic HD mouse model was generated by insertion of a yeast artificial chromosome (YAC) containing several copies of entire mutant HD gene comprising a 128 CAG repeat sequence driven by the human endogenous HD promoter [Slow et al., 2003]. A progressive loss of brain weight can be observed from an age of 6 months on, which appears due to atrophy of the striatum and the cortex. Unlike in the R6/2 mouse models, other brain areas are not affected in the YAC128 model. However, cognitive deficits can be observed at an age of 8 weeks, followed by motor deficits at 3 months [Van Raamsdonk et al., 2005]. Progression of striatal cell loss appears slower and the life span is longer compared to R6/2 transgenic mice, which makes the YAC 128 an attractive model to study long-term therapeutic strategies.

The transgenic rat model of HD expresses a rat *htt* cDNA fragment including 51 human-derived CAG repeats driven by the endogenous rat *htt* promoter [von Horsten et al., 2003]. Inclusions of the mutant truncated *htt* protein have been found predominantly in cells of the basal ganglia but also the cortex, the hippocampus, and the midbrain. Furthermore, the transgenic rat model shows enlarged lateral ventricles and focal lesions in the striatum. The relatively small number of CAG triplets in the transgenic rat model results in an adult-onset neuropathological phenotype. Motor function impairment can be observed from an age of 6 months for homozygous and 8 months for heterozygous animals [Nguyen et al., 2006]. Progressive course of symptoms in the rat HD model also includes gait abnormalities and involuntary head and neck movements which appear at 10–15 months. At 24 months, the animals are 20% lighter compared to the wild-type rats. The larger brain of the HD rat model facilitates examinations such as surgical approaches and transplantation studies and allows an expanded repertoire of complex motor and cognitive function tests compared to the smaller mouse models.

Due to insertion of the mutated *htt* gene at the actual genomic locus, knock-in mouse models have been considered as more precise models for HD. However, initial studies did not reveal a neuropathologic phenotype in knock-in models [Ramaswamy et al., 2007a]. Not until the creation of models with larger numbers of CAG repeats, were specific pathologic features observed in knock-in models. The mouse *htt* exon 1 has been replaced by a chimeric mouse/human exon 1 coding for CAG triplets of 94, 140, and 111 repeats in the HdhQ94, HdhQ140, and HdhQ111 knock-in models, respectively [Levine et al., 1999; Menalled et al., 2002; Wheeler et al., 2002]. All three models show progressive neuronal intranuclear formation of inclusions and develop behavioral abnormalities at certain stages. Striatal atrophy but no loss in the number of striatal neurons has been observed in the HdhQ94 mice, whereas neither alterations in striatal volume nor loss of neurons have been demonstrated for HdhQ140 and HdhQ111. Lin et al. [2001] created the Hdh(CAG)150 model by inserting approximately 150 CAG repeats into exon 1 of the murine *htt* gene. Presumably, the higher number of CAG repeats results in a more severe phenotype compared to other knock-in models. Hdh(CAG)150 mice show a late disease-onset with progressive motor and behavioral deficits, neuronal nuclear inclusion aggregates and increased gliosis in the striatum.

The creation of various HD animal models has aided in the investigation of the pathological mechanisms underlining HD, and has also enabled pre-clinical investigations to be performed using novel treatment approaches such as cell therapies.

STEM CELL-BASED TREATMENT FOR HD

Cell-based therapies for HD aim to restore brain circuitry and function by cellular replacement of lost neurons and/or by neurotrophic support of damaged and diseased tissue. There are several possible sources of tissues such as embryonic or fetal striatal tissue which have traditionally been used as grafts in pre-clinical transplantation studies with rodent and non-human primate models [Dunnett et al., 2000; Kendall et al., 2000]. These studies have demonstrated proof-of-principle evidence of neuro-anatomical integration that alleviates clinically relevant motor and cognitive deficits in HD animal models [Isacson et al., 1986; Pritzel et al., 1986; Sirinathsinghji et al., 1988; Clarke et al., 1988ab; Dunnett et al., 1988ab; Palfi et al., 1998; Nakao and Itakura, 2000; Freeman et al., 2000b; Dunnett and Rosser, 2007; Ramaswamy et al., 2007b]. As a result, primary fetal tissue transplantation for HD is currently undergoing clinical evaluation [Bachoud-Levi et al., 2000; Freeman et al., 2000a; Hauser et al., 2002; Rosser et al., 2002; Rosser and Dunnett, 2003; Gaura et al., 2004; Furtado et al., 2005; Bachoud-Levi et al., 2006; Farrington et al., 2006; Krystkowiak et al., 2007;

Gallina et al., 2008; Reuter et al., 2008]. In contrast to primary striatal tissue grafts, stem cells have the potential to resolve a number of technical issues associated with the use of human fetal tissue, including supply, pathogenic contamination, and viability of human fetal tissue [Isacson and Breakefield, 1997].

The following will give an overview of studies that have been undertaken to investigate whether stem cells can attenuate, delay or prevent disease progression, or provide a source of cellular replacement in HD animal models. While neural stem and progenitor cells have been investigated as possible sources for cell replacement therapies in HD, stem cells derived from origins other than the CNS and genetically modified stem cells have also been examined.

NEURAL STEM/PROGENITOR CELL TRANSPLANTATION

Studies from two independent groups looked at transplantation of human neural stem cells (huNSCs) into excitotoxic HD rat models [McBride et al., 2004; Ryu et al., 2004]. Ryu et al. [2004] generated immortalized huNSC lines from fetal telencephalon tissue and transplanted cells 1 week prior or 12 h after 3-NP lesioning. Interestingly, animals which received transplants prior to 3-NP treatment showed improved motor functions and reduced cellular damage compared to control animals, whereas those which were transplanted after lesioning did not benefit from cell therapy treatment. Immunohistochemical staining revealed partial differentiation of huNSCs into GABAergic neurons and astrocytes. Furthermore, the authors observed endogenous BDNF secretion from huNSCs prior to and following transplantation. Thus, the therapeutic potential of huNSCs in this study may result both from engraftment and functionality of transplanted cells as well as from neurotrophic support by secreted BDNF.

A second study examined transplantation of fetal huNSCs into the QA rat model of HD [McBride et al., 2004]. Dissociated primary human cortical neurospheres which were cultured and pre-differentiated in the presence or absence of ciliary-derived neurotrophic factor (CNTF) were injected into the striatum 1 week after receiving a QA lesion. Motor function was assessed for 8 weeks using the cylinder test and found to be significantly improved in the groups which received cell treatment compared to vehicle control group. Interestingly, similar survival and migration profiles were observed for both CNTF+ and CNTF− human neurospheres, however, only animals which received CNTF+ cells showed significantly reduced striatal atrophy compared with vehicle control rats.

A study by Lee et al. [2006] compared the migration of transplanted huNSC towards the lesion site after systemic administration of huNSC via tail vein injection or by direct injection into the ventricle in the QA model. Detection of huNSCs 3 weeks after intravenous injection revealed a large population of huNSC had migrated into the striatum of the lesioned hemisphere and were mostly found around necrotic tissue in the parenchyma and adjacent to vessels. Migration of huNSCs to the lesion site was also observed when cells were injected into the lateral ventricle on the contralateral side to the lesioned striatum. In another study, intravenous tail vein injections of huNSCs were carried out 1 week post-QA lesion and led to significant reduction in apomorphine-induced rotations over a period of 2 weeks after injection to the end of the study 9 weeks after injection [Lee et al., 2005]. Furthermore, QA-lesioned animals which received huNSC administration showed a significantly greater striatal volume than control animals.

The timing of cell transplantation in relation to cell loss or injury and the effect this has on transplant survival is an interesting question. Johann et al. [2007] addressed this by examining allotransplants of NSCs into a QA mouse model at 2, 7, and 14 days after QA lesioning. Striatal cells were isolated from E14 mice and neurospheres were transplanted either as whole spheres or mechanically dissociated cells. Intact neurospheres transplanted 2 days post-QA lesioning were found to be superior regarding cell survival and differentiated into $GFAP^+$ astroglial cells 3 months later. To explain reduced graft survival in animals which were transplanted 7 and 14 days post-lesioning, the temporal activation of endogenous microglia after QA injection was investigated. Indeed, the authors observed a moderate astroglial and microglial response to QA on Day 2 and a significant increase on Days 7 and 14 after QA injection which was in accordance with another study demonstrating up-regulation of a number of chemokines following QA striatal lesioning [Gordon et al., 2009]. Roberts et al. [2006] also investigated lesion progression and temporal engraftment of transplanted MHP36 cell line-derived NSCs in a 3-NP model of HD by serial magnetic resonance imaging (MRI). Striatal tissue declined steadily over a period of 6 weeks after systemic 3-NP administration but striatal degeneration could be spared by transplantation of NSCs compared to non-transplanted controls. This correlated with partially improved motor functions of transplanted animals compared to controls.

In conjunction with these studies, we have also demonstrated the survival and differentiation of allogenic transplanted neural progenitor cells (NPCs) in a rat QA lesion model of HD [Vazey et al., 2006]. NPCs were derived from the subventricular zone (SVZ) of adult rats and injected into the lesioned striatum 14 days after QA injection. Animals which received transplantation of adult NPCs demonstrated significantly reduced motor function impairment compared to sham transplanted rats. Immunohistochemical analysis revealed a survival rate of 12% of transplanted cells. While the majority of transplanted cells were found to differentiate into astrocytes within lesioned striatum, ~35% of transplanted cells, predominantly located adjacent to the lesion, exhibited a mature neuronal NeuN phenotype. Further, ~15% of these neurons expressed DARPP-32 or GAD_{67}, specific markers for striatal medium spiny projections neurons and interneurons. Further, by priming rat SVZ-derived NPCs with lithium chloride before transplantation a 34% increase in the number of $DARP32^+$ neurons was detected 12 weeks after transplantation in the striatum of the QA model compared to animals with received untreated NPCs [Vazey and Connor, 2010].

Cell survival and differentiation were further reported in a study in which NPCs derived from human embryonic stem (ES) cells were transplanted into the QA model of HD [Song et al., 2007]. NPCs were differentiated from ES cells, colonies were dissociated and single cell populations were injected unilaterally into the striatum 7 days post-QA lesioning. Animals demonstrated significant improvement in motor function 3 weeks after NPC transplantation compared to control rats which received vehicle injections. Transplanted cells

either migrated to the cortex or were found to form cell clusters in the lesioned area and expressed the early neural markers TuJ1 and nestin. A subpopulation of these cells committed to a GABAergic neuronal cell fate. Extending these observations, we have demonstrated that NPCs derived from human ES cells which were primed with noggin were superior in survival and neural differentiation over spontaneously derived NPCs when transplanted into the rat QA model [Vazey et al., 2010]. Interestingly, examination of the fate of transplanted ES cell-derived NPCs at 8 weeks post-transplantation revealed tumor formation of spontaneously derived NPCs which queries the safety profile of cells derived from ES cells for transplantation therapies. Survival, differentiation, and tumor formation from human ES cell-derived NPCs has been shown to be dependent on the differentiation stage of the transplanted cells [Aubry et al., 2008]. The further NPCs were differentiated towards a striatal DARPP32^{+} phenotype prior to transplantation the less likely the incidence for tumor formation at 4–6 weeks post-transplantation. However, when animals were examined 2 months post-transplantation large outgrowths of transplanted cells were still observed indicating the difficulties in preventing tumorgenesis from ES cell-derived NPCs.

TRANSPLANTATION OF NON-NEURAL STEM CELLS

It has been shown that multipotent adult cells from tissue other than the CNS, such as bone marrow-derived mesenchymal stromal cells (BM-MSC) and adipose-derived stem cells (ASC) can exhibit neuronal phenotypes under certain conditions [Choong et al., 2007; Erba et al., 2010; Zavan et al., 2010]. Another important feature of adipose- and bone marrow-derived stem cells is the capacity to secret growth factors and neuroprotective factors [Rehman et al., 2004; Wang et al., 2006]. Thus, several studies have examined the potential of these stem cells for transplantation into HD models as neuroprotective or cell replacement therapies. A major advantage of cells derived from bone marrow or adipose tissue is accessibility and the potential for autologous transplantation. However, the genetic component has to be taken into consideration for autologous transplantation therapies in HD patients.

The paracrine effects of human ASCs on HD pathology was investigated by Lee et al. [2009] in cell culture experiments and in vivo in two different rodent HD models. Characterization of human ASCs derived from subcutaneous adipose tissue revealed secretion of multiple growth factors, for example, BDNF, nerve growth factor (NGF), insulin-like growth factor 1 (IGF-1), epidermal growth factor (EGF), and CNTF. Transplantation of ASCs into the QA model of HD led to significant improvement in apomorphine-induced rotation tests over 4 weeks, which correlated with a reduced lesion volume and a lower number of apoptotic striatal cells compared to control animals. Transplantation of ASCs was also performed in the R6/2 mouse HD model. Both groups, untreated and transplanted R6/2 mice showed significantly poorer performance in the rotarod test compared to WT control. From 9.5 to 12 weeks post-transplantation, ASC-transplanted mice showed improved motor functions compared to untreated R6/2 controls, although the overall performance continued to decline. Interestingly, mice which received ASC transplantation displayed a significant longer survival time than untreated R6/2 control mice. To examine the potential of ASC transplantation in a long-lived HD model and further to assess the possibility of autologous ASC transplantation in HD patients, the same researchers compared transplantation of human HD patient-derived ASCs with normal human ASCs in the YAC128 model [Im et al., 2010]. RT-PCR analysis revealed similar expression levels of the growth factors BDNF, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and leukemia inhibitory factor (LIF) for human HD ASCs and human normal ASCs when cultured under the same conditions. When cells were transplanted prior to the appearance of disease phenotype there was no significant differences in motor function and body weight loss between transplanted YAC128 animals and YAC128 controls. However, differences in neurodegeneration were observed with decreased striatal volume in mice transplanted with HD ASCs and a volumetric increase of the striatum in animals transplanted with normal ASC compared to untreated control YAC128 animals. Transplantation of normal ASCs after onset of disease phenotype improved the performance on the rotarod, however, no improvement regarding striatal atrophy was observed. Transplantation of HD patient-derived ASCs after the onset of disease phenotype was not performed.

In another study autologous transplantation of bone marrow cells was conducted in the QA model [Lescaudron et al., 2003]. QA lesions were performed and 14 days later whole bone marrow cells we isolated from the tibia and transplanted into the striatum. Both, QA-lesioned rats which received transplantation of bone marrow cells and sham-operated animals performed significantly better in working memory test compared to QA-treated control animals. However, no significant differences in swim speeds, visual paw-placement task or in the lesion size could be observed between the three groups. Cells labeled prior to transplantation remained mainly in an undifferentiated state and only $\sim$1% of the cells exhibited a neuronal phenotype indicated by TUJ1 and $GAD_{65/67}$ expression. Two recent studies have also examined the effects of human mesenchymal stem cells (MSCs) after intrastriatal transplantation in different mouse models of HD [Snyder et al., 2010; Lin et al., 2011]. Interestingly, both studies demonstrated reduced striatal atrophy but observed differences in the survival of transplanted cells. Transplanted MSCs only survived for up to 7 days in N171-82Q transgenic HD mice [Snyder et al., 2010], whereas a small number of MSC survived and differentiated over a time period of 16 weeks in the QA mouse model, which might be attributed to the more acute quality of the QA lesion [Lin et al., 2011]. Both groups suggested a neuroprotective effect resulting from neurotrophic factors secreted by transplanted MSCs. The impact of an acute QA lesion on transplant survival was further demonstrated for both exogenous neural stem cells and MSCs in a study by Bantubungi et al. [2008]. A large fraction of transplanted cells were detected in animals which received a QA lesion, whereas very few transplanted MSCs or NSCs could be detected at either 3 or 8 weeks post-lesion in sham-lesioned animals. Stem cell factor (SCF) was identified as one candidate for mediating the survival of transplanted cells in the QA environment. In addition, Kwan et al. [2012] examined stem cell transplantation into a non-neuronal body compartment and performed allogenic bone marrow transplantation in two transgenic HD mouse models. BACHD, YAC128, and WT animals were lethally irradiated and

whole bone marrow cells of WT donors were administered intravenously by retro-orbital injection. Behavioral analysis revealed moderate improvement in bone marrow transplanted HD animals compared to untreated transgenic HD mice. Interestingly, serum levels of interleukin-6, interleukin-10, CXC chemokine ligand 1, and interferon γ were significantly higher in HD than WT mice, but were normalized in mice that received bone marrow transplants. This indicates that mutant htt may cause an immune response contributing to pathogenesis in HD and this may be amenable to therapeutic intervention through bone marrow transplantation.

TRANSPLANTATION OF GENETICALLY MODIFIED STEM CELLS

One of the earliest transplantation studies using genetically modified cells looked at the transplantation of neural stem cell lines derived from the embryonic hippocampus which were genetically modified to over-express either NGF or BDNF [Martinez-Serrano and Bjorklund, 1996]. Genetically modified and control cells were transplanted into the striatum 1 week prior to excitotoxic QA lesioning. The extent of cell engraftment and the effects of striatal NGF and BDNF secretion on the protection and survival of damaged tissue was assessed 5 weeks post-transplantation. All animals showed surviving grafts with mostly a glial-like morphology for NGF-, BDNF-expressing, and control NSC transplants. Whereas a loss of neurons was observed in QA-lesioned sham and control NSC-grafted animals, a significant number of surviving medium-sized spiny striatal projection neurons and even an increase in striatal cholinergic interneurons were found within the QA injection site for NGF-expressing cells and to a less extent for BDNF-expressing transplants. The increase in astroglial and microglial cells following the QA lesion was also significantly reduced in animals which received NGF-expressing transplants. Furthermore, QA-induced atrophy of the striatum was significantly reduced by 30% and the lesion size was smaller in animals transplanted with NGF-expressing cells.

In a recent study, MSCs engineered to stably over-expressing BDNF or NGF were transplanted into transgenic YAC128 mice and the long-term effects of the treatment was investigated [Dey et al., 2010]. After in vitro characterization, genetically modified MSCs were injected intrastriatally into both hemispheres of 4-month-old YAC128 mice. Animals received MSCs over-expressing either BDNF or NGF, a mix of MSCs over-expressing both BDNF and NGF, or a control MSC population. In contrast to the earlier study assessing the effects BDNF and NGF 5 weeks post-transplantation, histological analysis after 9 months revealed sparing of NeuN-positive and DARPP-32-positive neurons for animals which were transplanted with BDNF-expressing MSCs only. Neither NGF-expressing MSCs nor a combination of both BDNF- and NGF-expressing MSCs exhibited a protective effect. Interestingly, all mice which were transplanted with MSCs showed reduced clasping, whereas only mice which received BDNF over-expressing MSCs showed improved motor coordination on the rotarod performance test. This may indicate that MSCs have a beneficial effect on the cellular dysfunction underlying clasping behavior, whereas BDNF and subsequently the reduced striatal cell loss seem to be specifically beneficial for motor coordination performance.

An additional study examined the effect of glial cell line-derived neurotrophic factor (GDNF)-expressing neural stem cells (NSCs) following transplantion into QA-lesioned nude mice [Pineda et al., 2007]. One day after NSCs transplantation, the striatum was lesioned with QA and the engraftment and proliferation of NSCs was assessed by optical luminescent neuroimaging over a period of 15 days. Transplanted NSCs integrated into the brain tissue and proliferated in the QA-lesioned striatum. GDNF-expressing NSCs exhibited a protective effect on the survival of striatal neurons which also correlated with reduced amphetamine-induced rotational behavior compared to control mice.

CONCLUSION

HD is a progressive neurodegenerative disorder with no effective treatment available to date. Recent studies have demonstrated that neural stem/progenitor cells can survive and differentiate into replacement striatal neurons following transplantation into the brain of HD animal models resulting in an improvement of motor and cognitive functions. This appears to be dependent on the pathology of the rodent host brain with acute cell loss appearing to provide enhanced support of transplanted cell survival. Mesenchymal- and adipose-derived stem cells have also been demonstrated to secret neurotrophic factors and reduce pro-inflammatory cytokine expression in HD rodent models, and are therefore able to improve HD pathology. Further, stem cells genetically engineered to over-express a range of neurotrophic factors have been investigated for transplantation studies in HD models and have been shown to provide both protective and regenerative support. Further studies are now required to address important questions regarding the availability and safety of stem cells for clinical transplantation trials. However, improvements in motor function, survival, and histopathology observed in preclinical studies following transplantation of stem cells suggest that stem cell therapy holds a great potential to become an effective treatment strategy for HD in the future.

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