

Huntington's Disease: New Frontiers for Molecular and Cell Therapy

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Abstract: Huntington's disease (HD) is an incurable, adult-onset, dominantly inherited neurodegenerative disease, caused by a CAG expansion in the 5' coding region of the gene HD [encoding huntingtin (htt), which is ubiquitously expressed in all tissues]. The disease progresses inexorably with devastating clinical effects on motor, cognitive and psychological functions; death occurring approximately 18 years from the time of onset. These clinical symptoms primarily relate to the progressive death of medium-spiny GABA-ergic neurons of the striatum and in the deep layers of the cortex; during the later stages of the disease, the degeneration extends to a variety of brain regions, including the hypothalamus and hippocampus. The mechanism by which mutant htt leads to neuronal cell death and the question of why striatal neurons are targeted both remain to be further investigated. Certainly htt is required for cell survival and impairment of wild-type htt function can be involved in neurodegeneration, but considerable evidence also shows that trinucleotide repeat expansion into glutamine (polyQ domain) endows the protein with a newly acquired toxic activity. The increasing availability of HD animal models have allowed not only to investigate the function of htt, but also to screen and test potential therapeutic drugs in the promising area of neurotherapeutics. So, thorough analysis of these molecular and biochemical events, assessing the validity of candidate mechanisms, provides a means to identify effective therapeutic strategies for cellular repair. Here, the rationale and efficacy of different therapies are compared and alternative therapies are reviewed including intrastriatal transplantation of human fetal striatal tissue to support the cell replacement strategy in HD. Since functional restoration through neuronal replacement probably could be combined with neuroprotective strategies for optimum clinical benefit, *in vivo* and *ex vivo* gene therapy for delivery of neuroprotective growth factor molecules are also considered.

Key Words: Huntington's disease, huntingtin, trinucleotide repeat, cell therapy, excitotoxicity, neurotrophins, striatal grafts, stem cell transplantation, neuroprotection.

OVERVIEW

"Hereditary chorea.....confined to certain and fortunately a few families, and has been transmitted to them, an heirloom from generations away back in the dim past. It is spoken of by those in whose veins the seeds of the disease are known to exist, with a kind of horror. There are three marked peculiarities in this disease: 1. Its hereditary nature. 2. A tendency to insanity and suicide. 3. Its manifesting itself as a grave disease only in adult life" [1].

Over a century ago, George Huntington, a Long Island family practitioner, provided the first complete description of the disorder that now bears his name. He accurately reported in a paper presented to the Meigs and Mason Academy of Medicine in Middleport, Ohio, the salient clinical features of the disease, its pattern of transmission from parent to child, and its unfavourable prognosis. In effect, the delineation of the disorder was the outcome of the cooperation between three generations of doctors in the Huntington family [2]. Once considered a relatively rare disorder, HD has been historically important and continues to be at the frontier of human neurological diseases. Prevalence figures for HD vary depending on the geographical area, but the best estimate is

10 per 100,000. The disorder is reported in all races, although it is much more common in Scotland and Venezuela and less common in Finland, China, Japan, and black South Africans. Chorea, from the Greek word for dance, is the most common motor symptom in HD adult patients, usually present along with memory deficits, affective disturbance, and changes in personality; other forms of motor dysfunction such as parkinsonism, dystonia, and involuntary motor impairments may all be present [3]. Juvenile-onset patients show bradykinesia, rigidity, epilepsy, severe dementia, and a more rapidly progressing disease. Caudate atrophy is visible in computer tomography or magnetic resonance imaging scans. Certain symptoms of HD are likely explained by the anatomy of neurodegeneration. The most striking neuropathology in HD occurs within the neostriatum, in which gross atrophy of the caudate nucleus and putamen (together called the neostriatum) is accompanied by selective neuronal loss and astrogliosis [4]. Pathologic changes have also been described in deep layers of the cerebral cortex, lateral and medial globus pallidus, thalamus, subthalamic nucleus [5]. Other regions, including substantia nigra pars reticulata, and cerebellum, show varying degrees of atrophy depending on the pathologic grade. In juvenile onset cases, cell loss tends to be more pronounced, and can also be seen in the cerebellum. Within the striatum, HD differentially affects subpopulations of neurons, with projection neurons preferentially being lost rather than interneurons [6, 7]. The earliest and most extensively affected neurons (approximately 90%

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of striatal neurons) are the medium-sized striatal neurons [8, 9], the output projection neurons, that contain γ -aminobutyric acid (GABA) and enkephalin (Enk) or GABA and substance P as neurotransmitters. Medium-sized and large neurons containing the enzyme NADPH-diaphorase (identified in 1991 [10] as a nitric oxide synthase) remain intact [8]. These neurons contain somatostatin and neuropeptide Y as neurotransmitter and are localized in the striatal matrix [11, 12]. Consistent with the finding of loss of projection neurons was the early finding that GABA levels were markedly reduced in the caudate-putamen of HD patients [13]. Of the two populations of striatal projection neurons, the neurons of the indirect pathway (i.e. enkephalin/GABA-containing neurons) are affected first, thus providing an anatomical substrate for the increased movement which is the hallmark of HD [14, 15]. Consistent with this observation is the fact that in early grade HD cases, markers for these striatal neurons are decreased, including dopamine D2 receptors, adenosine A2a receptors and enkephalin. In early HD, thus, the indirect pathway is predominantly disrupted, and basal ganglia circuitry models predict an overall increase in movement, manifested as chorea and ballism [7]. In later stages of adult HD, both populations of striatal projection neurons are affected, with concomitant loss of markers of the direct pathway (i.e. substance P/dynorphin/GABA-containing neurons), including dopamine D1 receptors and substance P [15, 16]. The functional correlates of degeneration of both the direct and indirect pathways are a rigid bradykinetic state [7]. In juvenile HD cases which resemble Parkinson's disease, degeneration of both direct and indirect pathway striatal neurons is observed [17, 18].

HD became the first human disease gene mapped to a chromosomal location using only the approach of linkage analysis with polymorphic DNA markers, heralding a new era in human genetics [19, 20]. In fact, HD is the most prevalent disorder in a family of inherited neurodegenerative diseases (the trinucleotide repeat disorders [21] that are caused by expansion of a polyglutamine (polyQ) tract in the associated disease proteins). This family includes eight other neurodegenerative disorders: dentatorubral-pallidolusian atrophy (DRPLA), spinobulbar muscular atrophy (SBMA) and spinocerebellar ataxia (SCA) types 1–3, 6, 7 and 17 [22, 23]. With the exception of SBMA, these neurodegenerative disorders are dominantly inherited. All eight disorders are progressive, typically striking in midlife and causing increasing neuronal dysfunction and eventual neuronal loss 10–20 years after onset of symptoms. HD is caused by an unstable expanded CAG trinucleotide repeat in exon 1 of the huntingtin gene on the tip of the short arm of chromosome 4 (4p16.3). Wild-type genes with a stable CAG repeat have 6–34 repeat units; more than 36 repeats result in an unstable, expanded, disease-causing allele. Juvenile forms of HD are associated with alleles containing more than 70 repeats; the longest allele described to date contains 121 CAG repeats. Intergenerational instability shows a dramatic transmitting parent effect: 80% of juvenile patients inherit the mutant HD gene from their father [24, 25]. HD is a true dominant condition [26]. Homozygotes do not have an earlier onset or more severe form of the illness, suggesting that the disorder results from a toxic effect of the mutant protein, a so-called "gain of function." The gene controls the synthesis of

huntingtin, a large protein of more than 3,100 amino acids that bears no close similarity to any other protein [27]. The ubiquitous expression of htt in nearly all tissues and its widespread localization at the subcellular level also make it difficult to determine its function. Htt is associated with a variety of organelles, including the nucleus, endoplasmic reticulum, Golgi complex, synaptic vesicles and mitochondria [28–30]. An expansive repertoire of proteins have been shown to interact with wild-type htt, including transcription factors, proteins involved in endocytosis, synaptic vesicle recycling, intracellular transport and signalling, cellular metabolism [31]. The identification of these htt-interacting proteins suggests that htt might function as a scaffold involved in orchestrating sets of proteins for signaling processes and intracellular transport [32]. The polyQ expansion in the N-terminal of htt, which is responsible for the disease, dysregulates those interactions and promotes the abnormal binding to a variety of proteins. Indeed, in gel filtration and Western blot studies under non-denaturing conditions htt migrates in a large (>1000-kDa) complex, indicating the possibility of many direct or indirect interacting proteins [33]. Certainly, some htt-interacting proteins are associated with the normal functions of htt, whereas others are involved in pathological events. In any case, wild-type and mutant htt proteins are both expressed widely, inside and outside the nervous system, providing no obvious basis for the specificity of neuronal death [27]. However, there are significant physical differences between the two proteins. The mutant version displays a disproportionate decrease in electrophoretic mobility in SDS polyacrylamide gels due to the expanded glutamine tract, that begins 17 amino acids after the initiator methionine, in the extreme amino terminal region, suggesting an unusual conformation in this region [34]. The first experimental clues that the novel property of mutant htt can indeed promote self-aggregation came from transgenic mice overexpressing only exon 1 of the HD gene with an extreme CAG repeat [35]. Subsequently, anti-htt-reactive nuclear inclusions were detected in HD post-mortem brain [36]. Thus, it has been proposed that the HD mutation, by virtue of the expanded glutamine tract, confers a new and deleterious physical property on mutant htt [37]. But impairment of wild-type htt function may also contribute to the disease [38]. Htt is a cytoplasmic protein, but ubiquitinated, mutant, proteolytic N-terminal htt fragments form protein aggregates in the cytoplasm and nucleus of neurons. Mutant htt causes misfolded protein stress. Htt interacts with huntingtin-associated protein, a protein selectively expressed in the striatum, and glyceraldehyde-3-phosphate dehydrogenase, an enzyme essential for glycolysis, and other proteins (huntingtin-interacting proteins 1 and 2) [31]. Htt may also interfere with the function of postsynaptic density protein-95, a scaffolding protein associated with NMDA and kainate receptors, rendering these glutamate receptors hypersensitive [31]. Mutant htt also interferes with gene transcription, leading to an alteration in cell phenotype and disrupting many cell functions. Mutant htt, for example, may block the normal function of htt to upregulate brain-derived neurotrophic factor (BDNF) [39]. Nevertheless, whereas pathogenesis is clearly triggered by the presence of the abnormal glutamine tract in each CAG disorder, the disease pathway in HD as in each CAG disorder may begin differently, a) through an effect on the function of the host

protein, b) through an effect on existing or new interactions of the full-length host protein, or c) through the process or consequences of forming insoluble polyQ amyloid [40]. However, once neuronal homeostasis has been disrupted, it is likely that many other downstream changes occur. These changes may be shared across diseases, may be polyQ-independent, and some of them lead to cell death. Furthermore, the selectivity of neurodegeneration in HD, accompanied by observations that htt is widely distributed in the brain and in other compartments, suggests that the typical pattern of neuronal death is not only dependent on the mutated protein, but also reflects differences in the distribution of protective agents, such as trophic factors, free radical scavengers, Ca⁺⁺ binding proteins, and susceptibility to damaging processes, including decreased cellular respiration, decreased glucose availability and raised extracellular glutamate levels [9].

THERAPIES

To date pharmacological approaches to HD have produced very scant improvements, resulting in a slight symptomatic relief in the complex clinical course of affected patients [41]. Like other neurodegenerations, many potential types of interventions might prove useful, such as blocking transcription of the mutant gene, enhancing chaperone function, interfering with association and aggregation of the protein, improving cell bioenergetics and mitochondrial integrity, and interfering with the triggers of and the ultimate steps in the process of apoptosis. Other possibilities include the identification of molecules that mimic wild-type htt activity, upregulate wild-type htt or even alleviate excitotoxicity. Aspects of the human neuropathology can be well modeled by excitotoxic or metabolic lesions in experimental animals and in transgenic mice carrying the htt mutation, providing the basis for testing alternative therapeutic strategies. Recent progress shows that neurons suitable for transplantation can be generated from stem cells in culture, and that the adult brain produces new neurons from its own stem cells in response to injury. These findings raise hope for the development of stem cell therapies in human neurodegenerative disorders. Grafting immature neuronal precursor cells into the HD striatum, in order to restore functional circuitry, is currently a goal of a number of research programs.

MOLECULAR THERAPY

Excitotoxicity

The selective pattern of neurodegeneration observed in HD have stimulated the idea that endogenously produced excitatory amino acids (EAAs), or closely related substances, physiologically involved in neurotransmission, damage and kill neurons that are chronically exposed to their effects [6]. Neurons affected are those which possess receptors for EAA neurotransmitters, including the N-methyl-D-aspartate (NMDA), the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and the kainate receptor. EAA receptors are abundant in the striatum, and NMDA, AMPA, and kainate binding are significantly decreased in HD patients [42]. Some of the first evidence that overactivity of the glutamate neurotransmission system could lead to

neuronal death followed from observations that administration of glutamate receptor agonists reproduces certain neuropathologic features of HD [43, 44]. Indeed, excitotoxicity and metabolic impairment have been used extensively as models of HD, and they have helped to characterize the trophic requirements of the different neuronal populations in the striatum. Consistent with this hypothesis, the selective neuropathology seen in the human HD brain can be mimicked in experimental animals by direct striatal injection of excitatory amino acids such as quinolinic acid (QA) [44, 45].

Recently, several studies have linked htt with neurotrophic factors and excitotoxicity. Htt seems to regulate transcription of BDNF and could be relevant for maintaining corticostriatal connectivity. In transgenic HD mice, a decrease in BDNF with a concomitant increase in the sensitivity of NMDA-mediated excitotoxicity has been demonstrated [46, 47]. Moreover, excitotoxicity is able to regulate BDNF expression in cortical and striatal neurons [48, 49].

The endogenous expression of neurotrophic factors and receptors within the striatum and their altered expression in the HD human brain [50] and in response to excitotoxic striatal lesioning [51, 52] have led to *in vivo* studies investigating whether increased exposure of striatal neurons to neurotrophic factors may provide neuronal protection in animal models of HD. Using the QA lesion rodent model of HD, various neurotrophic factors have been shown to effectively prevent the loss of striatal GABAergic medium spiny projection neurons. Nerve growth factor (NGF) [53-55], ciliary neurotrophic factor (CNTF) [56-59], insulin-like growth factor-I (IGF-1) [60], and activin-A [61] have all been demonstrated to protect only partially GABAergic striatal projection neurons from excitotoxic cell death *in vivo* after their intraparenchymal administration or after the implantation of engineered cells producing these factors (see the "In vivo drug delivery" section).

Several observations have suggested that BDNF and glial cell line-derived neurotrophic factor (GDNF) may also protect vulnerable GABAergic striatal neurons from cell death. *In vitro* studies have demonstrated that BDNF is a potent neurotrophic factor for GABAergic striatal neurons [62, 63] and has been shown to protect GABAergic striatal neurons against excitotoxic stress [64] and to inhibit apoptosis in striatal cells engineered to express htt with an expanded trinucleotide CAG repeat sequence [65].

However, despite the promising neuroprotective actions of BDNF observed *in vitro*, both direct infusion of BDNF protein into the rat striatum [59] and the localized striatal production of BDNF following *ex vivo* gene delivery [53, 66] have failed to effectively protect striatal neurons against QA lesioning. GDNF has also been shown to provide striatal neurons with neuroprotective support against excitotoxic lesioning, although these previous studies examining both intraventricular GDNF protein infusion [67] and *ex vivo* gene delivery [68, 69] of GDNF have demonstrated only partial protection of the GABAergic striatal neurons from QA-induced cell death. These observations suggest that, while neurotrophic factors such as BDNF and GDNF may provide trophic support for striatal neurons, difficulties in

achieving efficient delivery of these factors to striatal neurons *in vivo* may be limiting the true potential hold by these factors in counteracting the neurodegenerative process of HD. Indeed, the need for continuous, long-term supply of neurotrophic factor expression across the entire striatum makes the delivery mechanism of central importance for the treatment of HD. This challenge may be met by the use of *in vivo* gene delivery that allows for the continuous and targeted biosynthesis of neurotrophic factors in the striatum, thereby increasing their bioavailability to selectively vulnerable striatal neurons (see the “*In vivo* drug delivery” section).

Metabolic Toxicity

A number of lines of evidence point to impaired mitochondrial function in HD, including abnormalities in complex I, II, III, and IV in caudate nuclei of affected brains [70]. PET studies show reductions in striatal glucose metabolism and loss of dopamine D2-receptor-bearing neurons in the striatum and MRS studies have suggested increased brain lactate levels [71, 72]. Systemic administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) models the disease in animals. Garcia and colleagues describe activation of the c-Jun N-terminal kinase (JNK) pathway during chronic 3-nitropropionic acid infusion leading to dorsolateral striatal cell death. In addition, they found that the activation of JNK pathway caused phosphorylation of c-Jun *in vivo* and *in vitro* models support the role of phosphorylated c-Jun in causing selective striatal cell death [73]. Intrastriatal administration of the excitotoxins kainate and QA also reproduces the striatal lesions of HD. One theory that ties these animal models together is that of indirect excitotoxicity. Mitochondrial energy failure increases the vulnerability of the cell to excitotoxic injury because the resulting change in cell membrane potential results in loss of the magnesium ion from the NMDA-receptor-associated ion channel, allowing ligand-associated depolarization of the postsynaptic receptor and excitotoxic-mediated damage. A model that has attracted increasing attention is the use of toxins such as 3-NP or malonate that disrupt mitochondrial energy metabolism, leading to striatal cell death and functional deficits [74, 75] by a mechanism that may reflect similar deficits in cellular metabolism observed in HD [70].

For this reason molecules that function to increase energy by boosting ATP stores such as creatine, carnitine and cyclocreatine have been studied for their potential neuroprotective effects. In rats treated with 3-NP, oral creatine supplementation produced significant protection against malonate- and 3-NP-induced lesions [76].

In addition, transgenic HD mice given oral supplementation with creatine exhibited improved survival, delayed atrophy of striatal neurons and delayed formation of huntingtin-positive aggregates [77].

These data support a role for metabolic dysfunction as a pathogenic component of HD and suggest a potential role for ATP repletion via creatine supplementation of compounds related to mitochondrial energy production as a therapeutic strategy. Human clinical trials of creatine in HD patients are underway.

Apoptosis and Transglutaminase

Currently, there are only few evidences about the role played by apoptosis in human HD, although the demonstration that inhibiting caspase-1 could extend lifespan in transgenic HD mice gave credence to the idea that apoptosis was a legitimate target for therapeutics [78]. DNA fragmentation characteristic of apoptosis has been described in HD brain [79-81]. However, it is not yet clear whether apoptosis is the primary pathologic event, or merely the final mode of exit for neurons, which have been damaged by some other process. Minocycline, a second-generation tetracycline used in humans, which effectively crosses the blood-brain barrier, was demonstrated both to inhibit caspase-1 and inducible nitric oxide synthetase (iNOS) upregulation, and to decrease the infarct size after experimental ischemia [82, 83]. As a detrimental role for caspase-1 and iNOS has been proposed in HD, recent researches evaluated minocycline using the R6/2 HD mouse [78, 84, 85]. The results showed that in R6/2 mice, minocycline delayed disease progression and death, as well as inhibited caspase-1 and iNOS activation. In addition, caspase-1 and caspase-3 gene expression, which is upregulated in this mouse model, is remarkably inhibited by minocycline [86].

Cystamine, a caspase inhibitor, has also been studied in transgenic HD mice. Cystamine was originally tested as a therapeutic candidate given its ability to inhibit transglutaminases. Transglutaminases have been implicated as a possible mechanism of aggregate formation, by possibly cross-linking molecules of mutant htt. Because mutant htt aggregation appeared to correlate with disease phenotype, a transglutaminase inhibitor such as cystamine was tested for its potential ability to reduce aggregate formation and, in turn, extend survival [87]. Both oral and intraperitoneal administration of cystamine extended survival in transgenic HD mice and reduced the number and size of aggregates [88, 89]. Recently, cystamine has been found to inhibit caspase-3 activity *in vitro*, suggesting that cystamine may work through a variety of mechanisms, including caspase inhibition, to prolong neuronal survival in HD [90].

Transcriptional Dysregulation

The role of transcriptional dysregulation in HD has been an exciting area of research over the last few years. New studies indicate that transcriptional repression is the main result of transcriptional dysregulation and could be explained by the recruitment and sequestering of transcription factors by mutant htt [91]. These new findings have potential therapeutic implications.

Recently, it has been found that a fragment of mutant htt interacts directly with CREB-binding protein (CBP), which contains an acetyltransferase domain and is a co-activator of numerous promoters [92]. Moreover, the polyglutamine-containing domain of htt, Htt exon 1 protein (Httex1p), directly binds the acetyltransferase domains of two distinct proteins: CBP and p300/CBP-associated factor (P/CAF) [93]. In cell-free assays, Httex1p also inhibits the acetyltransferase activity of at least three enzymes: p300, P/CAF and CBP. Expression of Httex1p in cultured cells reduces the level of acetylated histones H3 and H4, and this reduction

can be reversed by administering inhibitors of histone deacetylase (HDAC). *In vivo*, HDAC inhibitors arrest ongoing progressive neuronal degeneration induced by polyQ repeat expansion, and they reduce lethality in two *Drosophila* models of polyQ disease [93]. These findings raise the possibility that therapy with HDAC inhibitors may slow or prevent the progressive neurodegeneration seen in Huntington's disease and other polyglutamine-repeat diseases, even after the onset of symptoms [94]. As matter of fact, Zuccato *et al.* (2003) found that the transcriptional effects of wild-type and mutant htt on the BDNF promoter are mediated through the neuron-restrictive silencer element (NRSE) [39], a silencer of gene expression typical of several neuronal genes [95-97]. So, wild-type htt acts in the cytoplasm of neurons to regulate the availability of repressor element-1 transcription factor (REST)/NRSF to its nuclear NRSE-binding site and that this control is lost in the pathology of HD [39]. The authors suggest that an alternative strategy to reducing HDAC activity in general is to target REST/NRSF activity so as to restore neuronal gene transcription in the disease.

Finally, another recent study tested the effects of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) in a transgenic mouse model of HD [98]. Mice treated with SAHA demonstrated improved motor impairment and less striatal neuronal loss, though there was no significant effect on weight or polyglutamine aggregation [98].

CELL THERAPY

The efforts based on the use of cells of different origin for the production of neuroprotective compounds or for the direct replacement of neurodegenerating neurons, fall into the generic definition of "cell therapy". The beneficial effect of cell therapy is explained by two strategies facing different aspects of the disease: the *in vivo* drug delivery approach aims to interfere with the ongoing neuronal degeneration, trying to protect cells destined to die by direct supply of neuroactive compounds, while the cell replacement strategy is intended to reconstruct brain neural circuits exploiting the plasticity of neural cells, with all the limits imposed by the complexity of human brain cytoarchitecture and interconnections. It is clear that the combination of these approaches by an overlap between cell therapy, gene therapy, tissue engineering, and regenerative medicine would be of extreme interest and could provide HD patients the means to slow the progression of the disease and to recovery, at least in part, their motor, cognitive and psychiatric functions. From the first attempt of neural grafting at New York University - cerebral cortex derived from adult cats into adult dogs - that was unsuccessful, neural transplants have become clinically relevant over the past 17 years for two major neurodegenerative diseases, namely Parkinson's disease (PD) and HD. [99, 100]. The efficacy of cell therapy approaches to HD has been tested on animal models and clinical trials are underway [101].

Cell Replacement: Striatal Grafts

The hypothesis that grafts of fetal striatal tissue could survive, differentiate toward a functional phenotype and be integrated into the host circuitry has been validated by

several studies in mice, rats and non-human primates [102-106]. This level of reconstruction of corticostriatopallidal circuitry is sufficient to reverse motor and cognitive deficits in rats and monkeys [106-108]. Transplantation of human striatal neurons in QA treated rat models of HD were then demonstrated to fill a substantial portion of the lesioned striatum with both mature and immature cells and to display heterogeneous acetylcholinesterase activity. Moreover NADPH-diaphorase positive cells of donor origin were detected by histochemistry and host afferent dopaminergic fibres penetrating into the transplant were observed [109]. The migration of primary neural cells from human striatal graft in an athymic rat model of HD has been shown in a xenograft situation: cells stained with markers to detect human neurons and astrocytes were found significantly dispersed throughout the brain six month after transplantation [110]. The authors speculated that this observation could be a consequence of the xenograft situation and that this property may be of interest to reach regions of distant pathology in neurodegenerative diseases [110]. Further improvements in fetal striatal graft techniques came from studies showing that, although the highest proportion of striatal-like cells is obtained from grafts comprising the lateral ganglionic eminence (LGE) [111], grafts deriving from the whole ganglionic eminence (WGE) show the largest volume of striatum-like tissue, more striatal neurons respect to grafts deriving from the LGE [112, 113] and result in better cell survival, indicating that medial ganglionic eminence (MGE)-derived cells may provide trophic support [114]. Moreover the age of donors also seems to be crucial, with optimal results occurring when donor ages range from Stage 19 to 23 [115]. Several studies showed that fetal striatal grafts indeed promote recovery from motor and cognitive deficits associated with cytotoxic striatal lesions in rat models of HD [116-121], with behavioural recovery possibly being influenced by postoperative training-mediated host-graft connections [122]. Intrastriatal grafts were also shown to ameliorate the cortical atrophy observed in later stages HD patients [123]; whether this cortical neurodegeneration is secondary to striatal atrophy or is a direct consequence of the disease is not yet clear.

However, experiments in transgenic mouse models of HD produced poor results: although surviving and differentiating into the host tissue, nor striatal [124] neither cortical grafts [125] did promote clinically relevant behavioural benefits. This is probably due to the fact that mouse transgenic models show a pattern of neurodegeneration more severe and widely distributed than the human pathology [119].

The first long-term demonstration that striatal allografts could survive, differentiate and integrate in the host striatum in a non-human primate model of HD came from the group of Dunnett, which also described a recovery of motor skills [107]. Then, Palfi *et al.* corroborated the hypothesis that fetal striatal grafts could promote improvements in motor and cognitive deficits of HD primate models [108]. Primary human fetal striatal cells were also demonstrated to survive, migrate and differentiate into both neurons and glia when used for xenograft experiments in the adult rat central nervous system [110].

Useful noninvasive methods to study the development of striatal grafts, such as clinical MR scan [126], and techniques enabling to store transplantable tissues in hibernation medium as long as 8 days without loss of viability and consequently without differences in terms of graft volume and expression of striatal phenotypic markers after transplantation [127] gave further input to the growing field of fetal tissue transplantation.

Based on the encouraging results obtained in different animal models, several clinical trials have been undertaken [120, 128]. Most of these trials demonstrated the feasibility of human fetal striatal transplantation in patients with HD and reported on safety of the graft procedure. Except for one study, where three patients with a moderately advanced stage of the disease developed subdural hemorrhages after surgery [129], no serious side effects, which were mostly related to the immunosuppression therapy associated with tissue implantation, were described [119, 130-133]. The grafts survived without typical pathology, contained striatal projection neurons and interneurons, and received afferents from the patient's brain [130]. In one trial, an HD patient who had received a fetal cell transplant died of presumably unrelated causes 18 months later. Autopsy analysis demonstrated that the implanted cells had survived, expressed appropriate neurochemical markers, and received innervation by host dopaminergic cells, demonstrating that transplanted tissue could persist for 18 months [130]. In another study, grafting fetal neuroblast cells into 5 patients with HD appeared to improve motor and cognitive function in 3 [134].

Although slightly differing in some parameters of the transplantation procedure (age of the donor, dissected tissue, implantation track, immune treatment), and the limited number of patients involved in the studies, MRI and PET imaging clearly demonstrated that grafted human fetal striatal tissue survives, grows and is integrated in the host brain of HD affected patients [119] and does not show protein aggregates of mutated huntingtin, typical of HD pathology [130]. Furthermore, clinical improvement is associated with reduction of striatal and cortical hypometabolism, suggesting that the grafts restore function in striato-cortical neural loops [135]. However, the efficacy of transplantation in promoting improvements in the clinical course of HD patients is still on debate, with some studies reporting motor and cognitive benefits or stabilization, associated with increased metabolic activity in the striatum of some patients [134, 136], whereas the outcome was unchanged in another experimentation [129].

The use of human fetal brain for transplantation, mostly deriving from elective abortions, is limited by ethical, practical and regulatory concerns and is dependent upon availability of donor tissue. In an effort to circumvent these drawbacks, Fink and collaborators performed clinical trials with porcine xenografts [137]. However, the procedure, which was shown to be safe, did not produce improvements in the clinical outcome of 12 HD patients [137].

Besides the problems correlated to the limited supply of human fresh fetal tissue, the strategy of striatal transplantation is further complicated by the lack of standardization inevitably correlated with the use of such a source [138]. For these reasons, other cell therapy approaches are currently

under investigation, based on the *in vitro* amplification and subsequent differentiation of stem cells and neural precursors.

Cell Replacement: Stem Cell Transplantation

Substantial benefit following cell therapy will require that many more grafted striatal neurons survive than the low numbers achieved in the trials with fetal tissue [130]. The stem cell technology could markedly increase the availability of such cells. The ideal candidate for stem cell transplantation would be a self-renewing cell population of easy access, amenable for *in vitro* amplification and readily prone to differentiate toward the desired phenotype. The search for such a population of stem cells is a growing field of scientific investigation and holds encouraging promises for future therapeutical applications [101].

Stem cells in mammals have been isolated both from the embryo and the adult. Human embryonic stem cells are pluripotent and have been demonstrated to be amenable to differentiation toward almost all the cell types of the organism. Their use for scientific purposes, however, has raised major ethical concerns and is forbidden in several countries. On the other hand, adult stem cells are in some way committed toward specific cell fates that usually reflect the phenotypes present in the tissue where they reside. Nevertheless new evidences have suggested that various populations of adult stem cells possess an unexpected capacity to be switched toward differentiated phenotypes not related to their tissue of origin [139]. This somatic plasticity could be exploited for cell therapy approaches.

Neural Stem Cells

The main goal in stem cell transplantation for HD is to obtain cells that acquire a striatal phenotype. Intuitively, stem cells of neural origin would be very useful for this purpose, even because the default pathway of neural stem cell differentiation *in vitro* seems to be biased toward the formation of GABAergic neurons [101].

Neural stem cells (NSCs) are referred to as cells derived from the nervous system that have some capacity for self-renewal and can give rise to cells other than themselves through asymmetric division [140]. NSCs have been found not only in the developing nervous system, but also in the adult nervous system of all mammalian organisms [141-147]. The subventricular zone (SVZ) of the lateral ventricles [141, 148] and the dentate gyrus of the hippocampus have been identified as neurogenic areas in the adult [149, 150] and an astrocytic identity for NSCs has been claimed [151, 152]. Neuroblasts originating from NSCs of the the SVZ migrate along the rostral migratory stream to reach the olfactory bulb [153, 154], where they differentiate into local GABAergic interneurons and are functionally integrated into the local neural network [155].

NSCs can be amplified in culture by epigenetic or genetic means. NSCs can then be induced to differentiate into neurons, astrocytes and oligodendrocytes, the percentage of which is influenced by several parameters, namely specie and age of the donor, region of origin, growth factors added, time in culture, culture density [156-161]. Doetsch *et al.*

observed that EGF is able to convert neurogenic precursors into multipotent stem cells [162] and studies on cultured NSCs have shown that growth factors can expand the differentiation potential of the cells [163]. Nevertheless it has been demonstrated that NSCs in culture maintain a certain degree of temporal and regional specification [164, 165]. Therefore, there is an intrinsic heterogeneity of the culture that should be taken into account when planning large-scale therapeutic experimentations. To avoid senescence of long-term grown NSCs, cellular immortalisation has been achieved by ectopic expression of proteins involved in cell-cycle regulation. The most extensively used technique is based on the transduction with replication-defective retroviruses driving constitutive or regulated expression of an oncogene. NSCs lines immortalised by expression of v-myc have been created [166, 167]. These cell lines have proven to be not tumorigenic and are able to differentiate upon transplantation [168].

While retaining a certain degree of heterogeneity that underscores the need for careful examination of each cell line, NSCs offer the advantage to be amenable to extensive propagation and to induction of the desired phenotype. Such characteristics have been exploited to drive tests on animal models of HD to ascertain the possible use of NSCs for cell therapy.

Differentiation of neural progenitors is a tightly regulated process, in which extrinsic epigenetic factors and intrinsic cell-autonomous mechanisms are integrated in a specific temporal pattern [169]. In this context, the creation of artificial extracellular matrix proteins represents a potentially powerful tool to custom design artificial niches to strategically control stem cell behaviour [170].

Whether differentiation should be induced *in vitro* prior to transplantation or the acquisition of a fully differentiated, functional striatal phenotype would be more effective when immature cells are influenced by the local microenvironment of the host brain is still an open question. This is a crucial issue, since differences in the maturation stage likely have major influences on the ability of grafted cells to survive, migrate, differentiate and establish functional connections with the host tissue [171]. Indeed, studies in a rat model of Parkinson's disease underscored the necessity of pre-differentiation of transplanted neural progenitor cells into dopaminergic neurons to alleviate behavioural impairments of the diseased animals [172], whereas other experiments suggest that region-specific differentiation of multipotent neural progenitors may be supported by local cues also in regions outside the neurogenic zones, at least under injury conditions [173-175].

Fricker *et al.* showed that neural progenitors, obtained from the embryonic human forebrain and transplanted into neurogenic zones of the adult rat brain are integrated into the host tissue and migrate along the routes normally taken by endogenous progenitors. Moreover these cells displayed site-specific differentiation into both neurons and glia also within non-neurogenic regions, thus demonstrating their ability to respond *in vivo* to guidance cues and local differentiation signals [176]. Armstrong *et al.* then transplanted epigenetically propagated human neural precursors into a rat model of HD. Grafted cells survived transplantation and differentiated

into neuronal phenotypes, with some cells resulting positive for DARPP-32, a marker expressed by mature striatal neurons [177]. Researchers of the University of Barcelona took advantage of an immortalized striatal neural stem cell line to transplant predifferentiated GABAergic neurons into the adult brain of a rat model of HD [178]. This immortalized cell line, named ST14A, was created by retroviral transduction of the temperature-sensitive SV40 Large T antigen into primary cells derived from the primordial of embryonic rat striatum. The immortalizing oncoprotein promotes cell proliferation at 33 degrees, whereas it undergoes a conformational change that makes it unable to induce cell division at the nonpermissive temperature [179]. A nearly homogeneous population of functional GABAergic neurons was generated in culture by treatment with retinoic acid and KCl depolarisation, which induced neurite outgrowth and expression of GABAergic markers along with GABA release and uptake. The cells were then transplanted into QA lesioned rat striata, where they survived, conserved their GABAergic phenotype and developed neuritic processes with synaptic contacts with endogenous neurons [178]. These observations are encouraging, but do not analyse the therapeutic effect of grafted cells on motor and cognitive dysfunctions of the animal models. Two recently published papers report distinct conclusions on this crucial topic: Ryu *et al.* studied the effects of human NSC transplantation into the striatum of rats treated with the metabolic toxin 3-NP [180]. The authors showed that animals receiving intrastriatal cell implantation one week before the induction of the lesion display improved motor performance and reduced damage to striatal neurons respect to control animals, whereas these benefits disappear if transplantation is performed twelve hours after 3-NP administration. A protective role for BDNF, which is secreted by grafted cells, is also proposed [180]. On the contrary, another study demonstrated that transplanted human NSCs elicit behavioural and anatomical recovery in rat models of HD in which the striatum was lesioned with QA one week before NSCs transplantation. McBride *et al.* showed that undifferentiated or CNTF pretreated human NSCs are able to survive in the host brain and migrate toward areas of the basal ganglia receiving striatal projections. The treatment resulted in better performances in the cylinder test 8 weeks after transplantation and in increase in the striatal volume of grafts receiving animals respect to controls [181].

These discrepancies underscore that different outcomes are strictly dependent upon the grafted cell line and the type of lesion. For these reasons, thorough characterization of the cells to be used and careful evaluation of disease-related neuropathology are necessary for planning therapeutic interventions based on cell transplantation.

The existence of adult NSCs has also prompted researchers to investigate the endogenous ability of the brain for regeneration. A recent study points to an unsuspected source of neural stem cells: the Huntington's disease brain itself. In fact, Curtis *et al.* [182] demonstrated enhanced proliferation of cells stained for both neural and glial antigens in the subependymal layer adjacent to the caudate nucleus in postmortem human brains from HD patients respect to control brains. Furthermore, the degree of cell proliferation increased with pathological severity and with increasing

CAG repeats in the HD gene [182]. These observations raise the possibility that the HD brain itself could be coaxed into generating replacement neurons, especially if the rate of disease pathogenesis could be slowed by other means.

Other Sources of Cells for Transplantation

In the need to obtain huge quantities of transplantable neural cells, researchers have explored the possibility to turn other cell types into neurons. These studies were focused both on immature multipotent embryonic stem cells and on adult stem cells from non-neural districts showing the ability to be reprogrammed toward cells with neural properties.

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the pre-implantation blastocyst. ES cells are maintained in culture in an immature state in the presence of leukaemia inhibitory factor (LIF) (at least for ES cells of rodent origin) or with a feeder layer of embryonic fibroblasts, while they differentiate and form embryoid bodies when this support is withdrawn [183]. Cell populations highly enriched/homogenous in neural precursors have been achieved using a variety of chemical or biological inducing agents coupled with selective growth conditions. Methods that promote the differentiation toward a specific neuronal population or that selectively expand neural precursors present in culture have been claimed, while other approaches are based on the introduction of a fluorescent marker or a drug resistance into a gene that is expressed only in neural precursors and selection of the population of interest [184-188]. Mouse embryonic stem cells treated with retinoic acid (RA) have been shown to differentiate into highly enriched populations of GABAergic neurons [189]. These cells have been transplanted into the striatum of QA treated rat models of HD, where they have been reported to survive for up to six weeks and integrate into the host tissue [189].

Primordial embryonic germ (EG) cells obtained from the developing gonad are other promising multipotent cells, that form embryoid bodies in culture and differentiate into cell types of all three germ layers [190]. Cell lines predominantly expressing markers of the neural lineage can be obtained from EG cells, thus enabling the hypothesis of a clinical use of this population [191].

In order to remove any possibility for tumorigenesis, however, therapeutic use of ES and EG cells should be carefully checked to ensure that all cells are terminally differentiated.

Human embryonal carcinoma cells from the Ntera-2 cell line were also tested for transplantation, since they can be induced toward terminally differentiated neurons by treatment with RA [192]. Purified neurons from this cell line, shown to display biochemical properties similar to fetal striatal tissue, were transplanted into unilateral QA-lesioned rat striatum and promoted improvements in motor skills of treated animals [193].

Several studies reported the transdifferentiation of adult stem cells into phenotypes peculiar of cells of different tissues and even different germ layers [139]. Some researchers have also claimed that a stem cell should not be thought as a discrete entity but should rather be considered a

dynamic functional status that virtually any cell could reach in the proper environmental conditions and that undergoes a progressive but reversible restriction as differentiation proceeds [194].

Among adult stem cells, marrow stromal cells (MSCs) have been extensively studied, due to the ease by which they can be harvested and propagated in culture. MSCs usually differentiate into cell types of mesenchymal origin, such as cartilage, muscle, bone, fat [195]. However, it has been demonstrated that MSCs can be induced to differentiate into neurons, astrocytes and oligodendrocytes both *in vitro* [196-202] and *in vivo* [203-206]. Therefore, Lescaudron *et al.* transplanted autologous MSCs in the QA-lesioned striatum of rat models of HD and observed a reduction of cognitive deficits [207]. However, this beneficial effect was not ascribed to differentiation of MSCs into neurons, since the majority of the grafted cells displayed an immature phenotype, but rather to the release of surviving factors that promoted compensatory responses [207]. Similarly, Sertoli cells, which have immunosuppressive and trophic properties, were demonstrated to ameliorate locomotor abnormalities in the 3-NP treated rat model of HD [208].

Human umbilical cord blood (HUCB) cells have also been shown to be amenable to neural differentiation [209]. Based on these findings, experiments were carried out to study their behaviour when injected into the brain. Walczak and colleagues observed that HUCB cells grafted into the SVZ of rat brains migrated along the rostral migratory stream and a few cells expressed markers of endogenous neural progenitors [210]. Moreover, transplanted HUCB cells were shown to significantly increase the life span and decrease the rate of weight loss in a transgenic mouse model of HD [211].

IN VIVO DRUG DELIVERY

The idea that neuroactive compounds could rescue neurons from death has been pursued by several groups and has led to a large body of evidence that this therapeutical approach indeed holds promises for the future.

Since the precise molecular pathways that promote the pattern of striatal and cortical degeneration observed in HD patients are still largely unknown, in an effort to diminish ongoing neurodegeneration and/or promote regeneration scientists have driven their attention to neurotrophins and cytokines, substances known for their trophic and survival activity on cells of the nervous system [212].

These compounds are naturally occurring peptides that accomplish their biological function through interaction with specific receptors. Thorough studies have investigated the pattern of expression of neurotrophic factors and their receptors in both physiologic and injury conditions [212], thus providing the basis for a rationale approach to experimentation in preclinical studies on animal models of HD.

However, attempts to supply these factors through systemic administration are not practicable since, besides potential side effects, these peptides do not cross the brain-blood barrier, thus evidencing the need for direct and continuous administration of the neuroactive compound into the lesioned site of the brain. Therefore, alternative delivery

systems have been developed, based on transplantation of cells engineered to allow synthesis and secretion of therapeutically relevant substances or direct injection of viral vectors to induce overexpression of these compounds in endogenous cells. These methods are designed to improve spatial spread and long term delivery of the compound of interest. Different strategies emerge from a review of the literature: some researchers have developed viral vectors to ensure ectopic expression of the substance of interest in the degenerating area, while others focused their efforts to produce engineered cell lines to promote expression and release of the trophic factor. Each approach has some drawbacks. For example viral vectors are often toxic and expression of the exogenous protein may be difficult to regulate, while grafted cells can be rejected or expression of the transgene downregulated. Therefore, technical improvements are being ideated to direct the choice toward an effective strategy. New generation viral vectors may offer more safety and the introduction of systems of regulated expression could permit standardized intracerebral gene transfer. On the other hand, defined properties of transplanted cells are being exploited. For example, NSCs could be of interest for their ability to migrate through the brain, their proposed tropism for areas of damage and their possible integration into the normal cytoarchitecture of the brain, which could result in delivery regulated by physiological mechanisms [156]. Instead, to avoid immune responses and risks for tumour production, other researchers have managed to isolate grafted engineered cells from the host tissue in semi permeable structures that only allow diffusion of soluble molecule.

Early experimentations were based on the well-known activity of NGF to promote survival and differentiation of several populations of neurons in the central and peripheral nervous system [213] and on previous work indicating that intracerebral infusion of NGF into the rat striatum displayed a protective effect against excitotoxic damage [214]. The group of Isacson developed a delivery system involving fibroblasts engineered to secrete high levels of NGF, implanted into the striatum before infusion of excitotoxic compounds, such as QA and quisqualate [54]. The authors observed smaller lesions in the treated animals [54], but also demarcated the spontaneous decrease of *in vivo* peptide synthesis by grafted cells [55]. Subsequently, NGF was shown to protect against neuronal death by inhibiting the production of oxidative agents in the 3-NP rat model of HD [215]. Then, NSCs genetically modified to produce NGF transplanted to the striatum of rats one week before the injection of QA were proven to induce a reduction of the lesion size and of the loss of DARPP-32 positive neurons [53]. Similar results were obtained by grafting EGF responsive NSCs, derived from transgenic mice carrying the coding sequence for NGF under the promoter for the glial fibrillary acidic protein (GFAP), in rats receiving intrastriatal injection of QA nine days posttransplantation [216].

Other peptides of the neurotrophin family were also tested for neuroprotection in HD animal models. BDNF was proven to be effective against QA-induced striatal lesions, as revealed by smaller size of the lesion and increased survival of DARPP-32 positive striatal output neurons [217]. In this work, BDNF expression was induced by stereological

injection of adenoviral vectors two weeks before the administration of the toxin [217]. While a previous work had shown that BDNF secreted by genetically modified NSCs in the striatum of rats one week before the injection of QA into the same area resulted only in mild protection [53], other researchers confirmed the beneficial effect of BDNF in protecting from excitotoxic striatal injury *in vivo* and compared it to the effects of the other neurotrophins NT-3 and NT-4/5 in an experimental framework where the neurotrophins were secreted by transplanted engineered cell lines [218, 219]. The authors studied the effects of the neurotrophins on the phenotype of striatal projection neurons before the induction of the lesion, finding that BDNF, NT-3 and NT-4/5 differentially regulate the soma area of prepro-tachykinin A, preproenkephalin and prodynorphin positive neurons, without affecting the levels of their mRNA levels. Moreover, they demonstrated that transplantation of cell-lines secreting these neurotrophins differentially protect striatal projection neurons from subsequent degeneration induced by QA injection: while BDNF increased the survival of all types of striatal projection neurons, NT-3 and NT-4/5 were not effective in protecting prodynorphin-expressing neurons [218, 219]. The rationale for these results was then suggested by studies that highlighted the correlation between BDNF expression and mutant htt. Ferrer *et al.* showed that BDNF expression is reduced in the caudate and putamen of patients affected by HD [50], then Zuccato and colleagues demonstrated that wild-type htt up-regulates transcription of BDNF in cortical neurons, while the mutated form of the protein loses this activity, which results in an insufficient neurotrophic support for striatal neurons [46].

Studies similar to those performed with BDNF showed that GDNF and the related factor neurturin (NRTN) also possess specific protective activity for striatal neurons against excitotoxicity. Direct GDNF infusion before injection of QA into rat striatum was shown to reduce behavioural disabilities and neurochemical deficits [67], while GDNF overexpression, driven by striatal injections of adeno-associated viral (AAV) vectors, reduced motor impairments and promoted cell survival in the 3-NP rat model of HD [220]. Both GDNF and NRTN protected calbindin-immunoreactive striatal projection neurons, but not parvalbumin-immunoreactive striatal interneurons from QA- or kainate induced lesions, when secreted by a cell-line transplanted into the striatum, but only GDNF prevents the decrease of choline acetyltransferase activity [69, 218, 221]. A recent study examined whether enhanced expression of the neurotrophic factors BDNF or GDNF, achieved by *in vivo* gene delivery, could protect striatal neurons in the QA lesion rodent model of HD [222]. In this paper, recombinant AAV vectors for *in vivo* gene delivery to striatal neurons in the adult rat brain were used and the potential for BDNF and GDNF to provide neuroprotection to subpopulations of striatal neurons was evaluated. This is the first study to directly compare the *in vivo* neuroprotective effects of BDNF and GDNF using *in vivo* gene delivery and the results indicate that AAV-mediated gene transfer of BDNF or GDNF into the striatum provides neuronal protection in a rodent model of HD [222].

CNTF is a member of a cytokine superfamily, which also includes IL-6 and LIF, and is expressed by glial cells both in

the central and the peripheral nervous system, promoting survival and differentiation of several neuronal populations [212]. CNTF has been indicated as an important mediator of protection against neuronal damage and its expression is upregulated in astrocytes lining the injury site [212]. Its neuroprotective effect has been demonstrated *in vivo* by direct intracerebral administration followed by striatal injection of QA by Anderson and colleagues, who showed that CNTF promoted the recovery of striatal output neurons, while administration of BDNF, NGF or NT-3 did not provide protective effects [59].

An interesting approach to evaluate the neuroprotective effects of CNTF and to face some of the problems associated to the delivery of neurotrophic factors *in vivo* was described by Emerich *et al.* [57]. The authors used a system in which baby hamster kidney fibroblasts genetically engineered to produce CNTF were encapsulated in a semi-permeable polymer structure that was implanted in the striatum of the disease animal model, before the induction of injury by injection of QA into the same site. This system allowed the passage of CNTF to the lesioned area and the supply of nutrients and oxygen to the encapsulated cells and concomitantly impeded the rejection by the host immune system and any possible risk correlated to tumorigenicity of implanted cells. The neuroprotective effect of CNTF was assessed by the observation that several populations of both striatal and cortical populations destined to die were spared both in rodents [56] and non-human primates [57] receiving striatal injections of QA. An improvement in both cognitive and motor functions was later described in monkey models of HD receiving these implants [223]. A subsequent study performed with the same approach confirmed the protective role of CNTF in the QA rat model of HD, as compared to NT-4/5, which did not produce any improvement in the pattern of neurodegeneration [224]. These encouraging results led to the commencement of a clinical trial based on the use of such encapsulated CNTF secreting cells, the results of which have not yet been published [225].

The potential therapeutic use of CNTF has also been investigated by methods of delivery based on viral vector-mediated overexpression of this neurotrophic factor. Lentiviral vectors driving CNTF expression under the mouse phosphoglycerate kinase 1 promoter produced a decrease in the lesion volume, sparing of several striatal populations and motor function improvements in the QA rat model of HD [58]. This approach was further improved by constructing tetracycline-regulated lentiviral vectors driving CNTF delivery [226].

Similar results were reported with an adenoviral vector-mediated system, which provided widespread, long-term delivery of CNTF into the rat striatum and produced effective neuroprotection against 3-NP induced toxicity [227].

While convincing, all these evidences were obtained in animal models based on the use of toxins that mimic the pattern of neurodegeneration observed in HD. Therefore, Zala and colleagues analyzed the effects of long-term CNTF expression, mediated by striatal injection of lentiviral vectors, in a transgenic mouse model of the disease. The authors observed that expression of CNTF was still observed one year after viral injection and produced complex effects

on different striatal neuronal subpopulations, with decreased expression of DARPP-32 and NeuN and upregulated expression of GFAP. Data regarding beneficial effects of CNTF on HD, however, have still to be evaluated [228].

Interleukin-6 (IL6), a molecule of the same cytokine family of CNTF, was also tested for neuroprotection in the QA rat model of HD [229]. IL-6, or a chimeric molecule in which the cytokine and its soluble receptor are fused (IL6/IL6R), was expressed by injection of engineered lentiviral vectors in the striatum of rats by stereotaxical injections. Three weeks later, QA was administered and the effects of the cytokine were analysed. The chimeric molecule IL6/IL6R and, to a lesser extent, IL6 both promoted a significant decrease of striatal damage, as assessed by the volume of the lesion and the number of surviving striatal interneurons [229].

Attempts to develop new therapies for HD based on the expression of neurotrophic factors has raised new hopes for the treatment of this devastating pathology. However, improved methods are needed to ensure long lasting, regulated delivery of these compounds to specific neuronal populations. The study of the expression of neurotrophic factors and their receptors in physiological and pathological conditions and the elucidation of the survival pathways promoted by these peptides is providing further indications as to which substance could be beneficial for different damages [212]. For this purpose, *in vivo* results on animal models have been of great interest and have clearly demonstrated the effectiveness of several compounds to interfere with the loss of various neuronal subpopulations. However, some discrepancies emerge from the great amount of preclinical data reported and a careful evaluation of differences among protocols should be undertaken to direct the choice toward the most effective substance and delivery method. Precise molecular mechanisms should also be thoroughly investigated before planning human trials.

Most experiments aimed to study the protective effects of neuroactive compounds have been driven in conditions where the neurotrophic agent was administered before the injection of the excitotoxic or metabolic toxin. This experimental design has raised some criticisms, but it should be noted that HD has a progressive clinical course with an onset in middle age and that, after the identification of the responsible genetic defect, the risk to develop the disease can be diagnosed before the onset of symptoms. Thus, this therapeutic approach could be applied in individuals at risk before neurodegeneration and atrophy occur [230].

The combination of *in vivo* drug delivery and replacement of lost cells through transplantation may constitute the ideal therapeutic strategy to slow ongoing degeneration and promote restoration of functional circuitry.

ABBREVIATIONS

3-NP	=	3-nitropropionic acid
AAV	=	Adeno-associated virus
AMPA	=	-amino3-hydroxy-5-methylsoxazole-4-propionic acid
BDNF	=	Brain-derived neurotrophic factor

CBP	=	Creb-binding protein
CNTF	=	Ciliary neurotrophic factor
DRPLA	=	Dentatorubral-pallidoluysian atrophy
EAA	=	Excitatory amino acid
EG	=	Embryonic germ cell
Enk	=	Enkephalin
ES	=	Embryonic stem cell
GABA	=	-aminobutyric acid (GABA)
GDNF	=	Glial cell line-derived neurotrophic factor
GFAP	=	Glial fibrillary acidic protein
HD	=	Huntington's disease
HDAC	=	Histone deacetylase
Htt	=	Huntingtin protein
HUCB	=	Human umbilical cord blood
IGF-1	=	Insulin-like growth factor 1
IL6	=	Interleukin 6
iNOS	=	Inducible nitric oxide synthase
JNK	=	c-Jun N-terminal kinase
LGE, MGE, WGE	=	Lateral, medial, whole ganglionic eminence
LIF	=	Leukaemia inhibitory factor
MRI	=	Magnetic resonance imaging
MSC	=	Marrow stromal cell
NGF	=	Nerve growth factor
NMDA	=	N-methyl-D-aspartate,
NRSE	=	Neuron-restrictive silencer element
NRTN	=	Neurturin
NSC	=	Neural stem cell
NT-3/4/5	=	Neurotrophin 3, 4, 5
P/CAF	=	p300/CBP-associated factor
PD	=	Parkinson's disease
PET	=	Positron emission tomography
QA	=	Quinolinic acid
RA	=	Retinoic acid
REST	=	Repressor element-1 silencing transcription factor
SBMA	=	Spinobulbar muscular atrophy
SCA	=	Spinocerebellar Ataxia
SVZ	=	Subventricular zone

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