Molecular Targets and Therapeutic Strategies in Huntington's Disease

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Abstract: This article provides an overview of the molecular mechanisms associated with striatal neuronal degeneration in Huntington’s disease (HD), the most studied of the diseases caused by polyglutamine expansion. We discuss the current status of research in cellular and animal models of HD, in which protein aggregation, excitotoxicity, mitochondrial dysfunction, transcription deregulation, trophic factor starvation and the disruption of axonal transport appear to be key features for selective striatal neurodegeneration. We further emphasize some of the most promising current strategies in HD treatment. We delineate the molecular and cellular rationale underlying the development of new pharmaceutical interventions that offer new hope of future treatment for HD patients worldwide.

Keywords: Huntington's disease, huntingtin, CAG, neuronal death, protein aggregates, polyglutamine diseases, trinucleotide repeat diseases.

1. REPEAT DISEASES

In the last decade, a new kind of mutation underlying previously unrelated, inherited neurodegenerative disorders was unravelled by molecular genetic analysis [1]. In the human genome there are repetitive sequences of three nucleotides, once considered benign, that above a certain number become pathogenic [2]. The members of this family, called triplet repeat disorders, were first described in 1991 [3]. Since then 15 different representatives have been described [4]. Moreover, an expansion disease with a larger repeat unit (CCCCG) has been described [5]. These disorders are all neurological or neuromuscular and are characterized by somatic and germline instability of the mutation [6]. From generation to generation, there is a tendency towards expansion of the repeat length in paternal transmission. The increase in the repeat number within generations results in earlier onset and increased severity of the diseases, a phenomenon known as anticipation. Simultaneously, an expansion of repeat number is observed in post-mitotic cells in some regions. Although the mechanisms that cause the different diseases remain largely unknown, the repeats may interfere with DNA structure and transcription leading to either a loss or a gain of function of the corresponding transcript [6]. According to the position of the trinucleotide repeats within the human genes, repeat disorders can be classified into two groups: a) diseases with repeats in non-coding sequences and b) diseases with CAG repeats in coding sequences that transcribe and translate into mutant proteins with expanded polyglutamine tracts.

Dysfunction and degeneration in different tissues are observed in diseases with repeats in non-coding sequences. The sequence that is expanded is variable – CGG, GCC, GAA, CTG or CAG - as well as its gene location and repeat numbers. These are generally high, compared to the coding repeat diseases [2]. The repeat nature, number, and protein context affect the mechanism of degeneration associated with each disease.

In 1991, the group of Kenneth Fischbeck first identified a polyglutamine repeat as the cause of a genetic neurological disease - spinal bulbar muscular atrophy (SBMA) [3]. Since then, the number of diseases identified as polyglutamine disorders has been increasing [7] (Table 1). In all these pathologies, an expansion of CAGs within the coding region of a gene, specific for each disorder, results in an expansion of consecutive glutamine residues in the translated protein (Table 1). The number of repeats is comparatively smaller in the polyglutamine group of diseases than the diseases with repeats in non-coding sequences. The age of onset of the pathology correlates, partly, with the polyglutamine number. All these polyglutamine diseases present similarities that suggest some overlap of mechanisms of pathogenesis [8].

The translated polyglutamine repeats presumably alter the proteins conformation and the interactions with other proteins. This leads to neuronal dysfunction, usually in mid-life which later progresses to neurodegeneration with decline in motor and cognitive functions. According to some authors, polyglutamine pathogenesis is not due to the loss of function but, instead, to a toxicity of the proteins carrying a polyglutamine expansion leading to a gain of function. Similarly, an ectopic expression of a polyglutamine tract in an unrelated mouse gene induces a pathology [11]. Moreover, loss of function of the androgen receptor does cause feminisation, but no weakness or motor neuron degeneration as happens in SBMA [8].

Notably, although the genes with the polyglutamine expansions are ubiquitously expressed, the neurodegeneration is limited to specific brain areas. The gene carrying the repeat expansion confers this regional selectivity. With the exception of SBMA, an X-linked
Table 1. Polyglutamine Expansion Diseases

<table>
<thead>
<tr>
<th>Polyglutamine disorder</th>
<th>Protein product/ Locus</th>
<th>Normal and Pathogenic repeat number</th>
<th>Primary target of neuropathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentatorubro-pallidolusian atrophy (DRPLA)</td>
<td>Atrophin 1 12p12</td>
<td>3-35 49-88</td>
<td>Cerebellum (dentate nucleus), red nucleus, globus pallidus (external segment), subthalamic nucleus</td>
</tr>
<tr>
<td>Huntington’s disease (HD)</td>
<td>Huntingtin 4p16.3</td>
<td>6-35 36-121</td>
<td>Caudate nucleus (medium spiny neurons), putamen, globus pallidus (external segment)</td>
</tr>
<tr>
<td>Spinal and bulbar muscular atrophy (SBMA; Kennedy disease)</td>
<td>Androgen receptor Xq11-q12</td>
<td>9-36 38-62</td>
<td>Motor neuron in anterior horn cells of the spinal cord and brainstem</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 1 (SCA1)</td>
<td>Ataxin 1 6p23</td>
<td>6-38 39-83</td>
<td>Cerebellum, red nucleus, inferior olive, pons, anterior horn cells and pyramidal tracts</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 2 (SCA2)</td>
<td>Ataxin 2 12q24</td>
<td>14-31 32-77</td>
<td>Cerebellar Purkinje cells, cytoplasmatic inclusions</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 3 (SCA3)</td>
<td>Ataxin 3 14q24.3-q31</td>
<td>12-40 54-86</td>
<td>Cerebellar dentate neurons, basal ganglia, brain stem, spinal cord</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 6 (SCA6)</td>
<td>Ca²⁺ channel 19p13</td>
<td>4-19 20-30</td>
<td>Cerebellar Purkinje cells, dentate nucleus, inferior olive, cytoplasmatic inclusions</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 7 (SCA7)</td>
<td>Ataxin 7 3p21.1-p12</td>
<td>4-35 37-200</td>
<td>Cerebellum, brain stem, macula, visual cortex</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 17 (SCA17)</td>
<td>TATA-binding protein (TBP) 6q27</td>
<td>29-42 47-55</td>
<td>Cerebellum, cortex (diffuse atrophy), caudate and putamen. NII</td>
</tr>
</tbody>
</table>

Adapted from [1,9,10]

recessive disease, all other polyglutamine disorders are autosomal dominant [6].

2. HUNTINGTON’S DISEASE

Huntington’s disease (HD) is the most prevalent polyglutamine disease. The disorder was named after George Huntington who, based on the observation of patients from his father’s practice in Long Island, published in April 1872 at The Medical and Surgical Reporter, a detailed description of the disease symptoms. More than one century later, in 1993, the mutation in the huntingtin gene causing the disease was isolated by a multicenter study, organized by the Hereditary Disease Foundation. This opened the way for a molecular study of the disease [12]. The last years were marked by an intensive research on Huntington’s disease, which has enlightened on some of the mechanisms that may be involved in the disease pathogenesis. Despite this, there is currently no treatment able to block the progression of this devastating disorder.

HD symptoms involve motor, cognitive, and emotional disturbances [13,14]. Although the disease can manifest at any age, generally the first signs appear by 35-50 years of age, progressing inexorably to death, 15-20 years after the onset. The most common symptoms are uncontrolled and uncoordinated involuntary dancing–like movements of limbs and distal and proximal muscles of the trunk, designated as chorea, memory deficits, affective disturbances, change of personality, depression and dementia [15].

In early stages, subtle coordination changes with clumsiness and difficulty with balance can be observed. In addition, minor involuntary movements, difficulty in problem solving, irritability and depression are common [13]. Later, chorea, difficulty with voluntary motor activities, dysarthria, dysphagia, and cognitive deficits develop. In late stages, the patients develop severe rigidity and bradykinesia along with dementia. In juvenile cases (onset <20 years), the disease progresses faster and symptoms are more severe with bradykinesia, rigidity, seizures, and severe dementia, with little or no chorea [16]. Swallowing difficulties and complications associated with immobility are generally involved in the cause of death [9]. Loss of body weight and lack of muscle bulk have been reported, even though patients have high calorie intake [17]. Rubinsztein reports a prevalence of 1 in 10 000 in UK against a lower prevalence of 1 in 1 000 000 in Japanese and African populations [18].

The “interesting transcript 15” (IT15), presently known as the huntingtin gene [12], contains 67 exons in both mice and men. Its locus is located between the regions D4S127 and D4S180 on chromosome 4p16.3 spanning over a 200 kb genomic region, and is transcribed in two RNA differing in the 3' terminal untranslated region. The open reading frame encodes a large protein of 350 kDa without homology to other proteins [12].

The CAG expansion codes for a tail of polyglutamines, which are located in the N-terminal region of the huntingtin protein, 17 amino acids downstream of the ATG initiator codon [19,20]. The protein N-terminal is capable of interacting with several proteins. Adjacent to the polyglutamine tract, the protein is comprised of a polyproline tract. Otherwise, the protein is entirely composed of several HEAT repeats, a motif of 40 amino acids of largely unknown function, but which may play a scaffolding role in the formation of particular protein-protein interactions.
interactions at its C-terminal [21]. The number of CAG repeats determines the presence or absence of HD, its severity, and the time of onset, according to an inverse relationship between CAG repeat number and the age of onset [22,23].

As shown in Figure 1, a number of CAG repeats between 8 and 29 CAGs is within the normal range. An intermediate length, between 29 and 35 polyglutamine residues is non-phenotypical but prone to instability and expansion. A number of repeats over 36 is considered pathologic and will cause the disease [24,25], although between 36 and 40 repeats the penetrance is incomplete [26-28]. The penetrance is considered incomplete when, at the age of 65, the person does not present the disorder symptoms [9]. Between 40 and 50 repeats, the disease attacks patients in midlife. A number of repeats over 60 causes severe juvenile cases of HD, with an appearance of the symptoms before the age of 20 [14]. Numbers up to 240 repeats have rarely been identified in HD patients [29]. It should be noted that the age of onset only partially correlates with repeat size. Therefore, no accurate predictions of the age of onset can be made based on the polyglutamine repeat length [30,31]. It has been suggested that other factors, such as genetic polymorphisms adjacent to CAG repeat, may have significant contributions [32].

The relationship between CAG repeats length and clinical progression rate is more controversial [33,34]. Some authors found an inverse correlation between CAG number and the speed of progression of the disease [35-37]. Others saw no correlation [38], although pathological changes in post mortem tissue were described to correlate with polyglutamine length [39].

The repeats are unstable and length varies both in somatic tissues and during transmission. Paternal transmission is responsible for an increase in polyglutamine length leading to anticipation, an increase in severity, or a decrease of age of onset from generation to generation [40]. Accordingly, paternal transmission is responsible for most of the juvenile cases [41,42], with a mean advance of 8 years in successive generations [9].

HD is an inherited autosomal dominant disorder. This has suggested that the disease is caused by a gain of function mechanism. Accordingly, the huntingtin gene upon mutation (trinucleotide expansion) gains a new toxic function that is sufficient to cause the disease [18]. Supporting this gain of function theory, homozygote patients have been described who develop phenotypes that are identical to heterozygotes. Moreover, loss of one allele of the huntingtin gene does not cause HD [43,44]. Nevertheless, loss of the normal huntingtin function, due to the presence of a single copy of mutant huntingtin, could contribute to the pathogenesis. Normal huntingtin appears to have a neuroprotective function, and up-regulate trophic factor (brain derived neurotrophic factor (BDNF)) support in the striatum. The effect is lost in the presence of mutant huntingtin [45]. Therefore, a loss of function mechanism may also be important in defining HD pathogenesis. Normal huntingtin appears to be essential for embryogenesis (knock out mice for huntingtin show early embryonic mortality), playing an important role in the development of the forebrain and increasing the survival of adult neurons in the forebrain [46]. Mutant protein may sequester wild-type huntingtin and critically alter its normal function due to increased proteolysis, or to changes of protein-protein interactions. Therefore, understanding more about the function(s) of wild-type huntingtin may bring about new therapeutic perspectives that prevent the blockade of normal huntingtin function.

The previously described symptoms that occur in HD have been correlated with the pathological changes in the patient’s brain [9]. Accordingly, the motor changes can be attributed to the disconnection of the neuronal circuitry between the cortex, striatum, and globus pallidus. The striatum in humans designates the ensemble of caudate and putamen, and is the structure first and most extensively affected by atrophy and neuronal loss in HD. Degeneration follows an anatomic pattern progressing from dorsal medial regions to ventral and lateral regions [47]. Within the striatum, a selective neuronal loss involves γ-aminobutyric acid (GABA)-ergic medium spiny neurons (enkephalin and GABA positive) with preservation of large aspiny cholineric neurons. Also involved are the sparse medium spiny neurons containing somatostatin, neuropeptide Y and NADPH diaphorase [48,49]. Similarly to other neurodegenerative disorders, neuronal death in HD is accompanied by the development of gliosis. The Vonsattel

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**Fig. (1).** Representation of the huntingtin gene and the impact of polyglutamine repeat size on the onset of Huntington’s disease. The huntingtin gene is transcribed and translated into a 350 kDa protein.
scale, a semi-quantitative scale, is commonly used to grade the severity of HD pathology into five grades (0-4). Interestingly, patients in grade 0, without any apparent pathology in post mortem observation, have been described as symptomatic, suggesting that early neuronal dysfunction induces behavioural changes per se, before neurodegeneration [50,51]. The initial mild cognitive changes have also been attributed to basal ganglia pathology, while the severe global dementia observed in late stage HD is attributed to gross striatal neuronal atrophy and loss, associated with cortical neurodegeneration and gliosis. Within the cerebral cortex, large projection neurons in cortical layers III, V and VI are the most affected [52-54]. The cortical neurodegeneration has been associated with the changes in personality and dementia occurring in late HD [55].

One of the most striking aspects of HD is the presence of protein aggregates in the brain. Although previously reported, relatively little attention was given to this feature until the presence of intranuclear huntingtin aggregates were identified in the transgenic R6/2 mice [56]. Immediately afterwards, N-terminal fragments of mutant huntingtin were identified in neuronal intranuclear inclusions and dystrophic neurites in HD cortex and striatum [57]. Aggregates are present both in striatum and cerebellar cortex, ultrastructurally, both in the cell nucleus, neurites and neuropil [57-61]. Within the striatum, only 1 to 5% neurons are stained with N-terminal targeted antibodies [57,61] in both projecting neurons and interneurons [62]. In adult-onset HD the predominant form of aggregation is neuritic, attaining a large percentage of neurons. From the adult-onset to juvenile cases, aggregation shifts from neuritic to a predominantly nuclear pattern of aggregation [57]. Neuritophil aggregates were detected in the cerebral cortex of autopsied pre-symptomatic individuals, suggesting that their presence in the brain precedes the occurrence of HD neurological symptoms [57,61]. Despite intensive research on this subject, the function and relevance of aggregates remain controversial, and is discussed further in section 3.4.

3. MOLECULAR TARGETS AND THERAPEUTIC STRATEGIES

3.1. Excitotoxicity

Several lines of evidence suggest that chronic exposure of neurons to excitatory amino acids, like glutamate may induce neurodegeneration. Glutamate is released by cortical efferences in the striatum, where it can activate ionotropic N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid (KA) subtype of glutamate receptors, as well as metabotropic glutamate receptors. The inability to respond properly to elevations in synaptic concentrations of glutamate overexcites neurons, leading to neuronal death [63]. Activation of the NMDA receptor allows high levels of calcium entry in neurons. Calcium triggers different downstream events, such as the activation of calpains, protein kinase C, DNase, phospholipases and the consequent production of reactive oxygen and nitrogen species, which induce cell death by necrosis and/or apoptosis [64,65]. Thus, the different NMDA receptor (NMDAR) subtypes have been proposed to induce different excitotoxic death pathways.

HD is generally considered to be a slow form of excitotoxicity. A disproportionate loss of glutamate receptors, in particular NMDA receptors, is seen in HD post mortem tissue of symptomatic and pre-symptomatic patients. This suggests a role for these receptors in the disease pathogenesis [66-68]. Based on this hypothesis, different models have been developed. Upon intracranial injection of quinolinic acid (QA), an agonist of the NMDA receptors, neuropathological changes reminiscent of HD were observed in rodents and non-human primates, further supporting the importance of excitotoxicity in the pathogenesis of the disease [47,69,70].

In contrast, pre-symptomatic transgenic R6/1 mice showed a decreased sensitivity to QA-induced striatal excitotoxicity [71]. Interestingly, the resistance to QA or malonate (a reversible inhibitor of mitochondrial complex II) neurotoxicity (described in section 3.2) was shown to be dependent on the age of the mice and the number of CAG repeats. It occurred earlier in R6/2 (150 CAG repeats) than in R6/1 (115 CAG repeats) mice [72]. It has been suggested that the C-terminal portion of huntingtin, not expressed in the R6 mice, is required for functional interaction with the NMDA receptor [73]. Alternatively, early expression of highly toxic huntingtin fragments during development can induce unknown developmental modifications that protect R6 mice from QA toxicity.

The importance of NMDA receptor subunits on HD pathogenesis has been largely demonstrated by Lynn Raymond and Michael Hayden. Expression of full-length mutant huntingtin and NR2B in HEK 293 cells, but not the NR2A, was accompanied by an increase in apoptosis [73,74]. This is particularly interesting as the striatum, the structure most severely affected in HD, was reported to contain high levels of the NR2B NMDA receptor subunit [75], thus justifying the preferential degeneration of striatal medium spiny neurons in comparison with other regions and neuronal subtypes.

Moreover, the YAC transgenic mice carrying a full-length huntingtin transgene with 72 CAGs (YAC72) [76] were shown to be more prone to NMDA mediated cell death (via intrastriatal injection of QA) and NMDA receptor-mediated current amplitude than YAC transgenic mice expressing full-length huntingtin with 46 CAGs (YAC46) or wild-type mice [77]. Moreover, measurement of excitatory postsynaptic currents (EPSCs) in corticostriatal slices from YAC72 mice and wild-type littermates showed that the ratio of NMDA receptor- to AMPA receptor-mediated EPSC amplitude was increased in YAC72 compared to wild-type mice [78]. Thus, mutant huntingtin may increase NMDA receptor activity and, consequently, changes in intracellular calcium, mitochondrial dysfunction, caspases activation and cell death. These occur through the intrinsic apoptotic pathway, as reported recently by Zeron et al. (2004) [79], although, curiously, these results were prominent for YAC46. How mutant huntingtin increases NMDA receptor activity is still debatable, but could result from a change in the interaction with cytoskeletal proteins that modulate receptor function and localization [80]. In fact, expression of polyglutamine expanded form of huntingtin was shown to induce tyrosine phosphorylation of NR2B subunits through the interaction with PSD-95 (a protein mostly associated
with microtubules that mediates the binding of NMDA receptor with wild-type huntingtin) and the activation of Src tyrosine kinase, a PSD-95 associated protein [81,82].

Recently, the type 1 inositol-1,4,5-triphosphate receptor (IP$_3$R), an intracellular calcium release channel located at the endoplasmic reticulum, was shown to form a ternary complex with HAP1A (huntingtin-associated protein 1A) and huntingtin. In accordance with the mechanism proposed by the group of Ilya Bezprozvanny, under normal conditions huntingtin is strongly associated with the NMDA receptor via PSD-95 and weakly associated with the IP$_3$R. In the presence of mutant huntingtin there is an increased activity of NMDA receptor composed of NR2B subunits (most probably due to a decreased interaction with PSD-95, as previously reported [81]) and an increased sensitization of IP$_3$R (due to the strong binding of mutant huntingtin to the C-terminal tail of IP$_3$R) by IP$_3$, largely increasing the levels of cytosolic calcium [83]. This may occur even if low levels of glutamate are released from the cortical neurons projecting to the striatum. This rise in intracellular calcium could result in enhanced neuronal toxicity due to mitochondrial dysfunction and increased activity of enzymes responsible for huntingtin proteolysis (see sections 3.2 and 3.3). Importantly, the protein HAP1, which is expressed in the medium spiny neurons, is required for changes in intraneuronal calcium levels and facilitates the activation of IP$_3$R in the presence of mutant huntingtin, as determined using HAP1 -/ - mice [84].

In more recent studies, mutant huntingtin was reported to increase NMDA receptor localization to the synapses by interacting with huntingtin interacting protein-1 (HIP-1), an endocytic protein previously shown to regulate clathrin-mediated endocytosis and to be required for AMPA receptor internalization [85]. HIP-1 appears to interact directly with α-actinin and NR2B. Furthermore, primary neurons isolated from HIP-1 -/ - mice are protected from NMDA-induced excitotoxicity [86].

3.2. Impaired Energy Metabolism and Mitochondrial Dysfunction

Impaired energy production is another potential mechanism involved in HD pathogenesis that is in close relationship with excitotoxicity [87]. Under compromised energy metabolism normal glutamate concentrations become toxic in what is generally designated as the "secondary excitotoxic mechanism". The selective lesions produced in the striatum of rodents and non-human primates, following systemic administration of 3-nitropropionic acid (3-NP), support this putative mechanism [88-92]; reviewed in [93-95]. 3-NP irreversibly inhibits succinate dehydrogenase or mitochondrial complex II, leading to energy deficiency in vivo with ATP depletion, followed by the activation of excitatory amino acid receptors and the generation of free radicals [88,89,96-98]. According to this hypothesis, a loss of ATP leads to membrane depolarisation and removal of the voltage-dependent Mg$^{2+}$ block of the NMDA receptor, allowing subsequent activation of NMDA receptor, the influx of calcium and activation of cell death pathways [99]. In vitro experiments involving primary rat hippocampal neurons suggested that 3-NP induced cell death through an initial acute excitotoxic necrosis, resulting from NMDA receptor activation, which was followed by NMDA receptor-independent delayed apoptosis [100]. The involvement of apoptotic cell death in 3-NP neurotoxicity has been largely documented. Chronic administration of 3-NP caused selective striatal degeneration, analysed by DNA fragmentation, which involved the activation of the c-Jun N-terminal kinase pathway [101]. Other studies showed 3-NP-mediated mitochondrial apoptotic features, related with the translocation of cytochrome c from the mitochondria, changes in mitochondrial membrane potential, oxidative stress or an increase in Bax/Bcl-2 in vivo and in vitro (e.g. [102,103]). The involvement of caspase-1, a class of cysteine proteases (section 3.3) that are mediators of apoptosis in 3-NP neurotoxicity was also demonstrated using a caspase-1 dominant-negative mutant mice, which showed smaller 3-NP striatal lesions [104].

In the striatum of 3-NP-treated rats, a predominant activation of calpains over caspase-3 was observed, partially occurring due to calpain-mediated proteolysis of active caspase-3 [105]. Although calpain activation could be confirmed in primary striatal neurons treated with 3-NP, no significant changes were observed in primary cortical neurons [106,107]. Moreover, in cortical neurons, mitochondrial-dependent apoptotic features mediated by 3-NP, including cytochrome c release and caspase-3 activation, were precluded by treatment with FK506, a known inhibitor of calcineurin or protein phosphatase 2B [107]. FK506 also decreased mitochondrial Bax and enhanced mitochondrial Bcl-2 levels [107], an anti-apoptotic protein shown to prevent 3-NP mediated cell death [108]. Furthermore, increased vulnerability of striatal cells derived from mutant huntingtin knock-in mice to 3-NP appeared to be related to a non-apoptotic type of cell death involving mitochondrial deregulation [109].

According to the 3-NP model, mutant huntingtin would impair energy metabolism leading to neuronal dysfunction and death [110]. A considerable amount of data supports mitochondrial dysfunction in HD brain. Patients experience weight loss despite high caloric intake, high concentrations of lactate in striatum and cortex, concomitant with increased lactate/pyruvate in the cerebrospinal fluid, decreased muscle phosphocreatine/inorganic phosphate ratio, decreased N-acetylaspartate/creatine and decreased activity of mitochondrial complexes II/III and IV in basal ganglia [111-113]. A significant reduction in the activity of mitochondrial complex IV was observed both in the striatum and cerebral cortex of brains from 12-week-old R6/2 transgenic mice [114]. Importantly, significant changes in mitochondrial membrane potential and reduced mitochondrial calcium retention capacity were observed in lymphoblasts from HD patients and in brain mitochondria from the YAC72 transgenic mice [115]. These changes were correlated with the association of N-terminal huntingtin with the mitochondria of YAC72 mice, as determined by electron microscopic immunocytochemistry using the EM48 antibody, which recognizes amino acids 1-256 of huntingtin and labels aggregated huntingtin [115]. Using isolated mitochondria from cultured cells, Choo and collaborators (2004) demonstrated that full-length huntingtin is localized to the outer mitochondrial membrane [116]. They also demonstrated that mitochondria incubated in the presence of mutant huntingtin or isolated from knock-in HD mice had a...
reduced calcium threshold for opening of the mitochondrial permeability transition (MPT) pore and resulting cytochrome c release, which could be prevented by MPT blockers, such as cyclosporin A, ATP or EGTA [116]. A decrease in ATP/ADP ratio in striatal cells derived from knock-in mice expressing mutant huntingtin further elucidates the relevance of mitochondrial dysfunction in HD pathogenesis [117].

Nonetheless, no changes in electron transport chain activity (complexes I, III and IV), oxidative stress or calcium homeostasis were detected in HD compared to control cybrid cell lines, derived from the fusion of HD or control human platelets and human neuroblastoma SH-SY5Y p0 cells [118]. Since no data on platelet mitochondrial functionality was reported by these authors, we may envision the possibility that the platelets from those HD patients had no deficiencies in mitochondrial function, as previously described by Gu and collaborators [111].

In order to counteract the mitochondrial dysfunction and the bioenergetic defects associated with HD, mitochondrial neuroprotective agents, namely coenzyme Q10, an essential molecule for the electron transport chain in the inner mitochondrial membrane, and creatine, have been tested. As reported by Koroshetz and collaborators (1997), treatment of 18 HD patients with coenzyme Q10 resulted in a decrease in lactate concentration in the cortex, which reversed upon therapy withdrawal [113]. In 2001, the Huntington Study Group carried out a multicentre controlled trial in 347 patients with early HD, for 30 months, to examine the effects of coenzyme Q10 and the NMDA antagonist remacemide. None of these compounds, at the dosages used, had significant effects in slowing the functional decline observed in early HD [119], although untreated HD patients were recently shown to have lower serum coenzyme Q10 levels than treated HD patients and controls [120]. Nevertheless, the combination of coenzyme Q10 with remacemide was effective in increasing the survival of R6/2 transgenic mice by 32% [121]. Supplementation with creatine, known to increase brain phosphocreatine levels and stabilize the MPT, was also tested in 41 HD patients, with stage 1 to 3 [122]. No improvement in functional, neuromuscular or cognitive status was observed after one year of creatine intake. However, creatine was neuroprotective in transgenic HD mouse models, improving survival and slowing brain atrophy, the development of motor symptoms and weight loss [123,124].

3.3. Caspases Activation and Cell Death

The involvement of apoptosis has been proposed in HD neurodegeneration by several groups [125-127]. DNA strand breaks were detected in affected regions of HD patient brains [128-130]. Moreover, several studies reported that polyglutamine expansion in huntingtin mediates apoptosis through caspases activation, in particular caspases 1, 3, 8 and 9 [131-134]. Accordingly, caspase inhibition decreased polyglutamine-expanded huntingtin toxicity in cells [135] and slowed the progression of the pathology and mortality of transgenic R6/2 mice [134,136].

Caspases activation has been suggested to occur through oligomerization of caspase 8 in inclusions [132]. The involvement of caspase-8 was further reported by Gervais and collaborators [137]. These authors showed that formation of heterodimers of HIP-1 and a HIP-1 protein interactor (Hippi) in cells expressing huntingtin with 128 CAG repeats recruited procaspase-8, thus promoting apoptosis through the extrinsic pathway. However, increased susceptibility of HD lymphoblasts leading to activation of initiator caspase-9 and effector caspase-3, but not caspase-8, was largely associated with mitochondrial depolarisation [131]. Additionally, NMDA receptor stimulation leading to activation of caspases -9 and -3 (not caspase-8), was attenuated by cyclosporin A in cultured striatal neurons isolated from the YAC46 transgenic mice [79]. Polyglutamine-mediated apoptosis occurring through the intrinsic (mitochondria and caspase-9 mediated) pathway was recently demonstrated by Ilya Bezprozvanny in striatal neuronal cultures from YAC128 transgenic mice, following submitted to prolonged exposure to 100-250 μM glutamate [138].

Proteasome blockade is also strongly implicated in HD pathogenesis (section 3.5) and previous reports demonstrate that proteasome dysfunction leads to caspase-dependent apoptosis [139-142]. Jana and collaborators found a direct correlation between polyglutamine expansion in huntingtin or lactacystin blockade of proteasome with a cytoplasmatic disruption of mitochondrial membrane potential, release of cytochrome c from the mitochondria, and activation of caspases -9 and -3 [143].

Alternatively, polyglutamine expanded huntingtin fragments have been shown in PC12 cells to increase the expression of caspase-1 (at the transcriptional level), which may act as an initiator for the activation of caspase-3, leading to apoptosis [133]. Similarly, working with R6/2 mice, Chen and collaborators demonstrated that mutant huntingtin induces, by an unknown mechanism, caspase-1 upregulation and activation, followed by inducible nitric oxide synthase (iNOS) and caspase-3 upregulation and toxicity [134]. The time-course of caspases activation and changes in pro-apoptotic proteins of the Bcl-2 family was further elucidated in the R6/2 mice. In this HD model caspase-1 is the first to be activated, at 6-9 weeks, followed by the release of cytochrome c, activation of caspases -9 and -3, increased tBid and Bax translocation at 9-12 weeks, and further activation of caspase-8, increased Bim and decreased phospho-Bad after 12 weeks [144,145]. This caspase cascade could be blocked by administration of minocycline, a second-generation tetracycline analogue that is able to cross the blood-brain barrier [134]. Minocycline was also shown to inhibit both caspase-independent (occurring through the apoptosis-inducing factor) and caspase-dependent (occurring through Smac/Diablo and cytochrome c) mitochondrial apoptotic pathways in HD models, highlighting the broad neuroprotective effect of this compound [146].

Activation of caspase-3, up to certain levels, was previously suggested to be a normal process that cleaves substrates without causing apoptosis. Nevertheless, caspases have been largely implicated in huntingtin cleavage, which may be responsible for the production of toxic N-terminal huntingtin fragments. Activated caspases were demonstrated to cleave both normal and mutant huntingtin near the N-terminus, further supporting huntingtin as a caspase substrate [147-150]. Previously, an increased association of caspase-3 cleaved huntingtin fraction with the membrane...
fraction was found, suggesting an involvement in altered vesicle trafficking in HD [151]. Two caspase-3 cleavage sites were identified at amino acids 513 and 552 of huntingtin, whereas one caspase-6 cleavage site was identified at amino acid 586 [150]. The resulting neurotoxic fragments can further stimulate proteolysis [152]. Thus, polyglutamine expanded huntingtin increases caspase-3 activation, huntingtin aggregation and toxicity [148,149]. There is evidence of N-terminal fragments of huntingtin in both HD brains and transgenic mice expressing full-length huntingtin [57,76]. Working with human post mortem tissue, Kim and collaborators identified huntingtin fragments in HD brains with a molecular weight consistent with caspase-3 mediated cleavage, along with subsequent calpain proteolysis [151]. Accordingly, activated calpain was detected in the caudate of human HD tissue and cleavage of huntingtin by calpains was shown to be polyglutamine-length dependent [153]. The relevance of calpain activation in HD pathogenesis was recently demonstrated. Ellerby and collaborators have mutated two identified calpain cleavage sites in huntingtin (amino acids 469 and 536) showing decreased susceptibility to proteolysis, along with decreased aggregation and toxicity of mutant huntingtin in cell cultures. Interestingly, they showed that calpain/caspase-derived huntingtin fragments and activated forms of calpains 7 and 10 are located in the nucleus [154].

Overexpression of the anti-apoptotic molecules Bcl-xl and Bcl-2 was shown to be neuroprotective, further validating an apoptotic cell death pathway for HD pathogenesis [132,155]. Transgenic overexpression of Bcl-2, obtained from crossing R6/2 mice with mice overexpressing Bcl-2 in neurons, resulted in delayed onset of motor deficits and an extended survival by 10% in the R6/2 mice [145].

In addition, other evidence suggests that neurodegeneration in HD occurs neither by apoptosis nor by necrosis, as revealed in the R6 transgenic mice lines and in post mortem human HD brains [156]. Therefore, it is possible to assume that, depending on the stage of HD, cell death by autophagy, in which membrane-bound structures like the lysosomes engulf intracellular organelles and participate in the destruction of the cells, also plays a significant role in HD neurodegeneration. Previously, Keggel and collaborators reported that association of huntingtin with the lysosomal enzyme cathepsin D was polyglutamine-length dependent, suggesting the activation of the endosomal-lysosomal system and, consequently, of an autophagic cell death process [157].

Finally, the success of different anti-apoptotic and caspase-inhibition strategies in reducing or suppressing polyglutamine-induced toxicity reinforces its potential as therapeutic targets.

3.4. Aggregation and Neuronal Inclusions

One of the distinctive features of HD and polyglutamine diseases is the formation of neuronal inclusions. Cloning of the HD gene and production of R6 transgenic mice [56] enabled the identification of neuronal intranuclear inclusions first in mice and later in patient's tissue [57,59]. The systematic formation of intranuclear inclusions before the appearance of the phenotype, could indicate a causative role in HD pathogenesis [59]. Moreover, co-localization of ubiquitin with intranuclear inclusions suggested that the cell targets misfolded and aggregated huntingtin, through ubiquitination, to an ineffective proteasome degradation (described in section 3.5).

DiFiglia and collaborators confirmed the presence of accumulation of N-terminal fragments of mutant huntingtin in intranuclear inclusions and dystrophic neurites in human brain [57]. Schilling and collaborators also reported neuritic aggregates in their transgenic model of HD, a feature that more closely resembles the pattern described for HD tissue [57,61,158]. This information was confirmed in different cellular models of HD that documented the presence of nuclear aggregates of polyglutamine expanded protein and neurodegeneration [125,126,159,160]. Aggregates were also identified in all other polyglutamine-expansion diseases (reviewed in [161-165]). Moreover, transgenic models expressing truncated fragments of huntingtin [59,158] more readily and extensively formed inclusions than those expressing full-length huntingtin [76,166], suggesting a role of proteolysis in the disease process [158].

Interestingly, targeting the expression of polyglutamine expansion huntingtin towards the endoplasmic reticulum or the mitochondria was recently demonstrated to inhibit its ability to aggregate, suggesting that unknown cofactors in the nucleocytoplasmic compartment enable aggregation of abnormal proteins [167].

3.4.1. The mechanism of Aggregation and the Role of Transglutaminases

The mechanism of aggregation of huntingtin remains unclear. Perutz proposed that polyglutamine repeats, when over 40, destabilize proteins, and induce the formation of anti-parallel β-strands held together by hydrogen bonds (polar zippers) [168]. This process of polymerisation is a molecular property of proteins with more than 40 glutamine repeats. The resulting structures, designated as hairpins, would destabilize protein structures, which become substrates for proteolysis and consequently release N-terminal fragments of huntingtin. Nuclear translocation, ubiquitination and aggregation (inside and outside the nucleus) would follow [168]. Scherzinger and collaborators demonstrated that huntingtin aggregation was a self-driven process that occurred in aconcentration-dependent and repeat length-dependent manner in vitro, and the same polyglutamine length threshold was shown to cause aggregation and pathogenicity [161]. Interestingly, introduction of one additional proline residue in the center of a Q9 element within the PGQ9 peptide completely blocked its ability to aggregate [169].

Green (1993), by analogy to the role of transglutaminases in mammalian epidermis growth, proposed an alternative model. Involutin is a protein of the skin rich in glutamines that transglutaminases link to the ε-amino groups of lysine residues (isopeptide bonds) present in other proteins. Green suggested that polyglutamine cross-linking in the brain by isopeptide bonds might lead to the formation of multimers and ultimately to protein aggregation [170]. Transglutaminases are present in the brain as a tissue-type I transglutaminate and a synaptosomal membrane-bound transglutaminase, and have been found to promote polymerisation of polyQ expanded huntingtin [171,172]. Several evidences have been found related with the role of
transglutaminases in the formation of huntingtin aggregates: i) elevated levels of N(ε)-(γ-L-glutamyl)-L-lysine, a marker of di-isopeptides, were found in the cerebrospinal fluid (CSF) of HD patients [173]; ii) tissue transglutaminase was increased in HD brain [174]; iii) colocalization was observed between transglutaminase-catalyzed epsilon-(gamma-glutamyl) lysine covalent cross-links and nuclear aggregates of huntingtin in the frontal cortex of post mortem HD brains [175]; and iv) administration of the transglutaminase inhibitor cystamine in a cellular model of DRPLA (dentatorubral-pallidoluysian atrophy) (Table 1) and in R6/2 transgenic mice has therapeutic effects [176-178]. Nevertheless, it has been suggested that the therapeutic effects of cystamine may be related to its effect on chaperone overexpression and not to aggregation inhibition [177]. Apart from inhibiting transgluminasines, in more recent studies, cystamine was demonstrated to inhibit caspase-3 activity and increase the levels of glutathione, prolonging neuronal survival [179]. A role for cystamine in increasing the antioxidant activity was further shown through an increase in the levels of the cellular reducing amino acid L-cysteine in the R6/2 mice model [180].

Although the mechanisms involved in the formation of cortical and striatal perinuclear cytoplasmic aggregates and intranuclear inclusions of mutant huntingtin are still largely unclear, the existing data support the hypothesis that transglutaminase catalyzed cross-linking of mutant huntingtin is involved in the formation and/or stabilization of huntingtin protein aggregates in HD and that transglutaminase 2 cross-links mutant huntingtin through the regulation of calmodulin [175,181]. Thus, more studies are needed to evaluate the real potential of directly inhibiting transglutaminase activity or inhibiting the interaction of calmodulin with transglutaminase for HD therapy.

3.4.2. Are Huntingtin Aggregates the Cause of Pathogenesis?

The role of nuclear inclusions in HD pathogenesis remains controversial. Some authors considered that it could be the direct cause of the disease [57,59], while others believe the huntingtin aggregates are un-related to its mechanism or that it could even be a detoxification mechanism of the cell [155].

Initially, data pointed towards a pathogenic effect of aggregates. Production of transgenic mice expressing a 146-unit polyglutamine tract in the mouse hypoxanthine phosphoribosyltransferase gene (Hprt), a protein not related with any of the known polyglutamine disease, led to a phenotype characterized by the formation of nuclear inclusions and neurological dysfunctions [11]. More recently, the conditional mouse model of HD showed huntingtin inclusion body formation and progressive motor dysfunction when huntingtin expression was turned on. Administration of doxycycline eliminated huntingtin expression. The resulting elimination of both the behavioural phenotype and inclusions reinforced the idea of a close relationship between these two variables, demonstrating that the formation of nuclear inclusions is reversible [182]. A correlation between the number of inclusions and polyglutamine number in patients further suggested a role of inclusions in the pathogenesis of the disease [183].

Other studies acquitted nuclear inclusions from a direct responsibility in polyglutamine toxicity. In a HD cellular model, Saudou and collaborators (1998) blocked huntingtin ubiquitination, and observed a decrease of huntingtin aggregation with concomitant increase in toxicity, which led the authors to conclude that aggregates were part of a cell detoxifying mechanism [155]. Moreover, in models of SCA1 (Table 1), reducing aggregation by deletion of a self-association domain [184] or preventing ubiquitination, in mice with a mutation of the E6-AP ubiquitin ligase, increased toxicity [185]. In other studies it has been possible to reduce polyglutamine toxicity without affecting aggregation, strongly suggesting that aggregates were not the cause of the disease [186,187]. Kim and collaborators demonstrated that the formation of inclusions could be separated from events that control cell death depending on the caspase inhibitor used [135]. Furthermore, Muchowski and collaborators showed that disruption of microtubules avoided aggregation of huntingtin but increased toxicity, suggesting that free huntingtin is more toxic than nuclear inclusions [188]. Recently, the group of Huda Y. Zoghbi working with a knock-in mouse model that recapitulates the clinical features of spinocerebellar ataxia 7 (SCA7) (Table 1) found an inverse correlation between neuropathology and the degree of formation of ataxin-7 nuclear inclusions [189].

However, Sánchez and collaborators (2003) revealed that Congo red, known to inhibit oligomerization through the preferential binding to β-sheets, inhibited polyglutamine-expansion cell death, by preventing ATP depletion and caspases -3 and -8 activation, and disrupted preformed aggregates, as determined by FRET. Furthermore, infusion of Congo red in symptomatic transgenic R6/2 mice ameliorated the weight loss, increased motor function and increased polyglutamine clearance, further revealing the protective role of anti-amyloid compounds [190].

Moreover, Tanaka and collaborators, assuming that the aggregates are toxic, selected various disaccharides based on their ability to inhibit polyglutamine-mediated protein aggregation. The most active, trehalose, was orally administered to a transgenic mouse model of Huntington’s disease (R6/2 mice) and has been shown to decrease polyglutamine aggregates in cerebrum and liver, improve motor dysfunction and extend lifespan. This approach appears to be promising since no toxicity arising from oral trehalose administration was reported [191].

3.5. Proteasome Impairment

The ubiquitin-proteasome pathway (UPP) is involved in cellular quality control by degrading misfolded, unassembled, or damaged proteins that could otherwise form potentially toxic aggregates [192,193]. In most neurodegenerative diseases there is an accumulation of ubiquitinated protein aggregates suggesting a link between UPP and neurodegeneration [194]. Interestingly, wild-type ataxin-3, a poly-ubiquitin binding protein reported to have a deubiquitinating activity, is sequestered together with ubiquitin in protein aggregates of several polyglutamine expansion diseases [195-197]. A reduction of proteasome activity with aging could explain the late-onset of polyglutamine diseases [198].
Different findings support that aggregation of huntingtin, followed by ubiquitination leads to proteasome impairment. In polyglutamine disorders, proteasome components have been associated with intranuclear inclusions [57,59,143,199-202]. Moreover, drug-induced experimental proteasome blockade increases protein aggregation [143,203-205]. The work by Kopito’s group suggested that the presence of polyglutamine expanded huntingtin fragments inhibits proteasome function [206]. In fact, polyglutamine proteins appear to be trapped within the proteasome [207]. Furthermore, blocking huntingtin expression in Yamamoto’s conditional model of HD, either in vivo or in vitro, lead to rapid proteasome mediated clearance of both wild type and mutant huntingtin, disappearance of inclusions and later on, the complete remission of the phenotype [182,198]. 

The theory of proteasome impairment suggests that the blockade of proteasome by misfolded polyglutamine expanded proteins may induce the accumulation of other proteins and transcription factors, further impairing the proteasome function and promoting protein accumulation and aggregation by a positive-feedback mechanism. Moreover, sequestration of anti-apoptotic factors and transcription factors may contribute to cell death. Nevertheless, a recent work using a mouse model that expresses a green fluorescent protein (GFP)-based reporter substrate (Ub(G76V)-GFP) of the UPP and ataxin-7 found no evidence for general UPP impairment, or reduction of proteasome activity [189]. In spite of these observations, proteasome activity seems to counteract accumulation of both soluble and aggregated huntingtin [198], and therefore a logical therapeutic approach would be to boost its proteasome function.

In contrast to ubiquitination, SUMOylation (a post-translational modification resulting from the covalent attachment of small ubiquitin-like modifiers (SUMO) to lysine residues) of mutant huntingtin was recently proposed to interfere with HD pathogenesis by decreasing its ability to form aggregates [208]. SUMOylation can also promote transcription repression and exacerbate neurodegeneration, as demonstrated by mutation of lysine residues within the first 17 amino acids of huntingtin [208]. The SUMO pathway parallels the classical ubiquitination pathway, through activation involving enzyme E1, conjugation involving enzyme E2 and substrate modification through the cooperative association of E2 and E3 ligases. Thus, interfering with protein SUMOylation could bring about a new therapeutic candidate against neurodegenerative diseases.

Alternatively, chaperones may modify the mutant proteins solubility and promote their degradation [209].

3.6. Chaperones

Stress, like heat, ethanol, heavy metals, amino acid analogs and anoxia, cause denaturation of proteins, which tend to aggregate and precipitate. In response to these stresses, cells synthesize a set of proteins, collectively referred as heat shock protein(s) (Hsp) [210] and denominated according to their molecular weight [211]. The Hsp are proteins containing a functional element in their promoter that upon heat stimuli induce transcription [211,212]. Most of these proteins are designated as chaperones, because they assist protein folding, oligomeric assembly, transport to a particular subcellular compartment, controlled switching between active/inactive conformations, and recovery of native configuration after unfolding [213,214].

Mutated proteins, particularly with polyglutamine expansions, tend to unfold, aggregate, and deposit. Several lines of evidence support a role for chaperones in preventing polyglutamine toxicity: i) chaperones co-localize with aggregates in polyglutamine models and patients [202,205,215]; ii) overexpression of chaperones has been demonstrated to decrease polyglutamine toxicity in different models of disease [186,199,216-219].

Endogenous polyglutamine length-dependent induction of Hsp70 was shown in a HD mammalian cellular model expressing truncated N-terminal huntingtin. Overexpression of a Hsp40 member (Hdj-1), alone or in combination with Hsp70 (Hsc70) significantly reduced huntingtin aggregation and toxicity [215]. Muchowski and collaborators, working both in a cell-free system and with yeast, also observed that overexpression of chaperones (Hsp40 and Hsp70) suppress or reduce aggregation [218].

In contrast, suppression of toxicity without a decrease in protein aggregation was shown in a HD fly model [187], similarly to what had been previously described in an SCA-1 model [186]. The authors isolated two genes coding for molecules containing chaperone-related J domains, one of them homologous to human Hsp40/Hdj-1 and able to suppress huntingtin-induced toxicity in Drosophila. Therefore, chaperone detoxification is not always accompanied by a decrease in protein aggregation. A decrease in polyglutamine-induced cell death, with no changes in protein aggregation, was further associated with a suppression of formation of reactive oxygen species upon expression of Hsp27, in neuronal and non-neuronal cells [204]. Chaperone neuroprotection, mediated by Hsp 40, Hsp 70, and N-ethylmaleimide-sensitive factor, was also reported to involve an inhibition of caspases -3 and 9 activation by huntingtin, independently of the inhibition of aggregation, which was achieved only with Hsp 40 [220]. Recently, Schaffar and collaborators (2004) further supported the beneficial effects of Hsp70/Hsp40 by demonstrating that these chaperones inhibit the polyglutamine-mediated interaction of huntingtin with the transcription factor TATA box-binding protein or TBP (see section 3.7) [221].

Unexpectedly, chaperones can also promote inclusion formation [204]. Accordingly, in a yeast model of huntingtin, deletion of Hsp104 expression completely eliminated huntingtin aggregation, suggesting that this chaperone can have a propagation role in polyglutamine expanded aggregation [222]. Thus, the existence of polyglutamine tracts in a soluble form is prolonged in Δhsp104 yeast mutants. Nevertheless, the requirement of Hsp104 function for aggregate formation of short polyglutamine tracts was shown to be bypassed if long polyglutamine tracts were present [223].

Besides heat shock proteins, other small molecules have been reported to have a chaperone-like activity such as the osmolytes that unicellular organisms synthesize upon temperature or ionic strength stress. The previously
described success of trehalose administration to R6/2 mice may be attributed to a chaperone like-activity, which presumably allowed stabilization of the partially unfolded polyglutamine-containing protein [191].

Chaperones have multiple roles and its mechanisms of detoxification are complex and remain largely unknown. It has been suggested that chaperone could protect against formation of amyloid-like aggregates, which instead, in the presence of chaperones, give rise to amorphous aggregates amenable to proteasome degradation. Alternatively, chaperones could prevent aggregated protein from recruiting and inactivating other glutamine-rich proteins [218]. In more recent data, the group of Paul Muchowski reported that Hsp70 and Hsp40 can modulate polyglutamine aggregates by decreasing the formation of spherical and annular oligomeric structures induced by mutant huntingtin, as demonstrated by atomic force microscopy [224].

In conclusion, chaperones interact with polyglutamine-expanded fragments in the direct proportion of their polyglutamine length. They co-localize in model systems with aggregates and can suppress cell toxicity accompanied or not by simultaneous decrease of aggregation. Because in their normal stress response chaperones promote both solubilization of misfolded proteins and inhibit caspase activation, it remains unclear which is their dominant neuroprotective mechanism. They are, however, a promising therapeutic approach, which should be evaluated in mammalian models of HD.

3.7. Transcriptional Dysfunction

Polyglutamine recruitment of transcription factors may disrupt gene transcription [202,225] and have a determinant role in HD pathogenesis (reviewed in [226,227]). Different studies showed that N-terminal huntingtin fragments interact with, and recruit other proteins and that nuclear localization of mutant huntingtin contributes to its pathogenicity [155,228]. During the process of protein aggregation in the nucleus, mutant huntingtin appears to interact particularly with proteins containing polyglutamine domains, as it is the case of several transcription factors, such as the cAMP-responsive element-binding protein (CREB)-binding protein (CBP), TATA box binding protein (TBP), and specificity protein 1 (Sp1) [229-233].

TBP has been described to co-localize with huntingtin aggregates in HD post mortem brains [234] and an expansion of polyglutamine repeats within the TBP gene is responsible for a new polyglutamine disease, SCAl7 (Table 1) [235]. Furthermore, nuclear accumulation of monomers or soluble oligomers of mutant huntingtin, implicated in cell toxicity, were shown to interact with the benign polyglutamine tract of TBP, destabilizing its function [221]. A soluble form of mutant huntingtin also interacts with the transcriptional activator Sp1 thus reducing the expression of the corresponding Sp1-regulated genes [236,237].

An important mechanism leading to transcription blockade may involve acetyltransferases inhibition of activity. CBP is an acetyltransferase enzyme with an 18 glutamine domain [227,238] that has been shown to co-aggregate with huntingtin in vitro [239], in the R6/2 HD transgenic model and in post mortem brains of HD and DRPLA (caused by polyglutamine expansions in atrophin-1 - Table 1) patients [233,240] as well as in SBMA (caused by androgen receptor polyglutamine expansion – Table 1) models and human tissue [241]. Acetyltransferases promote transcription by acetylating histones, an effect that is in dynamic equilibrium with histone deacetylators, which have the opposite effect. Decreasing acetyltransferases activity results in reduced transcription of the genes under its control. Different studies identified decreased acetylation and CBP-mediated transcription in the presence of polyglutamine expansions in yeast and mammalian cell culture [233,241]. Furthermore, it has recently been shown that conditional disruption of CREB function in brain leads to neurodegeneration and a striatal phenotype reminiscent of HD further supporting CREB’s role in HD pathogenesis [242]. Unexpectedly, Obrietan and Hoyt (2004) found a significant increase in CRE-dependent transcription in the striatum, hippocampus and cortex, by cross breeding R6/2 HD transgenic mice with CRE-β-galactosidase reporter gene transgenic mice, resulting a HD transgenic mouse with pathological changes similar to the R6/2 [243].

Reasoning that up-regulating CBP would counteract the effects due to its sequestration, Nucifora and collaborators [233] and McCampbel and collaborators [241] reversed toxicity induced by polyglutamine expanded huntingtin or atrophin-1 by CBP overexpression, and further demonstrated the importance of this pathogenic mechanism. In addition, expression of Sp1 and the transcription coactivator TAFII130 (a human TBP-associated factor or TAF, known to interact with various cell activators, such as Sp1 and CREB) reversed the transcription deregulation of dopamine D2 receptor gene and protected from cell toxicity in striatal cell cultures expressing mutant huntingtin with 75 glutamines [236].

Using another strategy, Steffan and collaborators [244] compensated for the loss of acetyltransferase activity by using inhibitors of histone deacetylases (iHDACs). Histone deacetylases are classified into classes I (HDACs 1, 2, 3 and 8) and II (HDACs 4, 5, 6, 7 and 9), which have similar catalytic domains, and class III, composed of sirtuins (Sir), which are similar to the NAD⁺-dependent yeast SIR2 proteins and are structurally and catalytically different from classes I and II. Administering iHDACs reversed the decrease in acetylated H3 and H4, arrested neurodegeneration and reduced lethality induced by polyglutamines in vitro in cell lines and in vivo in Drosophila models [244,245]. Although many studies have reported the use of iHDACs as anticancer agents, these compounds have been tested in several models of HD to increase the levels of histone acetylation and gene expression. The iHDAC suberoylanilide hydroxamic acid (SAHA), which crosses the blood-brain barrier and could be administered orally in drinking water, increased histone (H2A, H2B, H3 and H4) acetylation and significantly improved the motor deficits of transgenic R6/2 mice [246]. Two other iHDAC, sodium butyrate and phenylbutyrate, administered by intraperitoneal injection to the R6/2 [247] and the N171-82Q transgenic mice models [248], respectively, were also shown to prevent the decrease in striatal neuronal atrophy, increase protein acetylation, alter gene expression, and increase survival. Administration of phenylbutyrate to HD mice further attenuated increased histone methylation, induced the expression of glutathione-
S-transferase and a proteasome subunit, and repressed the expression of caspases -8 and -9, overall having a neuroprotective role [248]. iHDACs were also reported to prevent oxidative stress-mediated neuronal death by enhancing Sp1 acetylation and Sp1-dependent gene expression [249], suggesting that these compounds may be used in other neuropathological situations associated with increased production of reactive oxygen species.

The transcriptional dysfunction hypothesis is also supported by observations of selective decreased mRNA levels, particularly of enkephalin and substance P messenger RNA in the striatum of early grade HD [250] and of dopamine D₁ and D₂ receptor gene expression in the striatum of HD patients [251]. These observations were confirmed in transgenic R6/2 mice [252,253], where decreased adenosine A₂A receptors mRNA levels were also observed [226]. Evidence of decreased gene transcription in HD mice comes also from gene expression arrays on DNA microchips [225]. Moreover, in studies using human fibroblasts from HD patients, it has been shown that N-terminal fragments of huntingtin repress transcript [254]. Wytenbach and collaborators [202], working with doxycycline inducible PC12 cells that express huntingtin exon-1 with increasing polyglutamine tract, also observed an early decrease in VGF8a gene expression possibly because it is CRE-regulated. VGF has an important role in energy homeostasis and therefore this observation is consistent with the depression in regulation of energy balance observed in HD patients. Under this perspective, Calcins and collaborators [255] have recently described that the nuclear factor erythroid 2-related factor (Nrf2)-dependent transcription, known to translocate to the nucleus and bind to the antioxidant response element (ARE), regulating the transcription of cytoprotective genes, is important for protecting against inhibition of mitochondrial complex II-mediated toxicity in vitro (using primary cortical cultures) and in vivo [255].

Importantly, Zuccato and collaborators [45] described the decrease in cortical transcription of BDNF in the presence of mutant huntingtin, thus reducing trophic support to the striatum [45]. This change in BDNF gene transcription was associated with the abnormal accumulation of the repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) in the nucleus, due to impairment of its retention by mutant huntingtin in the cytoplasm, in contrast to the ability of wild-type huntingtin [256]. As a consequence, in the presence of polyglutamine-expanded huntingtin, the transcription factor REST/NRSF binds to the neuron restrictive silencer element (NRSE), amplifying its activity and thus silencing BDNF expression [256]. Moreover, a microarray analysis of inducible striatal cells expressing the first 548 amino acids of wild-type or mutant huntingtin in a regulated manner, driven by a tetracycline/doxycycline-responsive element, were shown to differentially express genes involved in cell signalling, transcription, lipid metabolism (cholesterol and fatty acid) and vesicle trafficking [257], suggesting that changes in gene expression trigger polyglutamine cell toxicity. Premature decreased expression of genes involved in signal transduction and calcium homeostasis were also reported in transgenic mice models of SCA1, expressing polyglutamine-expanded ataxin-1 (Table 1), before the onset of symptoms, or in SCA1 human tissue [258].

In conclusion, transcription repression could be an important mechanism in the pathogenesis of HD. Accordingly, the regional selectivity of the pathology can result from the genes whose transcription is impaired or modified [253,259,260]. Nevertheless, it is important to note that the transcription deregulation hypothesis does not exclude other mechanisms of pathogenesis, as modifications of gene transcription of specific genes can potentiate other mechanisms of cell death, like excitotoxicity and apoptosis.

### 3.8. Trophic Factor Starvation and Deregulation of Axonal Transport

Neurotrophic factors are growth factors known to regulate survival and differentiation of neurons (reviewed in [261,262]). It has been suggested that neurotrophic factors could slow, arrest or even promote regeneration in several neurodegenerative diseases.

Since clinical genetic analysis allows the detection of people at risk of becoming HD patients prior to the development of symptoms, therefore, a neuroprotective approach with trophic factors, aiming at reducing or preventing the neuronal death occurring in HD, could be initiated before striatal neurodegeneration. Different neurotrophic factors, namely nerve growth factor (NGF), BDNF, glial cell line-derived neurotrophic factor (GDNF) or ciliary neurotrophic factor (CNTF), have shown potent protective effects in animal models of HD [155,263-268]; for review see [269].

NGF has been shown to protect cholinergic striatal neurons in the QA and 3-NP models of HD [270], and to preserve TrkA and choline acetyltransferase mRNA expression levels [271,272]. Unfortunately, NGF administration protects solely cholinergic neurons, the only striatal neurons expressing TrkA receptors, and therefore, the main striatal population attained in HD, the GABAergic neurons, are not protected by NGF delivery [270,273].

BDNF is another neurotrophin that has been suggested to have therapeutic value in HD. BDNF is anterogradely transported from the cortex to the striatum and its expression is increased upon striatal lesioning [274], suggesting that the corticostriatal circuitry is essential for survival of striatal neurons under normal and pathological conditions. Thus, under normal conditions, TrkB mRNA is present in the striatum, but BDNF mRNA is not [275]. Moreover, BDNF expression is upregulated by wild-type huntingtin, therefore suggesting that it provides trophic support for both striatal and cortical neurons. Importantly, this beneficial effect is lost with mutated huntingtin [45], and a selective BDNF loss of expression was observed in CNS areas degenerating in HD patients [276]. Moreover, wild-type huntingtin, previously shown to have an anti-apoptotic effect by inhibiting the cleavage of procaspase-9 [277,278], promotes the vesicular transport of BDNF along the microtubules through huntingtin-associated protein-1 (HAP-1) and the p150Glued subunit of dynactin [279]. In contrast, vesicular BDNF transport is impaired by mutant huntingtin [279], implying a decreased release of BDNF from the cortical projections and, consequently, a deleterious effect on striatal...
neurons. Interestingly, production of conditional BDNF mutant mice that lack cortical BDNF, by the Cre-loxP recombination system, recapitulated most symptoms of HD mice models [280]. Furthermore, this study showed that the absence of anterograde cortical BDNF leads to early striatal deficits and age-dependent neuronal loss [280].

Previous studies have clearly pointed out that the early degeneration of the corticostraiatal pathway may be due to the accumulation of mutant huntingtin and the consequent dysfunction of the axonal transport [281,282]. While wild-type huntingtin appears to be required for normal axonal transport, expression of mutant human huntingtin exon-1 in larval neurons of Drosophila caused the disruption of axonal transport and neuronal cell death [283]. In fact, cytoskeleton destabilization appears to precede the nuclear accumulation of mutant huntingtin, and taxol, a compound known to stabilize the microtubules, further demonstrated this by preventing polyglutamine-induced cell death [284]. Under this perspective, doxycycline-inducible expression of mutant huntingtin was shown to block calcium-induced exocytosis in non-differentiated PC12 cells, concomitantly with a depletion in complexin II, a protein that appears to control the SNARE complex [285].

Delivery of BDNF to the CNS, using either adenoviral or adeno-associated viral vectors—gene delivery, has shown neuroprotective effects in the QA rat model of HD [286,287]. These data suggest that neurotrophins and particularly BDNF may have a role in HD pathogenesis and could be used in HD therapy.

BDNF is a potent survival factor for dopaminergic neurons and also promotes survival of noradrenergic, cholinergic, sympathetic, parasympathetic and sensory neurons [288]. BDNF transmits its signal by interaction with a multicomponent receptor complex containing the receptor tyrosine kinase RET and a glycosyl-phosphatidylinositol (GPI)-linked receptor, GDNF family receptor alpha1 (GFR-alpha 1) [289].

Although most studies with GDNF have been focused on the study of neuroprotective effects on dopaminergic neurons [290,291], with a particular emphasis on the neuroprotection of Parkinson’s disease, it has been shown that GDNF also protects noradrenergic neurons, calbindin-immunoreactive and striatonigral neurons in various experimental paradigms. In the striatum, both RET and GFR-alpha 1 are expressed and GDNF has been shown to have neuroprotective effects in QA lesioned rats [292-294]. GDNF increased tyrosine hydroxylase staining in both intact and lesioned striata and protected a fraction of calbindin-positive, but not parvalbumin immunoreactive striatal neurons against QA lesion [295]. In addition, GDNF preserved neuronal expression of glutamic acid decarboxylase, preprotachynin A and prodynorphin [294]. Protective effects were also obtained when either recombinant GDNF [264] or an AAV vector encoding GDNF were administered in a chronic 3-NP model of HD in rats [296]. Finally, a neuroprotective effect was also reported with neurturin, a GDNF analog. The effect on striatal projection neurons was reported to be more specific and efficient with neurturin than with GDNF [297].

Moreover, work with cultured mouse astrocytes suggests that riluzole upregulates the expression of different neurotrophic factors, namely GDNF, contributing to the neuroprotective effect of this anti-excitotoxic agent [298].

CNTF belongs to the family of cytokines with multiple actions in the CNS and other tissues (reviewed in [299]). CNTF was first identified in avian ocular tissue and named for its ability to support survival of parasympathetic neurons from chick ciliary ganglion [300,301]. It was later shown to support survival and differentiation of central and peripheral neurons, including sympathetic, embryonic motor, striatal, thalamic and hippocampal neurons [302-305]. CNTF is considered an injury protein that upon damage to the brain is released from astrocytes, the brain sources of CNTF [306].

CNTF interacts with a tripartite complex receptor at the cell surface composed of CNTF-Ralpha and two beta subunits, gp130 and LIFR, which following formation of the CNTF/CNTF-Ralpha/gp130/LIFR complex activate a phosphorylation cascade involving the phosphorylation of transcription factors (STATs), which dimerize and translocate to the nucleus, resulting in the transcription of target genes, particularly anti-apoptotic genes of the Bcl family [307,308]. CNTF has also been implicated in activation of other cell survival pathways, particularly the phosphatidylinositol 3-kinase and the mitogen-activated protein kinase pathways [309]. In addition to its direct effects over cells, CNTF can also have indirect effects. CNTF is upregulated in astrocytes in response to injury and exogenously administered CNTF induces astrocyte hypertrophy and modification of phenotype [310-315]. Moreover, CNTF astrocyte activation induces the production of other trophic factors, particularly fibroblast growth factor (FGF-2) and NGF, and enhances astrocyte detoxification capacity, thus supporting neuronal survival [316]. The CNTF-Ralpha can also be released from the cell membrane, by phospholipase C-mediated cleavage. The resulting soluble form can promote binding to the gp130/LIFR dimer in cells that are not highly responsive to CNTF alone [317,318]. Furthermore, as it happens with other neurotrophic factors, CNTF is internalised at distal axons and retrogradely transported to cell bodies. This allows CNTF to produce effects in regions distant from the administration area [319].

Anderson and collaborators performed a systematic study of the therapeutic properties of various neurotrophic factors, using the QA model. BDNF, NGF and neurotrophin 3 (NT-3) did not elicit any protection of striatal output neurons against QA, while CNTF afforded marked neuroprotection [263]. Several studies confirmed the neuroprotective effect of CNTF at neurochemical, neuropathological and behavioural levels in different toxin based animal models of HD [263]. CNTF administration was also shown to produce neuroprotective effects in a genetic in vitro model of HD [155].

Previously described side-effects of systemic delivered CNTF, dry cough, weight loss and acute phase response, have driven research into alternative delivery systems, particularly into the use of macroencapsulated cells engineered to produce CNTF. This approach has been used by the group of Patrick Aebischer in a clinical trial for amyotrophic lateral sclerosis that proved the safety and feasibility of the approach [320]. The delivery of CNTF by
encapsulated genetically modified cells in HD has later been addressed. Encouraging results were obtained with BHK cells secreting the human CNTF, when implanted in the striata of rodent and non-human primate, in acute and semi-chronic toxic models of HD [265,321-323]. The preclinical studies led to a clinical trial using this approach which ended recently [324,325]. The study aimed at evaluating the safety of intracerebral administration of CNTF in subjects with HD, using a device formed by a semipermeable membrane encapsulating up to $10^6$ human CNTF-producing BHK cells, releasing 0.15-0.5 µg CNTF/day. Six subjects with stage 1 or 2 HD had one capsule implanted into the right lateral ventricle; the capsule was retrieved and exchanged for a new one every 6 months, over a total period of 2 years. Improvements in electrophysiological results and no toxicity were observed, in correlation with capsules releasing the largest amount of CNTF, although CNTF release was low in 13 of 24 cases. The study showed the safety, feasibility, and tolerability of the procedure, but also showed that technical improvements are still needed to overcome the heterogeneous cell survival and the resulting decrease in CNTF release. Furthermore, it remains to be elucidated if this is the best delivery system to ensure a very prolonged therapy.

An alternative approach consists in making the cells express CNTF locally, by transducing the targeted brain region with viral vectors encoding CNTF. Accordingly, transduction of the rat brain with lentiviral vectors encoding CNTF led to behavioural, neuroanatomical and immunohistochemical neuroprotection in the QA model of HD [268]. The approach has been refined to allow regulation of gene expression by engineering tetracycline-regulated lentiviral vectors encoding human CNTF. A dose-dependent neuroprotective effect was observed in this model of HD [326]. The effects of long term lentiviral-mediated expression of CNTF in the striatum of HD transgenic mice (YAC72 mice) were also evaluated [327]. CNTF expression reduced the over activity observed in YAC72 mice and the number of striatal dark cells [327]. The viral vector approach has considerable interest in a preclinical setting for research purposes. Nevertheless, there are safety concerns, which until now have precluded the clinical use of lentiviral vectors.

Other pro-survival effects may be related with the activation of the Akt signalling by the insulin-like growth factor 1 (IGF-1), which is involved in phosphorylation of huntingtin on serine 421, thus abrogating polyglutamine toxicity [328]. Nevertheless, phosphatidylinositol 3-kinase-dependent Akt activation in mutant striatal cells (derived from the knock-in mice) was associated with an early pro-survival response apparently associated with NMDA receptor activation and thus Ca$^{2+}$-dependent [329].

The putative mechanisms of HD pathogenesis are represented in Figure 2.

4. GENE AND PROTEIN SILENCING AND CELL REPLACEMENT THERAPY IN HD

Most of previously described strategies, as they envision therapy after huntingtin protein synthesis, can be considered late approaches. In opposition, early interventions aim at blocking the mutant protein production in the cell. Among the early strategies, the ideal therapy, but highly challenging, would involve gene repair [330]. Alternatively, gene-silencing techniques, aiming at reducing intracellular concentration of huntingtin or other polyglutamine carrying proteins, are a promising strategy for therapy of polyglutamine diseases. Downregulation of mRNA can be done through anti-sense technology [331,332] or RNA interference. A different, downstream intervention consists in promoting huntingtin removal through intrabodies.

The anti-sense (AS) approach allows downregulation of gene expression by blocking translation of mRNA, mainly through the action of RNase H ribonuclease. Boado and collaborators generated a series of AS oligodeoxynucleotides (ODNs) complementary to the huntingtin transcript, which markedly decreased the abundance of the huntingtin-green fluorescence fusion protein to 40-46% of the control levels [332]. Gene silencing by double-stranded RNAs (dsRNAs) designated as RNA interference induces the sustained down-regulation of the target gene by promoting mRNA degradation. This technology has recently been tested in mammalian and Drosophila cells carrying a polyglutamine expanded SBMA transgene [333]. Different dsRNAs were able to inhibit expression of non-repetitive sequence transcripts of the truncated human androgen receptor and to rescue mammalian cells from polyglutamine toxicity. The dilemma of silencing techniques is that it is not known if the cells can withstand a depletion of huntingtin that could compromise its normal role in the cell. A silencing modality that would allow a selective blockade of mutant huntingtin expression would be highly desirable. Such selective silencing has been achieved by the group of Henry Paulson with dsRNAs in cell models of Machado-Joseph disease/SCA3 (Table 1). The authors targeted a single-nucleotide polymorphism linked to the mutant SCA3 allele with small interfering RNAs and observed exclusive silencing of mutant ataxin-3 allele while sparing expression of the WT allele [334].

More recently, Davidson and collaborators showed that RNAi gene therapy for ataxin-1 can improve cellular and behavioural characteristics in a SCA1 mouse model. In the case of SCA1 the silencing of both wild-type and mutant ataxin-1 would probably not be so problematic as in other polyglutamine expansion diseases because ataxin-1 knockout mice do not display major pathologic features [335]. These studies suggest that RNAi is a highly promising technology for therapy of dominant neurodegenerative diseases.

Another approach involves modulating huntingtin toxicity and clearance through specific binding to other proteins. Intrabodies are a modality of intracellular immunization that allows suppression of specific proteins [336]. Intrabodies are engineered single-chain antibodies in which the variable domain of the heavy chain is joined to the variable domain of the light chain through a peptide linker, preserving the specificity and affinity of the parent antibody, and designated as single-chain variable region fragment antibodies (scFv). In an in vitro model of HD, Lecerf and collaborators (2001) showed that a human scFv antibody specific to the N-terminal 17 residues of huntingtin protein was able to counteract aggregate formation. The authors claim that the intrabody binds to huntingtin, decreases huntingtin propensity to form aggregates, and
Fig. (2). Potential mechanisms inducing HD pathogenesis include excitotoxicity, mitochondrial dysfunction, apoptosis, proteasome blockade and transcription impairment, either alone or combined with each other. The polyglutamine expanded huntingtin gene is transcribed and translated into a mutant huntingtin protein, which is cleaved by caspases into huntingtin fragments. Overactivation of caspases by mutant huntingtin can lead to apoptosis. Mutant huntingtin or its fragments can interact with the NMDA receptor promoting glutamate-mediated excitotoxicity. Mutant huntingtin or its fragments may also associate with mitochondria leading to mitochondrial dysfunction. The huntingtin fragments are ubiquitinated and aggregate in the cytoplasm and nucleus. Polyubiquitination targets the fragments to the proteasome, which is consequently blocked leading to cellular toxicity. In the nucleus, huntingtin fragments bind different molecules, particularly transcription factors that have polyglutamine tracts leading to transcriptional dysfunction.

forms a soluble complex that undergoes normal protein turnover [337]. Then, Ali Khoshnan generated scFvs antibodies that recognize the huntingtin polyproline domain and significantly inhibited huntingtin aggregation and cell death, demonstrating its potential therapeutic value in HD [338]. Analogous strategies with either a targeted peptide [339] or a monoclonal antibody [340], targeting elongated polyglutamine, showed efficacy on in vitro models. In vivo studies will be important to confirm the therapeutic potential of these protein-silencing strategies. Nevertheless, and as it happens with the previous described gene silencing approaches, binding huntingtin with intrabodies could block the normal huntingtin function. A promising alternative would involve the use of intrabodies that selectively recognize mutant huntingtin.

An alternative approach consists in HD restorative therapy through the transplantation of neural cells from several sources that replace the dying striatal neurons. Transplantation of human fetal striatal tissue into the striatum of one patient with clinical features of HD showed the survival of the fetal neural cells and the reconstitution of damaged neuronal connections, which were not affected by the genetically predetermined disease process. Unfortunately, the patient died 18 months after transplantation from cardiovascular disease [341]. Grafting of human fetal striatal neuroblasts into the striatum was further performed in five patients with mild to moderate HD. Three out of five patients showed increased metabolic activity, concomitantly with an improvement of motor and cognitive functions. Nevertheless, two other HD patients showed progressive decline upon transplantation [324]. The clinical improvement of the three HD patients was further associated with an increase in cortical metabolism, as analysed in a follow up studied by positron emission tomography measurements of brain glucose metabolism [342]. The feasibility and survival of grafts were further demonstrated in other studies after unilateral or bilateral transplantation of human fetal striatal tissue in symptomatic HD patients [343,344]. Although fetal neural transplant therapies have become clinically important, the establishment of their efficacy and their real benefits requires the analysis of a larger number of patients, and, in particular, a deep investigation into the basic mechanisms of neural survival. Nevertheless, the low availability of fetal cells and ethical concerns prevent progress in this area. Therefore the use of neural stem cells has been proposed as an alternative source.
of material for transplantation. In recent studies, intrastratial rat transplantation with human neural stem cells one week after QA injection [345] or one week before 3-NP systemic administration [346] was shown to restore motor deficits and delay neurodegeneration. Nevertheless, implantation of human-derived immortalized cell line in rats submitted to an excitotoxic lesion did not cause significant beneficial effects [347]. Additionally, predifferentiation of a neural stem cell line into a homogenous population of cells with a GABAergic phenotype and their further transplantation into the rat striatum subjected to QA injection showed a stable maintenance of the acquired phenotype and further neurite processing [348], supporting cell replacement strategies for HD treatment.

While neural (stem) cell transplantation still requires more intensive research, a complementary therapy could be based on boosting adult brain neurogenesis. Recent reports have looked at neurogenesis in human and animal models of HD. In the adult postmortem human brain, a significant increase in cell proliferation was observed in the subependymal layer in HD, compared with control brains [349]. An increase in cell proliferation in human HD brains increased with pathological severity and CAG repeats [349]. Concordantly, an increased cell proliferation and neurogenesis was observed in the subventricular zone of adult rats lesioned with QA, highly suggestive of formation of new neurons and their migration to damaged areas of the striatum [350]. Nonetheless, decreased neurogenesis has been observed in the hippocampus (dentate gyrus) of older (20-week-old) R6/1 [351] or 12-week-old R6/2 [352] transgenic HD mice.

**CONCLUSIONS**

The mechanistic insights described during the last years suggest different therapeutic approaches aiming at each of the cellular targets that contribute to HD pathogenesis, namely by: i) blocking excitotoxicity with NMDA antagonists; ii) improving the energetic state through metabolic enhancers; iii) blocking proteolysis and/or apoptosis with caspase inhibitors; iv) interfering with aggregation using transglutaminase inhibitors; v) reducing mutant huntingtin toxicity with chaperones or disaccharides; vi) upregulating transcription by inhibiting histone deacetylases; vii) silencing mutant huntingtin expression; or viii) replacing lesioned neurons by transplantation of neural stem cells.

Moreover, an active investigation in polyglutamine expansion diseases will bring about new molecular targets not only for HD therapy, but also for the cure of other neurodegenerative diseases. While some of the therapies against HD pathogenesis are still under experimental analysis or taking the first steps into clinical trials, an exhaustive study of combination therapies is required both in *in vitro* and *in vivo* HD models.

**LIST OF ABBREVIATIONS**

| 3-NP | 3-nitropropionic acid |
| AMPA | α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate |
| AS | Anti-sense |
| BDNF | Brain-derived neurotrophic factor |
| CBP | CREB-binding protein |
| CNTF | Ciliary neurotrophic factor |
| CREB | cAMP-responsive element-binding protein |
| FGF-2 | Fibroblast growth factor 2 or basic fibroblast growth factor |
| GDNF | Glial cell line-derived neurotrophic factor |
| GFR | GDNF family receptor |
| GPI | Glycosyl-phosphatidylinositol |
| HAP1 | Huntingtin-associated protein 1 |
| HD | Huntington’s disease |
| HIP-1 | Huntingtin interacting protein-1 |
| IGF-1 | Insulin-like growth factor 1 |
| iHDACs | Inhibitors of histone deacetylases |
| IP3R | Inositol-1,4,5-trisphosphate receptor |
| KA | Kainic acid |
| MPT | Mitochondrial permeability transition |
| NGF | Nerve growth factor |
| NMDA | N-methyl-D-aspartate |
| NRSE | Neuron restrictive silencer element |
| NRSF | Neuron restrictive silencer factor |
| ODNs | Oligodeoxynucleotides |
| QA | Quinolinic acid |
| REST | Repressor element-1 transcription factor |
| SAHA | Suberoylanilide hydroxamic acid |
| Sp1 | Specificity protein 1 |
| TAF | TBP-associated factor |
| TATA | Adenine- and thymine-rich promoter sequence |
| TBP | TATA box binding protein |

**REFERENCES**


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