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Slowing of neurodegeneration in Parkinson’s disease and Huntington’s disease: future therapeutic perspectives

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Several important advances have been made in our understanding of the pathways that lead to cell dysfunction and death in Parkinson’s disease and Huntington’s disease. These advances have been informed by both direct analysis of the post-mortem brain and by study of the biological consequences of the genetic causes of these diseases. Some of the pathways that have been implicated so far include mitochondrial dysfunction, oxidative stress, kinase pathways, calcium dysregulation, inflammation, protein handling, and prion-like processes. Intriguingly, these pathways seem to be important in the pathogenesis of both diseases and have led to the identification of molecular targets for candidate interventions designed to slow or reverse their course. We review some recent advances that underlie putative therapies for neuroprotection in Parkinson’s disease and Huntington’s disease, and potential targets that might be exploited in the future. Although we will need to overcome important hurdles, especially in terms of clinical trial design, we propose several target pathways that merit further study. In Parkinson’s disease, these targets include agents that might improve mitochondrial function or increase degradation of defective mitochondria, kinase inhibitors, calcium channel blockers, and approaches that interfere with the misfolding, templating, and transmission of α-synuclein. In Huntington’s disease, strategies might also be directed at mitochondrial bioenergetics and turnover, the prevention of protein dysregulation, disruption of the interaction between huntingtin and p53 or huntingtin-interacting protein 1 to reduce apoptosis, and interference with expression of mutant huntingtin at both the nucleic acid and protein levels.

Introduction

Parkinson’s disease is a common neurodegenerative disorder with an age-adjusted incidence of 13.5–13.9 per 100 000 person-years and an age-related prevalence of roughly 115 per 100 000 population. The frequency of the disorder is about 1·3 cases per 100 000 people younger than 45 years of age, 310 per 100 000 in those aged 75–85 years, and 13·5–13·9 per 100 000 person-years and an age-related frequency of about 1·3 cases per 100 000 people younger than 45 years of age, 310 per 100 000 in those aged 75–85 years, and 13·5–13·9 per 100 000 person-years and an age-related frequency of about 1·3 cases per 100 000 people younger than 45 years of age, 310 per 100 000 in those aged 75–85 years, and 13·5–13·9 per 100 000 person-years. The incidence of Parkinson’s disease is highest in people of western European descent (with the exception of the highest regional prevalence around Lake Maracaibo, Venezuela) with, for example, a prevalence of 12·3 per 100 000 people in the UK, and a lower frequency in the rest of the world (eg, one per million people of Asian and African descent). At present, management of the disease is limited to a few treatment options in the early stages for the control of the hyperkinetic movement disorders and psychiatric problems, but no treatment modifies the course of the disease. In both Parkinson’s disease and Huntington’s disease, neurodegeneration is progressive and leads to severe disability and reduced quality of life and life expectancy.

Treatments that slow or prevent disease progression in both disorders are a major unmet need. Recent scientific advances have identified new pathways in these diseases that suggest new targets for the development of neuroprotective drugs. In this review, we will summarise some of the most promising candidate targets for neuroprotection. Please see appendix for a list of supplementary references.

Challenges and solutions: Parkinson’s disease

Several drugs exist for the symptomatic relief of the dopaminergic motor features in Parkinson’s disease (figure 1). Nonetheless, disease progression is inexorable, and patients ultimately develop disability, which is largely related to the development of non-dopaminergic features such as gait disturbance and dementia. A crucial goal is the development of a neuroprotective treatment that slows
Disease progression and has beneficial effects on the full range of dopaminergic and non-dopaminergic features.

The development of neuroprotective agents evidently relies on an understanding of the causes and pathogenetic pathways responsible for the neurodegeneration that underlies Parkinson’s disease. Several reviews have summarised recent advances in this specialty. The process of neuronal degeneration probably progresses through various stages, all of which might be amenable to intervention (figure 2). The genetic composition and environment of a cell determines its biochemical signature and potentially its predisposition for degeneration—the molecular prodrome. Over time, these defects will adversely affect cell function, but initially to a degree that is insufficient to cause dysfuction because of the cell's own capacity or neighbouring cells' ability to compensate. During this phase, the cell is damaged but no abnormal neuronal activity is evident. Eventually, the compensatory mechanisms begin to fail and a clinical and pathological prodrome can be identified. This phase is called dysfunction. Finally, cell degeneration occurs and clinical features become evident. Each of these stages could potentially be targeted to protect or restore function to a cell (see reference 6 for a review). Improved insight into the genetic architecture of Parkinson’s disease and how gene mutations or risk factors initiate or contribute to the cascade of events that leads to neurodegeneration should provide new targets and enhance our ability to develop neuroprotective interventions. A key aim in the development of an effective neuroprotective drug is to target crucial pathogenetic pathways that are common to neurodegeneration and affect both dopaminergic and non-dopaminergic transmitter pathways, and would be anticipated to slow the development and progression of both motor and non-motor features of the disease.

Figure 1: Existing and evolving treatments for Parkinson’s disease

Drugs that are available or in development are shown according to their mechanism of action, target indication, and phase of development. Many compounds are presently known only by a code. For a complete list of ongoing and completed trials, see ClinicalTrials.gov. ER=extended release. JP=available in Japan.
Several clinical trials have investigated the potential neuroprotective properties of drugs in Parkinson’s disease, but so far such trials have had major limitations in their design and interpretation because of the heterogeneity of the populations studied and the potentially confounding symptomatic and pharmacological effects of study interventions. There is now great interest in the use of genetically defined populations (eg, carriers of LRRK2 or glucocerebrosidase mutations) or prodromal symptom-enriched groups of people (ie, those with a raised risk of developing Parkinson’s disease and a larger number of residual functioning neurons), to test putative neuroprotective treatments in a homogeneous group of patients to reduce variability and increase the chance of detecting a treatment effect. New study designs are now available for use in neuroprotective trials that enable a disease-modifying effect to be distinguished from a symptomatic effect.

The development of a biomarker that provides an objective measure of the underlying disease state, which could be used to ensure accurate diagnosis and as an outcome measure to provide an objective determinant of disease progression, would be a great advance in defining a neuroprotective treatment.

Challenges and solutions: Huntington’s disease
Unlike Parkinson’s disease, no major treatment has been developed specifically for Huntington’s disease. Some symptomatic treatment is available for some motor or psychiatric features. The cause of Huntington’s disease is a highly polymorphic CAG trinucleotide repeat expansion in exon 1 of the gene encoding the huntingtin protein. By contrast with Parkinson’s disease, neuroprotective strategies could be available immediately for carriers of mutant huntingtin identified by genetic testing in their prodromal phase. Although this idea seems straightforward, it is not without limitations because the natural course of the disease remains unclear. Since Huntington’s disease is a genetic disease, affected patients have abnormal huntingtin from the very first moment of the protein’s expression, which suggests that huntingtin-related biochemical or neuronal abnormalities might be present from the exact same point. Collectively, the evidence strongly supports a disease mechanism in which mutant huntingtin expression in several cell types within at least the striatum and cortex is probably needed for disease development and progression. The early period of normality is followed by 10–20 years of a prodromal phase of the disease, during which deleterious but subclinical events occur until the disease manifests itself and is diagnosed. The prodromal presymptomatic phase is the ideal time to intervene.

Targets for neuroprotection
Several exciting potential targets for drug intervention to lessen neurodegeneration in both Parkinson’s disease and Huntington’s disease are in development. Some of these targets are single molecules, whereas others involve the manipulation of biochemical pathways and several genes or proteins. Different approaches might be applicable at different stages of the disease and in different populations (figure 3). For example, primary prevention will be appropriate for the molecular prodrome, which is determined by, for example, genetic predisposition for Parkinson’s disease. Thus, LRRK2 kinase inhibitors might be suitable for people who carry the LRRK2 mutation and glucocerebrosidase protein.

Figure 2: Evolution of molecular pathology in Parkinson’s disease—the four putative phases towards neurodegeneration

Figure 3: Potential neuroprotective strategies
These strategies correspond with, and are adapted for, the four phases shown in figure 2. ROS=reactive oxygen species.
modulators for those with GBA mutations. Promotion or facilitation of biochemical compensatory mechanisms can be achieved with pro-mitochondrial, anti-inflammatory, anti-excitatory, or anti-oxidant strategies, which enhance specific pathways responsible for protein clearance or degradation and reduce toxic oligomers or aggregates. Multifunctional and multitarget approaches will probably be needed to restore neuronal health when cell dysfunction is present. Such approaches might take the form of any of the aforementioned mechanisms in combination, or trophic factors. Cell replacement therapies or trophic factors requiring direct intracerebral injection would be appropriate for the more advanced stages of disease and neurodegeneration.

Substantial evidence supports the involvement of mitochondrial dysfunction and oxidative stress, inflammation, autophagy, mitophagy, apoptosis, and changed protein handling in the pathogenesis of Parkinson’s disease, whereas present research in Huntington’s disease focuses on mitochondrial dysfunction, changed protein interactions (gain-of-function of mutant huntingtin), and reductions in huntingtin. We describe some of the newest and most promising targets and candidate neuroprotective therapies for these two disorders.

Mitochondrial function and oxidative stress pathways

Evidence suggests that defects of the respiratory chain (complex I), increased accumulation of mitochondrial DNA mutations, abnormal mitochondrial calcium homeostasis, defective autophagic removal of mitochondria (mitophagy), and increased oxidative stress are all involved in the pathogenesis of Parkinson’s disease, whereas present research in Huntington’s disease focuses on mitochondrial dysfunction, changed protein interactions (gain-of-function of mutant huntingtin), and reductions in huntingtin. We describe some of the newest and most promising targets and candidate neuroprotective therapies for these two disorders.

Attention has recently focused on glucagon-like peptide 1 (GLP-1), which originates in the L cells of the intestine. GLP-1 and the longer half-life GLP-1-like peptide exendin-4 (EX-4) have been used in the treatment of type 2 diabetes. GLP-1 and EX-4 promote cellular growth, increase mitochondrial biogenesis, reduce apoptosis, and might be anti-inflammatory, although the precise mode(s) of action remain uncertain. An open-label randomised study of exendin (exenatide) in Parkinson’s disease has just been completed and further studies are now planned.

Insights into the pathways for mitochondrial fission and fusion, turnover, and destruction by autophagy (mitophagy) have come from studies in which investigators have assessed the function of the proteins encoded by the parkin and PINK1 genes that cause autosomal recessive Parkinson’s disease. Mutations in parkin and PINK1 reduce turnover of mitochondria and of respiratory chain proteins. The accumulation of damaged respiratory chain proteins or mitochondria might contribute to the bioenergetic defects in neurodegenerative diseases. The observation that autophagy protein expression is reduced in the brain affected by Parkinson’s disease further supports the importance of autophagy–mitophagy pathways to pathogenesis. Treatments that enhance the removal of defective mitochondria could potentially improve or restore neuronal function. Impairment of mitochondrial motility as a consequence of defective fission and fusion, or abnormalities of axon motors for transport of mitochondria, could lead to impaired synaptic function and even a dying-back axonal neurodegeneration that might be of particular relevance to Parkinson’s disease.

Post-mortem studies show a substantial loss of dopamine terminal staining, with a complete loss of staining as soon as 4 years after diagnosis but with relative preservation of melanised substantia nigra pars compacta neurons, which is consistent with a dying-back process. In Huntington’s disease, in-vitro and in-vivo evidence obtained post mortem has indicated a deleterious effect of mutant huntingtin on mitochondrial function. First, mutant huntingtin interacts directly with mitochondria to impair calcium homeostasis and reduce membrane potential. Second, mutant huntingtin changes the balance between mitochondrial fission and fusion under the dynamin-related protein 1. Third, mutant huntingtin also decreases complex II or III activity. Mutant huntingtin also inhibits the expression of PGC-1a, which in turn will compromise mitochondrial biogenesis and function. Finally, increased N-methyl-D-aspartate (NMDA) receptor activity, which has been well described in Huntington’s disease, contributes to mitochondrial dysfunction.

Trafficking dysfunction, including vesicular transport of brain-derived neurotrophic factor (BDNF), a crucial
prosurvival factor of striatal neurons, is a contributor to neuronal death in Huntington’s disease. Autophagy pathways are important in the disease’s pathogenesis, and strategies that promote clearance or block formation of mutant huntingtin offer the potential for the treatment of Huntington’s disease, as might delivery of growth factors or gene therapy.

Calcium handling
Calcium homoeostasis, receptor activity, and calcium-evoked oxidative stress are recognised as potential contributors to the pathogenesis of Parkinson’s disease and potential targets for therapeutic intervention. Evidence suggests that over time, pacing of nigral dopaminergic neurons converts from reliance on sodium channels to L-type calcium channels (Ca\(^{\text{v}}\)1·3) to maintain autonomous activity. This dependence can be reversed, and protection against toxin-induced damage achieved, by the blockade of these channels with calcium channel-blocking agents, such as isradipine. The Ca\(^{\text{v}}\)1·3 channels generate mitochondrial-mediated oxidative stress during autonomous activity, which in turn induces mitochondrial uncoupling as a protective mechanism. Selective Ca\(^{\text{v}}\)1·3 channel inhibitors have recently been developed and offer a new approach to disease modification in Parkinson’s disease.

Kinase pathways
Several observations indicate that phosphorylation pathways are important in the pathogenesis of Parkinson’s disease and might be suitable targets for drug development. α-synuclein can undergo phosphorylation at several sites; phosphorylation at serine 129 constitutes the major form of the protein in Lewy bodies. The Ala53Thr α-synuclein mutation, which causes familial Parkinson’s disease, increases the amount of S-129 phosphorylation. S-129 phosphorylation of α-synuclein reportedly enhances its aggregation, modifies dopamine transporter function, and promotes toxicity to dopaminergic cells, which is increased further by oxidative stress and proteasomal inhibition.

Mutations in LRRK2 are quite common causes of familial and apparently sporadic cases of Parkinson’s disease. LRRK2 is a large and multifunctional protein kinase that includes a GTPase domain. Although pathogenic mutations have been identified at several sites, those at the kinase domain are the most frequent, and include the common Gly2019Ser mutation. Several mutations, including Gly2019Ser, cause increased phosphorylation, which can result in autophosphorylation of LRRK2. Inhibitors of LRRK2 kinase have now been developed and offer the opportunity to investigate whether prevention of autophosphorylation or phosphorylation of other target proteins can modify the deleterious effects of LRRK2 mutations. However, this strategy is limited by the absence of information about the physiological role of LRRK2 and whether inhibition could be confined to the CNS. Expression seems to be widespread, with especially high amounts of the protein in lymphocytes. Its role in the immune system is being actively investigated and could provide an alternative route to the development of a protective strategy for Parkinson’s disease.

LRRK2 kinase inhibitors are still in the very early stages of development for clinical use. The development of specific therapies such as kinase inhibitors, designed to treat defined subgroups of patients such as LRRK2 carriers, is an attractive prospect, and such interventions might have wider applicability in view of the possibility of overlapping pathogenetic pathways with different genetic causes. A LRRK2 kinase inhibitor has shown protection to toxins in inducible pluripotent cells derived from patients with mutations in LRRK2 and PINK1.

Changes in protein handling
Parkinson’s disease
Targeting of the formation and clearance of unwanted proteins is another logical approach to the development of a candidate neuroprotective therapy for Parkinson’s disease, since the disease is characterised by the accumulation of aggregated proteins in the form of Lewy bodies and Lewy neurites. Protein accumulation could result from increased production or impaired clearance. Unwanted proteins are usually cleared from the cell by the ubiquitin proteasome or autophagy–lysosomal systems, and defects in the ubiquitin proteasome and lysosomal function have been identified in patients with Parkinson’s disease, and mutations in this protein are associated with familial forms of the disease. Importantly, duplication or triplication of the wild-type protein also causes rare cases of Parkinson’s disease, which indicates that overexpression of the wild-type protein alone is sufficient to cause the disorder.

α-synuclein toxicity is thought to be related to protein accumulation with misfolding and the formation of toxic oligomers. Protein accumulation with misfolding might occur as a result of increased production (genetic cases), impaired clearance (abnormalities in lysosome or proteasome function), or stochastically. A vicious cycle could therefore exist, consisting of the formation of toxic oligomers, fibrils, and aggregates; secondary inhibition of ubiquitin proteasome and lysosomal systems by α-synuclein aggregates; further protein accumulation; and ultimately cell death. α-synuclein inclusions identical to Lewy bodies and Lewy neurites have been recorded in embryonic dopamine neurons that had been implanted into the striatum of patients with Parkinson’s disease. The embryonic tissue was derived from several unrelated donors, which makes it unlikely that these cells were the primary source of this pathology. Rather, the findings are consistent with transfer of α-synuclein from affected to
unaffected nerve cells. On the basis of these observations, it has been postulated that α-synuclein is a prion and that Parkinson’s disease is a prion-like disorder.54,55

α-synuclein can be transferred to unaffected nerve cells both in vitro and in vivo.56,57 Inoculates derived from the brains of elderly, clinically affected, α-synuclein transgenic mice accelerate the course of the disease when injected into the brains of young intact transgenic animals.58,59 More recently, misfolded α-synuclein filaments injected into the striatum have been shown to be taken up into nerve cells and induce behavioural abnormalities, Lewy pathology neurodegeneration in nigral dopamine neurons, and extension to involve anatomically related structures in non-transgenic wild-type mice.60 Importantly, these features are not recorded in α-synuclein-null mice, which suggests that host α-synuclein has an essential role in this process, possibly through a prion conformer reaction in which misfolded α-synuclein acts as a template to promote misfolding of native α-synuclein. Together, these observations suggest novel targets for candidate neuroprotective agents (figure 4).

The first of these new targets involved agents that reduce expression of wild-type α-synuclein and thus reduce the natural substrate for a prion or templating reaction; (2) upregulation of chaperones that promote refolding or clearance of abnormal proteins; (3) facilitation of UPS or autophagy/lysosomal function to promote clearance of unwanted proteins; (4) interference with the prion conformer whereby misfolded α-synuclein acts as a template to promote the conversion of wild-type α-synuclein; (5) agents or immune approaches targeted to remove toxic α-synuclein oligomers or aggregates; (6) increased glucocerebrosidase stability or trafficking through the endoplasmic reticulum to normalise α-synuclein metabolism and lysosomal function. These interventions (1–6) are designed to prevent or reduce the toxic effects of α-synuclein oligomers or aggregates on vital cell processes (eg, mitochondrial function and axonal transport). Intervention (7) represents agents that prevent release of α-synuclein from affected cells and/or the uptake of α-synuclein into healthy unaffected cells whereby the process might extend throughout the nervous system.6

Dashed arrows represent inhibition and solid arrows represent pathways of progression. UPS=ubiquitin proteasome system.
coupled with α-synuclein-positive inclusions. A recent study emphasises the reciprocal association between the activity of the lysosomal glucocerebrosidase enzyme (GCase) and α-synuclein concentrations. The GBA gene encodes this enzyme and mutations in the gene, which are a significant risk factor for Parkinson’s disease, are associated with reduced GCase activity and an increase in α-synuclein concentrations in the brain, whereas increased expression of α-synuclein in cell models induces a proportionate reduction in GCase. GCase activity is significantly decreased in sporadic Parkinson’s disease substantia nigra. The basis of this reciprocal association between GCase and α-synuclein (figure 5) suggests that in patients with GBA mutations, GCase activity could be enhanced, or mis-trafficking of protein corrected, by brain-penetrant small chaperone-like proteins, which might in turn be associated with a reduction in α-synuclein levels. Similarly, drugs like rapamycin that stimulate autophagy and protein clearance are protective in model systems. Other approaches could include gene delivery of defective or missing ubiquitin proteasome or lysosomal components and drugs such as glucocorticoids that help the clearance of abnormal proteins and prevent the cascade of continuing protein accumulation and cell death.

A further new target is to interfere with a prion conformer reaction. The molecular mechanisms responsible for α-synuclein templating have not yet been defined, but the identification of these signals could be important targets for putative neuroprotective agents.

Another potential target is to reduce the amounts of toxic α-synuclein oligomers, amyloid aggregates, or both. Exposure of cultured nerve cells to α-synuclein oligomers, fibrils, and aggregates can induce cell death. Therapeutic approaches could include kinase inhibitors (see above), agents that inhibit ubiquitination, and polyphenols such as curcumin that stimulate autophagy and protein clearance are protective in model systems. Other approaches could include gene delivery of defective or missing ubiquitin proteasome or lysosomal components and drugs such as glucocorticoids that help the clearance of abnormal proteins and prevent the cascade of continuing protein accumulation and cell death.

A general approach has been developed to reduce oligomer levels, and vaccination with human α-synuclein reduces aggregate formation in transgenic animals. More recent evidence shows that antibodies directed against α-synuclein specifically target and promote the clearance of extracellular α-synuclein by microglia, and not neuronal cells or astrocytes. Therefore, these antibodies might be of greatest value in preventing spread to neighbouring cells. A preliminary clinical trial (AFFiRiS), which is testing the AFFITOPE vaccine candidate PD01 that targets α-synuclein, has recently been initiated in patients with Parkinson’s disease (ClinicalTrials.gov identifier NCT01568099). However, in the planning of such studies, it must also be considered that Lewy bodies might represent an aggresome-like structure and could therefore be protective, and a treatment that removes α-synuclein aggregates might accelerate Parkinson’s disease progression. It is also notable that post-translational modification of α-synuclein (eg, nitration) might bypass or break immune tolerance, activate the immune system, and exacerbate Parkinson’s disease pathology. Manipulation of this immunological response could provide potential to modify the progression of Parkinson’s disease pathology. An alternative target might be the unfolded protein response, which upregulates in response to increased amounts of misfolded proteins and reduces protein translation through phosphorylation of the α subunit of eukaryotic translation initiation factor (eIF2α -P). Increased amounts of this factor have been recorded in patients with Parkinson’s disease, and agents that promote dephosphorylation are protective in models of prion disease.

Knockout of wild-type α-synuclein would restrict the amount of native protein available to participate in any prion-like reaction in which misfolded protein acts as a template to promote misfolding of the wild-type protein. Agents that knockout or reduce expression of wild-type α-synuclein might prevent continued α-synuclein aggregation, neuronal degeneration, and cell transfer. In this regard, the use of RNA interference (RNAi) or antisense oligonucleotides to reduce α-synuclein is a focus of present research. α-synuclein transfer does not induce aggregation or degeneration in α-synuclein-null neurons or animals. The precise physiological role of α-synuclein is not known, but knockout animals seem to be free of serious physiological deficits, and perhaps only a partial or short-term reduction is needed to interfere with the prion reaction and reset the protein equilibrium.

A final potential approach is to use agents that prevent transfer of α-synuclein. Braak and colleagues postulate that α-synuclein pathology develops in a sequential and predictable manner, and involves transfer from affected
to unaffected neurons. Similar findings have been noted in wild-type mice exposed to intrastriatal injections of misfolded α-synuclein.81 Although the precise mechanism by which transfer occurs is not known, agents that inhibit lysosomal function increase endosomal release and uptake of α-synuclein by recipient cells,84 and agents that block endocytosis reduce α-synuclein mediated damage in experimental models.57 It might therefore be anticipated that novel drugs that inhibit endocytosis could reduce transmission in patients with Parkinson’s disease.

None of these approaches has yet been formally tested in patients with Parkinson’s disease, and a huge amount of preclinical work will be necessary to identify the most promising compounds, define their safety profile, and test for efficacy in preclinical models before clinical trials can begin.

**Huntington’s disease**

A neuropathological hallmark of Huntington’s disease is the presence of neuronal nuclear inclusions and cytoplasmic aggregates of misfolded mutant huntingtin.81 As such, several strategies have been designed to reduce huntingtin mRNA expression or protein amounts in experimental models. The first of these strategies is to ablate mutant huntingtin expression. A reduction in the synthesis of mutant huntingtin should ameliorate toxicity if it is delivered to the key affected cells. The blockade of mutant huntingtin expression in a tet-regulated conditional mouse model of Huntington’s disease resulted in a behavioural improvement and a reduction of inclusion bodies.82 More clinically relevant methods include intracerebroventricular infusion to viral vector-mediated delivery.83–87 Transient infusion into the cerebrospinal fluid of symptomatic Huntington’s disease mouse models of antisense oligonucleotides that catalyse RNase H-mediated degradation of huntingtin mRNA not only delays disease progression but also mediates a sustained reversal of disease phenotype that persists longer than the huntingtin knockdown.86 Reduction of wild-type huntingtin, and mutant huntingtin, produces the same sustained disease reversal. Antisense oligonucleotides have also been developed for the CAG repeat region to decrease specifically the mutant huntingtin load without affecting the wild type.85 Plans for phase I trials in Huntington’s disease are underway with antisense oligonucleotides. As with antisense oligonucleotides, the RNAi are designed to target either the mutant huntingtin (allele-specific) or both forms (mutant and wild-type—i.e., nonallele-specific). Non-allele-specific safety studies in non-human primates achieved a significant reduction in striatal huntingtin load without any safety concerns.86,87 The developmental role of huntingtin88 and its unknown functions in load without any safety concerns.87 Plans for phase I trials in Huntington’s disease are underway with antisense oligonucleotides. As with antisense oligonucleotides, the RNAi are designed to target either the mutant huntingtin (allele-specific) or both forms (mutant and wild-type—i.e., nonallele-specific). Non-allele-specific safety studies in non-human primates achieved a significant reduction in striatal huntingtin load without any safety concerns.86,87 The developmental role of huntingtin88 and its unknown functions in unaffected cell types, however, necessitated the development of allele-specific RNAi as a more viable clinical method. This strategy was validated in a back-to-back comparison of miRNA versus siRNA—two RNAi methods taking advantage of different interference properties.89,90 Other strategies include targeting of the single-nucleotide polymorphisms occurring in some, but not all, mutant alleles of huntingtin.91

A second strategy is to counteract abnormal protein production with antibody fragments. Normalisation of protein homeostasis can be achieved with engineered intracellular antibody fragments—so-called intrabodies—that bind with high selectivity to the desired target. These intrabodies (also known as nanobodies) can be selected and manipulated as genes, allowing the full range of genetic engineering to produce multifunctional constructs that can change the folding, interactions, intracellular localisation, and turnover kinetics or concentrations of the target protein.92 An intrabody is a small antibody fragment that targets antigens intracellularly. These fragments are short, can be manipulated and delivered as genes or as proteins, and do not have the potentially inflammatory Fc region. Multiple recombinant single chain Fv (scFv) antibody fragments targeting domains of mutant huntingtin exon 1 have been selected and studied. Several intrabodies targeting different fragment of mutant huntingtin are at various stages of development.94

A third approach in Huntington’s disease is to use strategies that enhance clearance of unwanted proteins. Native or modified polyQ binding peptide 1 inhibits misfolding and aggregation of huntingtin in vitro,95 inhibits neurodegeneration in drosophila models,96 and is beneficial in a mouse model of Huntington’s disease.97 Other small peptides and molecules are being developed.98 None of the aforementioned strategies has yet been tested in patients with Huntington’s disease. A wide range of early-stage screening approaches on in vitro, cellular, and invertebrate models is increasingly being incorporated into drug discovery screening pipelines with the invaluable support of emerging technologies, such as high-content analysis, 3D culture models, and induced pluripotent stem cells.99

**Trophic factors**

Trophic factors are proteins that act on membrane receptors to activate protective signals (eg, phosphoinositide 3-kinase/Akt and extracellular-signal-regulated kinase) and promote cell growth and viability. The GDNF family of trophic factors, which act on both receptor tyrosine kinase (RET) and GFRα receptors, have been studied in Parkinson’s disease on the basis of their capacity in laboratory models to protect dopamine neurons from a range of toxins or restore function even when administered after an insult.98 However, despite promising laboratory studies in neurotoxin-based models, double-blind clinical trials in which growth factors were administered directly into the cerebral ventricle or through a catheter into the putamen have not shown any clinical benefit. Double-blind studies of gene delivery of the trophic factor neurturin to the putamen101
and more recently to both the putamen and substantia nigra pars compacta (Olanow CW, unpublished) did not show a benefit in comparison to a sham procedure. This outcome might be indicative of inadequate distribution throughout the target region, impaired transport from the putamen to the substantia nigra pars compacta because of axonal degeneration or dysfunction, or α-synuclein-induced downregulation of Nurr1 or RET that would prevent GDNF or neurturin signalling in nigral dopamine neurons.102,103

Counteracting apoptosis in Huntington’s disease

Although apoptosis is potentially a terminal pathway relevant to both Parkinson’s disease and Huntington’s disease, recent evidence suggests the value of targeting of pro-apoptotic pathways in Huntington’s disease—an approach that has been somewhat abandoned for Parkinson’s disease, especially since the failure of strategies such as CEP-1347, a semi-synthetic inhibitor of the mixed lineage kinase family.104 Neuronal cell death in Huntington’s disease is associated with neuronal apoptosis, in particular with the initiation of the intrinsic mitochondrial apoptotic pathway.105 In striatal neurons from both patients with Huntington’s disease and animals with the disease, markers for apoptotic cell death are activated. For example, in the brains of patients with Huntington’s disease, caspases 1, 3, 8, and 9, and are activated and cytochrome c is released from the mitochondria into the cytosol.106 Mutant huntingtin also binds more efficiently to p53 than does wild-type huntingtin,107 which causes an upregulation in nuclear p53, and consequently significantly higher amounts of downstream targets of p53, such as Bax and Puma, which are key effectors in the apoptotic cascade of events. Additionally, the interaction of huntingtin-interacting protein 1 and huntingtin decreases the polyQ length increases,108 thereby contributing to a reduced ability to sequester the pro-apoptotic huntingtin-interacting protein 1. This protein is then free to interact with apoptotic partners, such as procaspase 8, which allows initiation of the extrinsic apoptotic pathway.109 Such findings offer new targets for validation, such as the disruption of the interaction of mutant huntingtin with pro-apoptotic proteins—a strategy that does not necessarily require gene therapy but can also be achieved through the delivery of engineered peptides.

Conclusion

Despite the many therapeutic advances in Parkinson’s disease and Huntington’s disease, affected patients eventually experience intolerable disability and early death. A neuroprotective therapy that slows or stops disease progression and prevents the development of cumulative disability remains the highest priority in drug development. Although no agent has yet been established to have neuroprotective effects in either disease, advances in our understanding of the aetiopathogenetic basis of cell death have identified several promising therapeutic targets for future investigation. With the development of animal models that more closely represent the pathology of those disabling diseases, and clinical trial designs that allow the detection of an agent that slows clinical progression, we hope that great progress will be made in the detection of a neuroprotective agent in the coming decades.

References


