Gene therapy for Parkinson's disease

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Parkinson's disease (PD) is a debilitating neurodegenerative disorder arising from loss of dopaminergic neurons in the substantia nigra pars compacta and subsequent depletion of striatal dopamine levels, which results in distressing motor symptoms. The current standard pharmacological treatment for PD is direct replacement of dopamine by treatment with its precursor, levodopa (L-dopa). However, this does not significantly alter disease progression and might contribute to the ongoing pathology. Several features of PD make this disease one of the most promising targets for clinical gene therapy of any neurological disease. The confinement of the major pathology to a compact, localised neuronal population and the anatomy of the basal ganglia circuitry mean that global gene transfer is not required and there are well-defined sites for gene transfer. The multifactorial aetiology of idiopathic PD means that it is unlikely any single gene will cure the disease, and as a result at least three separate gene-transfer strategies are currently being pursued: transfer of genes for enzymes involved in dopamine production; transfer of genes for growth factors involved in dopaminergic cell survival and regeneration; and transfer of genes to reset neuronal circuitry by switching cellular phenotype. The merits of these strategies are discussed here, along with remaining hurdles that might impede transfer of gene therapy technology to the clinic as a treatment for PD.

Parkinson's disease (PD) is a prevalent neurodegenerative disorder affecting at least 1% of the population over the age of 65 (Ref. 1), and is characterised pathologically by the loss of dopaminergic neurons within the substantia nigra (SN) (reviewed in Ref. 2). The resulting disturbance in the circuitry of the basal ganglia results in the characteristic motor abnormalities of rigidity, bradykinesia (abnormal slowness of movement), tremor and gait disturbance that

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severely compromise a patient's quality of life. The current standard pharmacological treatment for this disease focuses on the direct replacement of dopamine by oral administration of its precursor, levodopa (L-dopa) (Refs 3, 4). However, prolonged systemic use of L-dopa can itself lead to motor side effects (Ref. 5), and use of this drug has not been shown to alter the progression of the disease.

Several features of PD make it particularly suited to a gene-therapy-based approach to treatment: (1) the identification of an active (and preventable) cell death process (apoptosis) occurring within the SN; (2) the confinement of the initial pathology of the disease to discrete locations within the brain; and (3) the progression of the disease over a long time-frame. However, the multifactorial aetiology of idiopathic PD means there is no single gene identified as the 'magic bullet' that will cure the disease and, as a result, there are at least three separate gene transfer strategies currently being pursued in an effort to combat this debilitating disease. The aim of this review is to outline the progress of research on these various gene therapy strategies and to identify outstanding issues that must be resolved if gene therapy for PD is to proceed to the clinic as a viable treatment option.

Parkinson's disease

The primary pathological hallmark of PD is death of dopaminergic neurons within the substantia nigra pars compacta (SNc) and loss of their axonal projections to the striatum. This depletion of dopaminergic tone in the nigrostriatal projection causes a cascade of modifications to the functioning of the basal ganglia circuitry, resulting in the characteristic motor symptoms observed in PD: tremor, muscle rigidity and bradykinesia (Ref. 6). This cell death process can take place over a time period of 20 or more years, and the clinical symptoms of PD are not exhibited until a loss of ~80% of striatal dopamine has occurred, representing a loss of ~50% of the dopaminergic cell bodies within the SNc (Refs 7, 8).

Some 90–95% of PD cases are idiopathic and several risk factors for PD have been identified, including exposure to well water (Refs 9, 10), pesticides (Refs 11, 12), herbicides (Ref. 13) and industrial chemicals (Ref. 14); however, no specific toxin has consistently been found in the brain of PD patients. The remaining 5–10% of PD cases have a genetic basis and are inherited in

an autosomal dominant pattern. Mutations in several genes putatively associated with familial PD including α-synuclein (Refs 15, 16), parkin (Ref. 17) and ubiquitin C-terminal hydrolase (UCH-L1) (Refs 18, 19) have been identified. The exact mechanisms by which these mutations might result in PD are still under investigation but involve aberrations in processing, folding and degradation pathways of several key proteins including α-synuclein.

There is an age-dependent cell loss from the SN during the normal aging process. It has been estimated that 4.5% of these cells are lost with each decade of life (Ref. 20) and this rate increases to up to 45% cell loss per decade in PD (Ref. 7), with a significant proportion of this cell death occurring before clinical diagnosis of the disease. It is conceivable that several distinct pathways – oxidative stress, mitochondrial dysfunction, apoptosis and excitotoxicity – are initiated by combinations of toxins and genetic risk factors to converge and interact with each other, resulting in the common outcome of dopaminergic neuronal dysfunction, atrophy and ultimately death.

The relative contributions of necrotic (passive) and apoptotic (requiring gene activation) cell death pathways to the degenerative process in PD is still an area of active investigation. Apoptosis can be initiated by a variety of insults to the adult brain and, as such, might have a role in the pathogenesis of PD. Post-mortem PD brains have shown evidence of apoptotic cell death in some studies (Refs 21, 22, 23, 24), but this is controversial and other groups have found no evidence of neuronal apoptosis (Refs 25, 26). The results of these studies rely heavily on the use of the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labelling (TUNEL) technique in which the free 3' ends of DNA generated after endonuclease digestion of DNA during apoptosis are labelled and detected immunohistochemically. The absence of positive TUNEL staining in post-mortem tissue does not automatically mean apoptosis is not involved in PD – considering the long time span that cell death occurs over (~20 years), it might be unrealistic to expect the presence of great numbers of apoptotic cells at any given time. Further independent evidence for the occurrence of apoptotic cell death in PD comes from the detection of the pro-apoptotic factor caspase-3 in the SN of post-mortem PD brain (Refs 27, 28): caspase-3 is

a key molecular player in programmed cell death pathways (Refs 29, 30).

Importantly, the realisation that loss of cells during PD might be the result of an active cell death programme opens up new avenues of therapeutic strategies for treatment of this disease. If the disease can be detected early enough, then the use of agents that inhibit initiation or propagation of this cell death cascade might be of benefit in arresting cell death and slowing disease progression.

Gene therapy for PD

Limitations of pharmacological therapy

Traditional pharmacological treatments for PD focus on direct replacement of dopamine by administration of the dopamine precursor L-dopa, or dopamine agonists, in combination with agents that act to prolong the action of dopamine at the synapse or prevent breakdown of dopamine. However, treatment with L-dopa becomes less efficacious over time and can lead to side effects such as dyskinesia, hallucinations and disorientation that are debilitating in their own right. Direct augmentation of dopamine in this way does nothing to halt the progress of the disease, and addition of exogenous dopamine to a compromised system might in fact contribute to the ongoing pathology (Ref. 31). In addition, as the disease progresses and nigrostriatal innervation is lost, fewer striatal terminals are available to metabolise the exogenously supplied L-dopa to dopamine. Thus, gene therapy strategies involving transfer of genes encoding factors that increase dopamine production and enhance dopaminergic cell phenotype and survival represent an innovative approach to attack this disease.

The side effects observed with prolonged L-dopa treatment can be attributed to the availability of dopamine to neurons other then the nigrostriatal pathway, and the fluctuating levels of dopamine produced following oral administration of L-dopa (Refs 32, 33, 34). Gene therapy could be used to increase dopamine production in localised cell populations within the nigrostriatal projection, thereby avoiding side effects resulting from the availability of dopamine to other systems. Thus, the production of dopamine following gene transfer would occur in a more physiological, continuous manner and would eliminate the effects of pulsatile dopamine production following ingestion of L-dopa. Gene transfer of neuroprotective or regenerative agents that prolong cell survival might also enhance the use of dopamine-replacement strategies by prolonging the time window during which cells are able to metabolise exogenous L-dopa. Thus, gene-based therapies could be used alone or in combination with current pharmacological paradigms.

Gene therapy in the central nervous system (CNS)

Any potential gene therapy strategy, regardless of the disease it targets, poses several technical considerations regarding parameters such as the optimal gene to transfer, the appropriate vector system to transfer that gene, the regulation and measurement of transgene expression, and the use of the most appropriate animal model to measure outcomes and assess whether the gene therapy intervention is working. Gene therapy treatments for diseases of the CNS present an additional set of constraints that affect these parameters, including the post-mitotic nature of neurons, the heterogeneous nature of the cell population, the arrangement of these cells into specific functional, interacting circuits, the encasement of the brain within the skull (leading to problems of access and volume constraints) and the presence of the blood–brain barrier (which restricts access of components in the blood to the brain).

Many of these problems have been addressed in recent years with regard to the potential treatment of PD (Refs 35, 36). As discussed in this article, the optimal gene for transfer in PD is still an area of debate, with several strategies currently being pursued. Efficient vector systems for transduction of neurons have been developed, such as recombinant adeno-associated virus (AAV) (Refs 37, 38, 39), adenovirus (Refs 40, 41, 42, 43) and lentivirus (Refs 44, 45, 46, 47). This has been coupled with optimisation of manufacturing methods for these vectors to produce pure, high-titre vector stocks suitable for use in human brain (Refs 48, 49), thus enabling infusion of a small volume into brain tissue to transduce a maximal number of cells. Advances in research into efficient vector-injection paradigms in animal models – for example, the use of mannitol to enhance vector spread and uptake (Ref. 50) or convection-enhanced delivery (Ref. 51) – also ensures transduction of a maximal number of cells following infusion of a minimal volume of vector.

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Gene therapy for Parkinson's disease

Why use gene therapy for PD?

Of all the neurodegenerative disorders, PD is particularly amenable to treatment using a gene therapy strategy for several reasons. First, the observation that the symptoms of PD become apparent only when an estimated 50% of nigral neurons have already been lost suggests the presence of an inherently large reserve and compensation capacity within the nigrostriatal system (Refs 1, 52, 53, 54, 55) and, as such, it is possible that even a minimal increase in dopamine production via gene therapy might be sufficient for amelioration of symptoms. Second, the initial pathology is confined to a particular, well-characterised subtype of neurons, and these are located within a small compact area in the brain (Ref. 56). Thus, attaining global gene transfer to large brain areas is not an issue in PD – a single injection of a high-titre vector stock could transduce a large proportion of the dopaminergic cell population within the human SN and have a large impact on the disease phenotype. There are potentially two sites for gene transfer in PD, depending on the gene to be transferred: the dopaminergic cell bodies in the SNc or their terminals within the striatum. Third, the disease is progressive, worsening over a time period of 10–20 years (Refs 29, 53) and so offers a large window of time for undertaking a therapeutic intervention. Fourth, neurodegenerative diseases like PD are, by definition, chronic, so any treatment options need to be long-lasting or permanent. This makes PD particularly suited to treatment with viral vectors, where a single application of vector can result in prolonged, stable transgene expression, with production of physiologically relevant levels of enzymes involved in the dopamine synthesis pathway or prolonged growth factor production over several months to promote reinnervation of the damaged nigrostriatal pathway.

In addition, PD is a well-characterised disease, with many animal models available for evaluation of the benefits of any gene therapy strategy. Despite the fact that the initiating factor in prompting the cell death observed in PD has not been fully elucidated, the pathways implicated in this cell death, and the effects of cell death on the functioning and innervation of the nigrostriatal pathway, can still be mimicked in animal models of the disease. For instance, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Refs 57, 58), the pesticide rotenone (Ref. 59) and the toxin 6-hydroxydopamine (6-OHDA) (Refs 60, 61) can evoke PD-like symptoms and neuropathological changes in various species including rodents and primates, and can provide insights into oxidative stress, excitotoxicity, necrosis and apoptotic cascades within the SN (reviewed in Ref. 62). There are a large number of standardised behavioural tests defined for both rodent and primate models of PD that can be used to gauge the effects of any gene therapy strategy (Refs 63, 64, 65, 66, 67, 68, 69). However, the relevance of measuring behavioural parameters such as amphetamine- and apomorphine-induced rotation to gauge the efficacy of potential human PD treatments remains unclear. Screening of potential PD gene therapy treatments requires comprehensive studies encompassing use of clinically relevant animal models (to mimic prolonged cell degeneration processes accurately) coupled with measurement of meaningful behavioural indices (e.g. spontaneous forepaw use or forepaw-adjusting steps as well as drug-induced rotation), and any changes in behavioural deficits need to be correlated with parallel quantitative measures of dopamine metabolism and examination of nigrostriatal innervation.

In summary, PD represents an achievable target for clinical gene therapy. In part this relates to the anatomy of the circuitry and the prolonged time period for development of pathology of PD, but it is also related to the development of appropriate viral vector systems for efficient gene transfer to neurons. There are some shortcomings in the animal models of PD currently used to test putative gene therapy treatment strategies. It is conceivable that gene transfer technology can be used not only for the development of therapeutics but also to generate more-accurate animal models of PD (e.g. one that has localised overexpression of α-synuclein) (Refs 70, 71).

Strategies for gene therapy intervention in PD

The multifactorial aetiology of PD means that intervention in the disease process via gene therapy is possible on many different levels, and there are at least three separate gene therapy strategies that might be used to attack this disease. First, transfer of genes involved in dopamine biosynthesis might help with the immediate motor symptoms of the disease by sustained local production of dopamine in the same way as

systemic administration of oral L-dopa. Second, transfer of growth factor genes such as glial-cell-line-derived neurotrophic factor (GDNF) might prevent further dopaminergic cell death (neuroprotection), restore atrophic neurons to a normal state and promote reinnervation of the damaged nigrostriatal system (neurorestoration). Third, transfer of genes involved in inhibitory neurotransmission might be used to dampen the activity of brain nuclei that become overactive in PD.

Within each of these separate strategies, both ex vivo (transplantation of cells that have been genetically modified in vitro) and in vivo (direct transfer of the gene into brain tissue) approaches could be used. This review focuses on in vivo gene delivery approaches used for these strategies.

Use of gene transfer to replace enzymes involved in dopamine synthesis

Many of the side effects caused by the fluctuating levels of L-dopa obtained after oral dosing with this drug can be abolished by the use of continuous intravenous infusion of L-dopa (Refs 32, 72, 73). This suggests that steady-state levels of the drug such as obtained by continual infusion might be of benefit clinically, with a reduction in the occurrence of phenomena such as 'wearing off' or 'on–off' motor fluctuations related to the pulsatile nature of dopamine production after oral L-dopa. However, it is not practical for many patients to receive continuous intravenous infusions and this method of administration still does not overcome the problem of unwanted mental side effects caused by the availability of L-dopa to dopaminergic regions of the brain other than the nigrostriatal projection. Thus, the targeted delivery of L-dopa in continuous, physiological quantities specifically to the striatum as could be achieved by localised gene transfer would be an advance in the treatment of PD.

Dopamine synthesis

The dopamine synthesis and storage pathway involves several enzymes and cofactors (Fig. 1), any one of which could be manipulated genetically to yield increased dopamine levels. The rate-limiting enzyme in dopamine production is tyrosine hydroxylase (TH), which converts the amino acid tyrosine to L-dopa. L-dopa is then metabolised to dopamine by aromatic amino acid decarboxylase (AADC) (Ref. 74). Another factor

that influences this pathway is the essential TH cofactor 6-tetrahydrobiopterin (BH_4), the level of which is limited by availability of the enzyme GTP-cyclohydrolase I (GTPCHI) (Refs 75, 76). Modification of the levels of any of these three key enzymes (TH, AADC or GTPCHI) through gene therapy could significantly impact on striatal dopamine levels and, as discussed here, many studies have been published on the use of genes encoding these enzymes in both rodent and primate PD models.

Tyrosine hydroxylase

The original studies in this area suggested beneficial increases in dopamine production could be achieved by use of ex vivo gene therapy approaches. Cells engineered to produce L-dopa by introduction of the TH gene were implanted
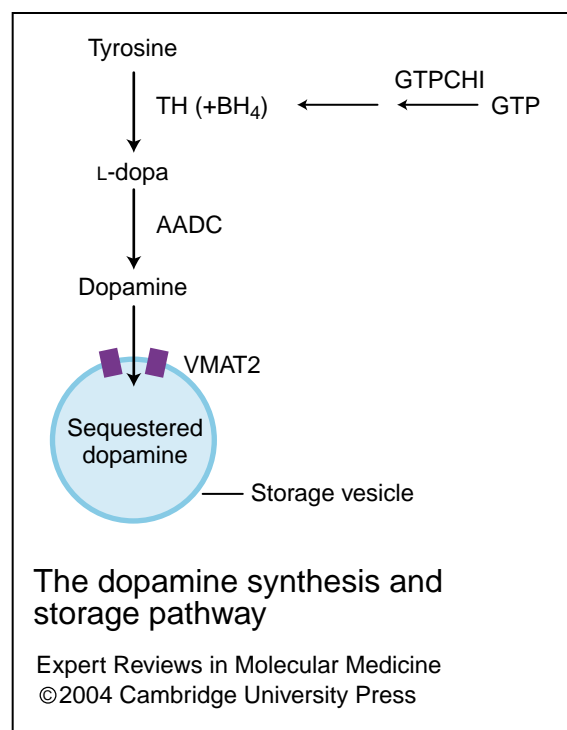

The dopamine synthesis and storage pathway

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Figure 1. The dopamine synthesis and storage pathway. Tyrosine is converted to L-dopa by the enzyme tyrosine hydroxylase (TH), a reaction that also requires the TH cofactor 6-tetrahydrobiopterin (BH_4). Guanosine triphosphate cyclohydrolase I (GTPCHI) is the first and rate-limiting enzyme involved in BH_4 synthesis. Conversion of L-dopa to dopamine requires the enzyme aromatic amino acid decarboxylase (AADC). Dopamine is sequestered into storage vesicles by vesicular monoamine transporter 2 (VMAT2).

into lesioned animals, and this correlated with increases in L-dopa levels (Ref. 77) and some recovery from behavioural deficits (Refs 78, 79). However, this indirect approach to increasing striatal dopamine levels means that there are two significant technical hurdles that must be overcome: cells must not only be modified to produce L-dopa but must also survive transplantation into the host animal and show long-term stability and integration into host circuitry.

Use of the more-direct in vivo approach (injection of viral vectors encoding for TH into the denervated striatum) removes one of these technical variables. Initial experiments demonstrated that direct transfer of the TH gene to the striatum of parkinsonian rats using AAV (Ref. 37), herpes simplex virus (HSV) (Ref. 80) or adenoviral vectors (Ref. 42) resulted in long-term expression of TH and some phenotypic correction of behavioural deficits in these animals. However, the use of the partial-lesion 6-OHDA model in some of these studies meant the presence of residual endogenous dopamine synthesis capacity, and, as such, determination of the amount of dopamine produced as the direct result of gene transfer was not possible. Also, the use of apomorphine-induced rotational behaviour as the main indicator of transgene function might have given limited information. Nevertheless, these pioneering studies provided proof-of-principle that increasing TH levels in the striatum via gene transfer could lead to some behavioural recovery, but further studies using alternative behavioural tests and concurrent measurement of biochemical and histopathological markers were needed. Also, the optimal gene (or gene combination) that would result in physiologically relevant levels of dopamine required further investigation.

GTPCHI and AADC

Reports on the use of ex vivo gene therapy approaches to modify cells genetically prior to grafting in PD animal models suggested that cotransduction of target cells with both TH and GTPCHI resulted in greater L-dopa production (Refs 81, 82) than use of TH alone. Furthermore, direct striatal AAV–TH transduction with addition of exogenous BH_4 or cotransduction with AAV–GTPCHI also resulted in increased L-dopa production (Ref. 83), suggesting that significant amounts of L-dopa following transfer

of the TH gene are not produced unless BH_4 levels are also increased. A further study, utilising regulatable adenoviral vectors (Ref. 84), showed that residual levels of BH_4 found in denervated animals might not allow production of the maximal amount of dopamine following TH gene transfer, and this might be achieved only by cotransduction with GTPCHI or application of exogenous BH_4.

Gene transfer of AADC to increase dopamine levels has also been investigated in both rat and primate PD models (Refs 51, 85, 86). Transduction of striatal tissue with AAV–AADC combined with an exogenous dose of L-dopa in dopaminergically denervated animals resulted in the phenotypic correction of motor deficits. This was proposed to be due to increased decarboxylation of L-dopa to dopamine as a result of increased AADC levels, and suggests that gene transfer of AADC by itself might be beneficial in PD treatment by reducing the dose of L-dopa required to give relief from motor abnormalities, thereby avoiding or delaying the development of side effects from L-dopa administration.

Gene combinations

Other studies have reported recovery-promoting effects after gene transfer of various combinations of these three key enzymes, using differing vector systems and various PD models. Cotransduction of rat striatum with AAV–TH and AAV–AADC resulted in increased dopamine production and greater behavioural recovery than that observed in rats receiving AAV–TH alone (Ref. 87). Use of this double enzyme combination with a bicistronic AAV vector in MPTP-treated monkeys also resulted in increased dopamine production (Ref. 88). These findings were extended to triple transduction of striatal tissue with three separate AAV vectors, expressing genes for TH, AADC or GTPCHI. In these studies in rat (Ref. 89) and primate (Ref. 90) models of PD, gene transfer of all three enzymes was achieved and resulted in increased behavioural modification above that seen in the double transduction study, suggesting that expression of all three of these factors might be necessary to obtain biologically relevant levels of dopamine. However, direct comparisons between different studies is difficult given the variety of models and transduction paradigms investigated – for example, the use of bicistronic vectors encoding for both TH and AADC compared with injection of two of three separate

vectors, one encoding for each enzyme. Use of a tricistronic lentiviral vector containing all three genes (TH, AADC and GTPCHI) increased striatal dopamine production and led to a reduction in behavioural deficits in 6-OHDA-treated rats (Ref. 91), and the inclusion of all genes into one vector meant that transduced cells produced all three enzymes in close proximity to each other, overcoming the need to transduce a single neuron with multiple vectors. However, studies that directly compare behavioural outcomes and dopamine levels following transduction with multiple vectors versus bi- or tricistronic vectors are needed to determine the relevant importance of each enzyme to increasing dopamine concentration.

A recent study using transduction of striatal tissue with AAV vectors containing genes for TH and GTPCHI in 6-OHDA-denervated rats (Ref. 92) highlighted the importance of determining levels of dopamine produced after gene therapy treatment, and the necessity of measuring appropriate behavioural outcomes in animal models. Results from this study suggest a threshold level of 1.5 pmol L-dopa/mg striatal tissue must be produced in order to give a quantifiable effect on spontaneous and drug-induced behaviours in parkinsonian rats. Although significant recovery was observed in both completely and partially lesioned animals, this recovery was most pronounced in the animals with partial preservation of striatal dopaminergic fibres, suggesting that spared fibres might play an important role in storage and release of newly synthesised dopamine.

VMAT2

One other possible intervention strategy in the dopamine synthesis and storage pathway is use of gene therapy to modify the levels of the vesicular monoamine transporter 2 (VMAT2). VMAT2 plays a crucial role in storage of dopamine by packaging of the neurotransmitter into synaptic vesicles and regulating vesicle release. Use of VMAT2 in combination with AADC has been reported (Ref. 93). In this study, fibroblasts that had been genetically modified to produce both VMAT2 and AADC were transplanted into parkinsonian rats. Following systemic administration of L-dopa, these animals contained higher levels of striatal dopamine, as measured by microdialysis, than those treated with AADC-modified cells alone. The impact of this treatment on restoration of behavioural deficits was not measured; however, this study underlines the fact that while increased dopamine production is required (perhaps via exogenous L-dopa), the ability to store and release this neurotransmitter in a gradual fashion is also beneficial.

Conclusions

Thus, intervention by gene therapy in the dopamine synthesis and storage pathway offers several possible strategies with the common aim of increasing dopamine production and release in a site-specific manner. Gene therapy could be used to augment current pharmacological treatments (e.g. use of vectors for AADC and/or VMAT2 to enhance decarboxylation and storage of exogenously supplied L-dopa). This might be of use in early stages of the disease where some striatal dopaminergic innervation remains intact. In other cases, where therapeutic efficacy of L-dopa has already been lost as a result of irreplaceable loss of nigrostriatal afferents, a gene therapy strategy involving replacement of multiple enzymes involved in dopamine synthesis and release might be beneficial. However, whatever the approach used to increase dopamine levels, the fact remains that this strategy is palliative only – it will correct the motor symptoms produced as the result of dopaminergic cell loss, but will have no effect on the progression of the disease.

Use of growth factor gene transfer for neuroprotection and neurorestoration

The progressive nature of the cell loss in PD offers opportunities for therapeutic intervention aimed not only at the prevention or slowing down of cell loss (neuroprotection) but also at the regeneration of dysfunctional or atrophic dopaminergic cells (neurorestoration). This might be achieved by gene transfer of trophic factors with effects on the dopaminergic phenotype.

Effects of GDNF on the nigrostriatal system

Various neurotrophic factors with putative effects on the nigrostriatal dopamine system have been evaluated for their therapeutic potential in PD. These include basic fibroblast growth factor (bFGF) (Ref. 94), epidermal growth factor (EGF) (Refs 95, 96), brain-derived neurotrophic factor (BDNF) (Ref. 97) and sonic hedgehog (Ref. 98). These factors displayed promising attributes

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in dopaminergic in vitro systems, including enhancement of neurite outgrowth and protection from insults such as the neurotoxin 1-methyl-4-phenylpyridinium, but have produced variable results when translated to animal models of PD (Refs 99, 100, 101, 102, 103, 104, 105, 106).

Arguably the most intensively studied trophic factor to date is GDNF. Initial in vitro studies examining survival of mid-brain cultures identified this molecule as a potent trophic factor for dopaminergic neurons of the SN (Ref. 107). The in vivo potential of GDNF as a PD therapeutic agent was established following studies utilising the 6-OHDA rat model and infusion of GDNF recombinant protein. As outlined below, these reports suggested the dual abilities of GDNF not only to protect cells from death but also to promote regeneration of an already damaged system. Injection of 6-OHDA into the striatal terminals of dopaminergic cells causes axonal retraction and death of the cell bodies in the SN to occur over a period of several weeks (Ref. 61) and so this model offers the opportunity to study both the protection of degenerating neurons by GDNF during the acute cell death phase and the regenerative and recovery-promoting effects of GDNF during the chronic phase of the lesion, when spared dopaminergic neurons persist in a dysfunctional state. Cell death was prevented by infusion of GDNF protein in the region of the SN throughout this time period (Ref. 108), and the neurorestorative power of this molecule was demonstrated in a study in which a single infusion of GDNF into the SN of stably lesioned animals displaying behavioural deficits resulted in attenuation of apomorphine-induced rotation and an increase in dopaminergic cell number (Ref. 109). Reports on the use of various GDNF protein infusion paradigms in this partial lesion model have confirmed both the neuroprotective (Refs 110, 111) and restorative (Refs 112, 113, 114) powers of GDNF.

Recovery-promoting effects of GDNF protein have also been demonstrated in primate PD models. Infusion of GDNF into the SN of rhesus monkeys stably lesioned with MPTP resulted in improvement of behavioural deficits (Ref. 115). In addition, cells of the SN increased in mean cell size and density of fibres, and mid-brain dopamine levels increased (Ref. 115). However, a later study showed that discontinuation of GDNF administration resulted in the slow decline of animals back to baseline deficit levels (Ref. 116).

These encouraging results in rodent and primate models augured well for the clinical use of this trophic factor. However, the first reported use of intracerebroventricular (icv) GDNF protein in a PD patient did not produce the expected beneficial effects (Ref. 117). The administration of GDNF via the ventricles did not result in any reduction of parkinsonian symptoms, but did cause several adverse side effects including nausea, loss of appetite, hallucinations and depression. Post-mortem examination of brain tissue revealed no evidence for nigrostriatal regeneration. A large, multicentre, double-blind trial on the use of recombinant GDNF also reported many adverse effects and no impact on parkinsonian symptoms following icv infusion (Ref. 118).

A more-recent study on the action of GDNF in primate PD models examined the effect of prolonged infusion of GDNF directly into the striatum three months after MPTP treatment, and found a reduction in parkinsonian symptoms coupled with an increase in striatal dopamine levels and increased cell bodies in the SN (Ref. 119). This finding was extrapolated to the human disease condition in a further trial on human patients: chronic infusion of GDNF protein directly into the caudate putamen resulted in improvements in motor symptoms, and positron emission tomography (PET) scans demonstrated an increase in dopamine storage (Ref. 120).

Overall, the data accumulated on the effects of GDNF protein in both 6-OHDA and MPTP models show not only that GDNF protects dopaminergic cells from death, but also that it might promote axonal sprouting and regeneration of lesioned neurons, depending on the site and regime of GDNF administration. Use of the factor in primates and in human patients highlighted the need for an efficient route of GDNF delivery and suggested that sustained presence of GDNF could be required for maintaining any beneficial effects. A viral-vector-based gene therapy strategy resulting in prolonged, stable production of GDNF might be more applicable than long-term infusion of protein to the human clinical condition. The production of transgenic GDNF should also be focal as widespread distribution of GDNF to systems other than the nigrostriatal projection after icv administration has been observed (Ref. 121) and might account for some side effects.

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Gene transfer of GDNF

Various viral vector systems – adenovirus, lentivirus and AAV – have been used to introduce GDNF into brain tissue, and efficacious effects on both behaviour and nigrostriatal innervation levels have been demonstrated in models designed to assess both the neuroprotective and the neurorestorative properties of GDNF.

Adenoviral-mediated transfer of GDNF resulted in protection from 6-OHDA-induced cell death following infusion of the vector adjacent to the cell bodies of the SN (Ref. 122) and into the striatum (Refs 123, 124). A direct comparison between the benefits of infusing GDNF-encoding vectors into either the SN or striatum (Refs 125, 126) showed that infusion into the striatum (the site of terminal degeneration) resulted in greater prevention of behavioural deficits and greater preservation of striatal innervation.

Other studies have provided evidence of the ability of virally delivered GDNF to promote restoration of function and innervation in 6-OHDA-lesioned rats (Refs 127, 128). A potential problem with the use of adenoviral vectors is production of an inflammatory response and consequent downregulation of transgene levels over time following injection into the brain. Other groups have reported on the use of AAV- or lentiviral-mediated delivery of GDNF to ensure long-term transgene expression and have demonstrated both neuroprotection (Refs 129, 130, 131) and restoration of function and innervation in rat models of PD (Ref. 132), with transgenic GDNF still at maximal levels six months after gene transfer (Ref. 130).

Lentiviral GDNF gene transfer to both the striatum and SN prevented neurodegeneration in a primate model of PD (Ref. 133). Transgenic GDNF expression in the striatum of parkinsonian primates also resulted in an increased number of striatal cells expressing TH (Ref. 134), suggesting that one effect of this growth factor is to convert striatal neurons to a dopaminergic phenotype; alternatively, this observation could represent an effect of GDNF on neurogenesis and migration of cells from the subventricular zone, an area known to contain neuronal progenitor cells. However, the benefits of long-term striatal GDNF overexpression might not be clear-cut; such expression has been found to result in aberrant sprouting of fibres in the globus pallidus and entopeduncular nucleus and, although the nigrostriatal fibres were protected from degeneration, the expression of TH might in fact be downregulated over time (Ref. 135).

Critical issues in growth factor gene therapy

A large body of data on use of both GDNF recombinant protein and virally mediated GDNF gene transfer in rodent and primate models of PD demonstrates both neuroprotective and neurorestorative effects of this growth factor, and highlights issues that will be important in any growth factor gene therapy. Studies on the use of GDNF gene transfer have demonstrated crucial points such as the necessity of the continual presence of the growth factor at discrete striatal and SN locations, and have also highlighted the potential problems associated with long-term overexpression of this growth factor. In addition, the biological action of the growth factor might not be restricted to directly transduced cells – for example, a study on the use of GDNF for protection of motor neurons resulted in survival of a greater number of cells than were actually transduced (Ref. 136). This bystander effect might be beneficial in some cases, but could also be disadvantageous if high expression levels of the growth factor caused side effects.

Identification and assessment of efficacy of alternative trophic factors with putative effects on the nigrostriatal system should also be pursued if growth factor gene therapy for PD is to become a realistic treatment option. In pursuing use of growth factors with neuroprotective or anti-apoptotic properties for PD, it must be realised that interference with apoptotic cascades and prevention of cell death might be only half the story: neurons might be saved from death but that is only useful if normal physiological functions of that cell, such as dopamine production, are still intact. There are probably many distinct apoptotic cascades that can be initiated within the SN by a variety of genetic and environmental insults – a truly neuroprotective strategy that will protect cells regardless of the cause of PD might become a reality only if a common molecular mechanism leading to degeneration is identified.

Use of gene transfer to reset neuronal circuitry

A third potential strategy for gene therapy treatment of PD involves the use of genes encoding enzymes involved in neurotransmitter

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production to alter activity of selected brain regions. Loss of the dopaminergic nigrostriatal projections in PD results in profound disturbances in the circuitry of the basal ganglia. One consequence is that the excitatory neurons of the STN become disinhibited and overactive. These cells of the STN project to the major inhibitory output nuclei of the basal ganglia – the SN pars reticulata (SNr) and the internal segment of the globus pallidus – and the STN overdriving of these nuclei results in inhibition of the thalamus and downstream motor pathways, and ultimately in the motor disturbances seen in PD. This has led researchers to speculate that silencing of the STN in PD might improve the motor symptoms of the disease. Indeed, electrical inhibition (Refs 137, 138), ablation (Refs 139, 140, 141) or pharmacological silencing (Ref. 142) of the STN have all been reported to control PD symptoms. A recent report on the infusion of an AAV vector coding for glutamic acid decarboxylase (GAD), the enzyme involved in synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), into the overactive STN of parkinsonian rats (Ref. 143) provided proof-of-principle that gene therapy could also be used to modulate STN activity. Expression of transgenic GAD resulted in increased GABA production levels in the SNr following STN stimulation (as determined by microdialysis to measure GABA levels), and electrophysiological recordings from the brains of GAD-treated animals confirmed increased inhibitory responses in these animals. This translated to a decrease in behavioural deficits in unilaterally 6-OHDA-lesioned rats, and the phenotypic shift of STN neurons from excitatory to inhibitory following GAD gene transfer also protected against further cell death in the SN. This novel work is being pursued in the human clinical setting as an alternative to deep brain stimulation of the STN, with a Phase I clinical trial approved by the US Food and Drug Administration currently under way (Refs 35, 36, 144).

Outstanding issues in gene therapy for PD

Although PD is considered by many researchers to be the closest neurological target for treatment via gene therapy there are still several outstanding research issues that need to be resolved before this becomes a mainstream treatment option. These include development of improved animals models, development of systems for control of transgene expression, and implementation of standards to ensure that manufacture of vector preparations for human use is safe.

Identification of suitable agents for gene therapy treatment of PD in humans relies upon testing of putative therapeutic agents in animal models that mimic the human condition. At this stage, it is unclear how relevant to human PD are the models that make use of toxins (MPTP, 6-OHDA and rotenone). Development of models that better recreate the prolonged degeneration and secondary pathologies observed in PD would hasten transfer of successful gene therapy technology to the clinic. Identification of gene mutations associated with PD might lead not only to new gene therapy strategies being identified, but also to development of more relevant animal models (e.g. overexpression of α-synuclein) in the SN (Refs 70, 71).

Issues such as the regulation of transgene expression also need to be resolved. Uncontrolled transgene expression of enzymes involved in dopamine synthesis could result in side effects (in much the same way as continual excessive dopamine stimulation results in side effects in pharmacological treatment paradigms) or unregulated growth factor expression might lead to uncontrolled axonal outgrowth in brain. Regulatable gene expression systems are being developed (Refs 145, 146, 147, 148), but these remain leaky or poorly inducible in vivo and would require prolonged use of drugs like tetracycline, the long-term effects of which are unknown. Also, if the introduced gene causes unforeseen problems, there is currently no reliable method for excising the transgene from brain tissue, short of ablating transduced cells.

In the past few years, issues regarding manufacture and quality control of viral vectors have been largely addressed with the introduction of efficient and reproducible packaging systems for vectors such as AAV that eliminate the need for use of helper viruses and result in no detectable contamination of stocks with wild-type virus (Ref. 49). Development of affinity-based purification methods enables production of large amounts of high-titre, pure vector stocks (Ref. 48). Also, as mentioned previously, attaining global gene transfer in PD is not necessarily an issue as a single injection of high-titre vector stock could transduce a large proportion of cells within a given nucleus of the basal ganglia.

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Once a gene therapy strategy has been selected for use in humans, careful trial design must be undertaken to ensure accurate measurement both of appropriate clinical outcomes and of vector output. The selection of patients could include both early- and late-stage PD patients, depending on the specific gene therapy being evaluated. Endpoints that can be measured include clinical assessment of motor symptoms [e.g. using the Unified Parkinson's Disease Rating Scale (UPDRS)] (Ref. 149) and imaging using PET to assess the impact of treatment on striatal dopamine metabolism (although this is not necessarily representative of SN cell number) and brain glucose utilisation. A noninvasive measure of vector output would also be needed (e.g. concentration of L-dopa/growth factor/GABA produced, or dopamine metabolised) and correlated to the clinical data.

Gene therapy for PD based on supplementation of dopamine production, prevention of cell death or prompting reinnervation of the nigrostriatal system is more likely to succeed in patients who still have a moderate number of nigrostriatal afferents. Unfortunately, definitive diagnosis of PD occurs only after motor symptoms become apparent, meaning a large number of SN cells have already been lost. Successful gene therapy for PD will probably go hand-in-hand with improvements in imaging techniques leading to earlier diagnosis and hence the ability to implement neuroprotective strategies. Any trial using gene transfer of growth factors for reinnervation of the nigrostriatal system must proceed for at least several months to enable axonal regeneration to occur.

Concluding remarks

PD is a complex neurodegenerative disorder caused by loss of cells within the circuitry of the basal ganglia, resulting in debilitating motor symptoms. The exact cause of dopaminergic cell loss remains to be elucidated but probably involves a combination of both genetic and environmental factors. Current mainstay treatments for PD, such as administration of L-dopa, treat the motor symptoms but do little to alter the ongoing pathology. As a result, alternative, long-term treatment strategies such as gene therapy are being pursued.

Gene transfer to alter the course of PD is possible on many different levels, and at least three separate strategies are currently being pursued: gene transfer to increase local dopamine production in a physiological manner; gene transfer of growth factors involved in survival of dopaminergic cells to prevent further cell death or rejuvenate the damaged nigrostriatal pathway; and gene transfer to reset altered neuronal circuitry. These genetic manipulations could be used alone or to augment current pharmacological treatments (e.g. use of AADC gene transfer to enhance metabolism of exogenously supplied L-dopa). Gene transfer could also be used to stimulate neurogenesis and to facilitate neuronal survival during cell transplantation procedures in neurodegenerative diseases.

Viral vectors systems capable of efficiently transducing neurons, such as AAV, have been developed, along with protocols for manufacture of pure vector stocks suitable for use in human brain. However, issues regarding regulation of transgene expression and, if required, excision of transgene from transduced cells or other rescue procedures need to be addressed if gene therapy for PD is to become a viable mainstream treatment option. As our knowledge of the molecular basis of this devastating disease expands, further options for gene therapy of this disease will doubtless present themselves.

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Further reading, resources and contacts

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The National Institute of Neurological Disorders and Stroke website entry for Parkinson's disease provides information on symptoms and medication:

http://www.ninds.nih.gov/health_and_medical/pubs/parkinson_disease_htr.htm

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Features associated with this article

Figure
Figure 1. The dopamine synthesis and storage pathway.

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