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Review

Gene therapy strategies in neurodegenerative diseases

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Summary. Treatment of neurodegenerative diseases by classical pharmacotherapy is restricted by blood-brain barrier which prevents access to the brain of potentially therapeutic molecules. Recent progress in the knowledge of pathophysiological molecular processes, and in the development of molecular biotechnology have opened the way to new therapeutic interventions for these disorders. This chapter reviews the most recent gene therapy strategies using experimental models for neurodegenerative diseases.

Key words: Gene therapy, Neurodegenerative diseases, Adenoviruses, Retroviruses, Motoneurons, Alzheimer's disease, Parkinson's disease

Introduction

Neurodegenerative diseases are a heterogenous and complex group of chronic disorders with a progressive evolution sharing a common pathological event: neuronal death. Treatment of these neurological diseases by classical pharmacotherapy is restricted by constraints specific to the nervous system. In particular, the blood-brain barrier prevents access to the brain of numerous potentially therapeutical molecules. Delivery of such molecules requires intracerebral or intracerebro-ventricular injection or infusion using osmotic mini-pumps when long-term treatments are required.

An alternative strategy has been developed to replace degenerated neurons by transplantation of embryonic brain cells. These grafts are able to synthesize interesting neurotransmitter for each pathology: dopamine for Parkinson's disease, acetylcholine for Alzheimer's disease or GABA for Huntington's disease (Sinden et al., 1992). However, this strategy is limited by the restricted availability of fetal tissue and by ethical problems in a therapeutic perspective.

Recent progress in the knowledge of pathophysiological molecular mechanisms involved in the neurodegenerative processes have permitted the identification of some genetic causes of many of these diseases (Lee et al., 1996; Hardy and Gwinn-Hardy, 1998; Price et al., 1998; Tran and Miller, 1999). Moreover, the increased development of molecular biotechnology has opened the way to new therapeutical interventions for these neurodegenerative disorders (Neve, 1993; Harding et al., 1997; Yeh and Perricaudet, 1997; Corti et al., 1999).

Gene therapy should enable neurologists to overcome the limitations of pharmacological treatment and grafting of embryonic cells. Gene transfer involves the introduction of a functional genetic material into a given brain structure for replacing a deficient gene or for inducing the controlled expression and release of a new gene with therapeutic properties (Karpati et al., 1996; Sabaté et al., 1996).

Moreover, the recent development of the neuroimagery technology permits a precise and early anatomo-functional correlation for the clinical evaluation of patients.

Gene therapy approaches

Therapeutic genes can be transferred into the nervous system by two different approaches: by *directly* using an appropriate vector (*in vivo* gene therapy) or by intraparenchymal grafting of genetically engineered cells (*indirect ex vivo* gene therapy) (Svendsen, 1993; Fisher and Ray, 1994; Ridet and Privat, 1995; Karpati et al., 1996; Slack and Miller, 1996; Vivien et al., 1999).

In the *ex vivo* approach, the therapeutic gene is introduced *in vitro* into neuronal or non-neuronal cells, or into established cell lines, which are then transplanted to an appropriate region of the nervous system. Thus, this approach allows us to test the efficacy and toxicity of different gene vectors before cell transplantation (Fisher, 1995; Taylor, 1997).

Direct gene therapy allows a local and controlled expression and release of therapeutic products and prevents the side effects associated with other administration routes (Le Gal La Salle et al., 1993).

In the two approaches, the efficacy of the therapeutic effect resides in the optimal choice of the appropriate

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promoter and vector for gene transfer.

Vectors

Therapeutic genes may be transferred to the cells by different types of vectors, which can be classified into two main groups. On the one hand, synthetic macromolecules, liposomes, lipids or cationic polymers carrying specific ligands for cell surface receptors. On the other hand, viral vectors, which are a very interesting tool for gene transfer into the nervous system.

Synthetic vectors are classified in two groups: the cationic lipids and the cationic polymers. Both types of synthetic molecules are able to establish electrostatic links with nucleic acids (Vivien et al., 1999). Cationic lipids are amphiphilic molecules with a positive-charged hydrophil-head. These synthetic molecules, such as cholesterol derivatives have been shown to exhibit a good efficacy of infection both *in vivo* and *in vitro*. Cationic polymers can be peptides or other structures highly positive-charged. The most interesting characteristic of these molecules is that they can be associated to specific ligands allowing a cellular or nuclear targeting. However, they exhibit a low efficacy of transfection (Vivien et al., 1999).

A number of viral vectors have been developed for central nervous system (CNS) gene transfer. Herpes simplex virus1 (HSV1) adenovirus, adeno-associated virus (AAV), retrovirus. Recently, lentivirus has largely been developed (Naldini et al., 1996; Slack and Miller, 1996; Zufferey et al., 1997). All vectors are replication-deficient virus (Kremer and Perricaudet, 1995; Slack and Miller, 1996). There are several important points concerning the use of virus as vector for gene transfer (Karpati et al., 1996): 1) the viral tropism for certain cells; 2) the putative toxicity, antigenicity and tumorigenity of the viral genome; 3) the duration of expression of inserted gene; 4) the possibility of interaction or integration of the viral genome into the host genome; and 5) the facility of mass production at high titers for an efficient cell transduction.

Each viral type has advantages and disadvantages (Kremer and Perricaudet, 1995; Slack and Miller, 1996; Castro et al., 2000; Latchman, 2000). The most important advantage of adenovirus is the safety. They readily infect almost total cell types *in vitro* and *in vivo*, and infect dividing as well as quiescent cells with a high efficiency. AAV and HSV1 can also infect neurons with a high transduction frequency. Retrovirus only is able to infect dividing cells. The integration of the viral genome into the host genome, as is the case for AAV and retrovirus, can be interesting if the target is a dividing cell. However, this constitutes a potential risk of insertional mutagenesis of the transfected cells. Adenovirus and HSV1 do not integrate into host genome remaining as a non replicating extrachromosomal entity (Kremer and Perricaudet, 1995; Slack and Miller, 1996). At present, adenovirus constitutes one of the most efficient vectors for the gene transfer into the nervous system (Davidson and Bohn, 1997). Adenovirus deleted E1 and E3 regions can accommodate large inserts of a high number of kilobases and can be propagated to high titers. However, cytotoxicity due to the viral capsid leads to an immune and acute inflammatory response which destroys transfected cells and decreases the expresion of the inserted gene. New strategies are being developed to remove all transcription units from the viral backbone: "gutless adenovirus" (Yeh and Perricaudet, 1997).

Promoters

Gene expression can be targeted to specific cell types by using appropriate promoter sequences. In the nervous system, an ideal promoter of a therapeutic gene should be active for the long-term and it should be tissue- or cell-specific. Thus, one can use the promoters for neurofilament light chain, neuron-specific enolase, tyrosine hydroxylase (TH) or dopamine ß-hydroxylase for neurons, glial fibrillary acidic protein (GFAP) for astrocytes, myelin basic protein (MBP) for oligodendrocytes (Brennen et al., 1994; Karpati et al., 1996).

In some situations where the quantity of the protein product of a transgene is essential, the use of an externally regulated "inducible" promoter can be used. For instance, the control of gene expression in neurons or astrocytes can be achieved by using tetracycline-controlled transcriptional activation systems (tet-off system) (Gossen and Bujard, 1992; Corti et al., 1996, 1999; Ridet et al., 1999).

Route of administration

The route of administration is a major factor in determining the efficiency and safety of gene therapy.

Direct injection, preferably by stereotaxic guidance, has the advantage that restricted and precise regions can be targeted, since the spread of the vector-transgene construct is limited to a few millimeters.

In order to target a neuron population in a specific fashion, an indirect route that uses the retrograde axonal-transport system is appropriate for the transfection of spinal or brainstem motoneurons (Finiels et al., 1995; Ghadge et al., 1995). This approach permits a selective and targeted transfection of motoneurons by a precise intramuscular injection of the vector carrying a gene of interest.

Cell vehicles for gene transfer to the CNS

Early studies used neuronal or non-neuronal cell lines as vehicles for foreign gene expression due to their ability to proliferate *in vitro*. However, because these cells are immortalized, they continue to divide after transplantation and form tumors *in vivo* (Ridet and Privat, 1995; Slack and Miller, 1996). Afterwards, primary non-neuronal cells (fibroblasts, myoblasts, astrocytes) have also been successfully used as vehicles for gene transfer. In this regard, astrocytes constitute a promising cell vehicle for gene transfer to the CNS (Ridet et al, 1999).

However, primary neurons as transgene carriers present a major advantage: the ability to establish synaptic contacts with neurons of the host tissue. In this way, neurons can provide a necessary gene product combined with a cell replacement function. Primary neurons have been transfected using viral and non-viral delivery systems (Levallois et al., 1994; Vivien et al., 1999). At present, the studies are focused on the transfection of immature neural precursors or stem cells (Martinez-Serrano et al., 1996; Wagner et al., 1999).

Moreover, an other promising approach for CNS gene therapy is transplantation of polymer-encapsulated genetically modified cells. This technique improves graft survival and protects it from immune rejection (Aebischer et al., 1996).

In neurodegenerative diseases, these different gene transfer approaches can be used to intervene at several different time points in a neurodegenerative process. In a first phase, gene therapy can prevent neuronal degeneration. Once the degenerative process is underway, a neuroprotection strategy by neurotrophic factors can halt the progression of the disease. To encourage axonal regeneration from injured neurons, transplanted cells can be engineered to express growth factors or permissive substances. Finally, when neurons are irreversibly lost, cells engineered to produce neurotransmitters can be transplanted into denervated target areas to restore neuronal function.

Alzheimer's disease

Alzheimer's disease (AD) is the first most common neurodegenerative disorder. The disease is associated with the selective damage of brain regions and neuronal circuits critical for cognition and memory, including neurons in the neocortex, hippocampus, amygdala, basal forebrain cholinergic system, and brainstem monoaminergic nuclei. Dysfunction and degeneration of neurons in these neuronal circuits, mainly cholinergic innervation, lead to progressive loss of memory, resulting in dementia and death.

Affected neurons accumulate *tau* and *ubiquitin* immunoreactivities within neurofibrillary tangles in cell bodies and dendrites, and in dystrophic neurites. In addition, patients show numerous senile plaques composed of dystrophic neurites displayed around extracellular deposits of an amyloid-*β peptide isoform* (42 residues) that is derived from the *β-amyloid precursor protein* (APP) (Hardy and Gwinn-Hardy, 1998; Price et al., 1998).

This age-associated disorder is linked to several genetic risk factors. The majority of early-onset cases of AD are familial and inherited as autosomal-dominant disorders (Lee et al., 1996). Thus, mutations have been identified in several genes such as *presenilin* genes or APP gene. Moreover, inheritance of the *apolipoprotein E*, apo E4 allelo constitutes a risk factor for late-onset sporadic AD (Lee et al., 1996).

The first successful animal model of AD has been generated in mice with a plateled-derived growth factor-ß promoter to drive the expression of a human APP minigene that encodes the APP-V717F substitution (Games et al., 1995). These mice reproduce certain pathological and biochemical features of AD.

However, axotomy of the fimbria-fornix which induce degeneration of basal forebrain cholinergic neurons is the most commonly used experimental model of AD. Thus, the early approaches of gene therapy were directed to the neuroprotection of cholinergic neurons using this surgical model as well as aged animals where a memory impairment is correlated with cholinergic atrophy in basal forebrain nuclei.

Since nerve growth factor (NGF) has been shown to prevent degeneration of adult basal forebrain cholinergic neurons after injury (Hefti, 1986; Williams et al., 1986), the attempts of gene therapy have been focused on NGF gene transfer to the brain by using indirect *ex vivo* as well as direct approaches.

Indirect approaches have used primary fibroblasts, neural stem cells or progenitor cells which were genetically modified to produce NGF by retroviral trasduction. Then, cells were transplanted to the nucleus basalis of Meynert or to the medial septum of aged rats. Genetically modified cell grafts were able to prevent spontaneous age-associated cholinergic atrophy and to reverse cognitive impairments of these animals (Chen and Gage, 1995; Martinez-Serrano et al., 1996). The same approach has been tested in aged primates. Aged monkeys received intraparenchymal grafts of autologous fibroblasts genetically modified to secrete NGF into cholinergic basal forebrain. Three months later, the loss of subcortical cholinergic neuronal markers in aged animals was nearly completely abolished by human NGF delivery, indicating a prevention of cholinergic degeneration by NGF (Smith et al., 1999). Amyloid plaques deposition in aged monkeys, was not significantly modified by NGF delivery (Tuszynski et al., 1998). Similar results were observed using this approach in adult primates that underwent fornix transection to induce degeneration basal forebrain cholinergic neurons (Tuszynski et al., 1996).

Direct intraparenchymal NGF gene transfer by using a recombinant adenovirus or an AAV was also performed in aged rats. A significant increase in cholinergic neurons ipsilateral to the injection was observed by choline acetyltransferase immunodetection (Castel-Barthe et al., 1996; Klein et al., 1999).

A recent *in vitro* approach has evidenced the neuroprotective effect of the proto-oncogene protein Bcl-2 in this context. Cultured cortical neurons from transgenic mice expressing human Bcl-2 were partially protected against amyloid ß-peptide-induced neuronal death. This neuroprotection appears to be related to the inhibition of amyloid ß-peptide-induced apoptosis

(Saillé et al., 1999).

Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD. The disease is characterized by tremor, bradykinesia, rigidity and postural instability that result primarily from a degeneration of dopaminergic neurons of the nigro-striatal pathway. In addition to the loss of nigral neurons, PD is also characterized by the widespread distribution of intracytoplasmic eosinophilic aggregates or Lewy bodies. It has been suggested that Lewy bodies have a causative role in the degeneration.

Most cases of Parkinson's disease occur spontaneously, but in a small percentage of cases, this disorder can be inherited in an autosomal dominant fashion. One form of familial AD has been associated with a missense mutation in a protein called α-synuclein (Tran and Miller, 1999). The function of this protein is unknown, but it is found in high concentrations in the nervous system, where it is primarily localized in nerve terminals. It was subsequently demonstrated that α-synuclein is a major component of the Lewy bodies. In idiopathic PD, α-synuclein aggregation could be triggered by damage to the normal protein, through free-radical-mediated oxidation. The widespread detection of α-synuclein in many types of aggregates in different neurodegenerative diseases has led to the suggestion that it could be a common factor in initiating their formation (Clayton and George, 1999). However, curiously, one of the mutations in α-synuclein that has been linked to inherited PD occurs normally in rats without pathological evidences (Clayton and George, 1998).

Recently, another mutation in the enzyme ubiquitin carboxy-terminal hydrolase (previously associated with Lewy bodies) has been detected in some patients (Tran and Miller, 1999).

Because no spontaneous degeneration of dopaminergic neurons of the nigro-striatal pathway has been described in animal models, experimental models of PD are based on surgical techniques (axotomy of medial forebrain bundle) or by using specific neurotoxins such as 6-hydroxydopamine (6-OHDA) or 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine (MPTP).

Gene therapy for PD has been focused on two main strategies: (1) a substitutive strategy to restore levels of neurotransmitters by using gene encoding human tyrosine hydroxylase (TH), the limiting enzyme in catecholamine synthesis, and (2) a neuroprotective strategy to spare dopaminergic neurons of the substantia nigra by using neurotrophic factors genes. Both strategies can be achieved by either indirect or direct gene transfer approaches.

Early ex vivo gene transfer studies used cell lines modified to produce TH by retroviral transduction. After transplantation into the denervated rat striatum by 6-OHDA, these engineered cells were able to reverse the apomorphine-induced rotation which depends on striatal dopamine (Horellou et al., 1990).

More recently, primary myoblasts, fibroblasts or astrocytes have been the most commonly used cell types as vehicles for foreign gene expression. Astrocytes genetically modified by a retrovirus encoding TH have been shown to survive and express TH after transplantation and reduce apomorphine-induced turning behavior (Lundberg et al., 1996; Cortez et al., 2000). Primary astrocytes constitute a promising cell vehicle for *ex vivo* gene therapy for neurodegenerative diseases. A recent study has shown that human adult astrocytes can be maintained and expanded as long-term pure primary cultures, and can be efficiently transduced by an adenovirus carrying human TH gene with a tetracycline-controlled transcriptional activation system (tet-off system) (Ridet et al., 1999). This approach has been used for engineering human neural precursor cells which were then transplanted to rat denervated striatum (Corti et al., 1999).

A substitutive strategy for PD has also been achieved by direct gene transfer using synthetic or viral vectors of diverse nature.

Defective viral vectors (HSV1, adenovirus or AAV) or cationic lipids carrying the human TH gene have been directly injected into 6-OHDA denervated striatum of rats. The expression of the transgene in the striatum resulted in a reduction of apomorphine-induced turning behavior, suggesting that TH expression partially restores dopamine production and behavior (During et al., 1994; Horellou et al., 1994; Segovia et al., 1998). Some attempts to apply the same approach in primates have been performed (During et al., 1998).

A neuroprotective strategy has been developed by using trophic factors such as brain-derived neurotrophic factor (BDNF) or glial cell line-derived neurotrophic factor (GDNF), which are involved in the differentiation of mesencephalic dopaminergic cells.

Thus, fibroblasts or astrocytes have been genetically modified to produce BDNF by retroviral transduction, and then transplanted into a rat denervated striatum. The expression of BDNF was able to prevent degeneration of dopaminergic neurons (Levivier et al., 1995) and to attenuate amphetamine-induced rotation (Yoshimoto et al., 1995).

In the same way, the neurotrophic effect of an adenoviral vector encoding human GDNF has been evaluated by direct injection into the denervated striatum. The expression of GDNF was able to prevent the degeneration of dopaminergic neurons and the development of behavioral asymmetries which depend on striatal dopamine (Bilang-Bleuel et al., 1997; Choi-Lundberg et al., 1998)

Transplantation of embryonic dopaminergic neurons has been used as an experimental strategy for PD. In order to ameliorate its efficacy this graft can be combined with a treatment of GDNF. Thus, baby hamster kidney (BHK) cells transfected with GDNF gene and encapsulated were then co-grafted with embryonic dopaminergic neurons into the denervated striatum. The release of GDNF was able to improve survival and function of embryonic grafts (Tseng et al., 1997).

A recent and promising study has shown that neural stem cells derived from the mouse cerebellum can be transfected with the Nurr-1 gene (a transcription factor that mediates induction of mesencephalic dopaminergic neurons). Overexpression of Nurr-1 caused postmitotic cells to adopt a neuronal phenotype, but none of them exhibited TH-immunoreactivity. When Nurr-1 overexpressing cells were exposed to a soluble signal secreted by type1 astrocytes from ventral mesencephalon, they developed into dopaminergic neurons. A small number of them survived after implantation into the adult mouse striatum (Wagner et al., 1999).

Huntington's disease

Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by chorea, involuntary movements, dystonia, intellectual impairment and emotional disturbances. The disease is associated with autosomal-dominant trinucleotide-repeat mutations and exhibits a neuronal loss in the striatum and cortex (Reddy et al., 1999; Tran and Miller, 1999).

The gene responsible for HD has been shown to code for a large 350 kDa protein named *huntingtin*, which is widely distributed both within and outside the CNS. The molecular basis for the transmission of HD is associated with instability in the length of a CAG repeat (which codes for glutamine residues) near the N-terminal of the protein. Therefore, HD constitutes a good candidate for gene therapy strategies.

To investigate the normal function of the HD gene, knockout mice have been generated. Targeted disruption of the murine homolog of the human HD gene was found to be lethal in homozygous embryos. However, knockout studies suggest that *huntingtin* is functionally indispensable for neurogenesis since a regionalized apoptotic cell death in the embryonic ectoderm has been observed (Reddy et al., 1999).

In an attempt to study the basis for the instability of CAG trinucleotide repeats, several transgenic mice were created. However, some of them did not show any degeneration or behavioral abnormalities. In a fascinating experimental study carried out by Ordway et al. (1997), transgenic mice were generated with 146 CAG repeats by targeting into the mouse phosphoribosyltransferase gene, which is not involved in any CAG-repeat disorder. These mice developed a neurodegenerative disease, clearly indicating that expanded polyglutamine repeat has a toxic gain-in-function effect.

Although transgenic technology has recently developed animal models of HD, the most commonly used animal model is obtained by excitotoxic lesions of the striatum with quinolinic acid, which preferentially destroys medium spiny GABAergic neurons (Kordower et al., 1999).

An antisense gene therapy strategy has been used to reduce the *in vivo* expression of *huntingtin* protein. However, repeated intrastriatal infusions of antisense oligodeoxynucleotides did not significantly reduce the levels of *huntingtin* (Haque and Isacson, 1997).

Although the genetic basis for the HD is known the mechanisms involved in neuronal death occurring in this disease are unknown. Thus, initial gene therapy approaches have been focused on a neuroprotective strategy.

Studies in which NGF has been infused into the striatum prior to or concurrent with injections of quinolinic acid result in a sparing of cholinergic interneurons. However, cellular delivery of NGF potently protect both cholinergic and noncholinergic neurons from degeneration by excitotoxic lesion (Kordower et al., 1999).

Three different cell types (fibroblasts, progenitors cells and stem cells) have been used for transfecting with the human NGF gene by retroviral transduction. Then, cells were grafted into the striatum prior to or after a quinolinic acid lesion. NGF-secreting grafts were able to protect diverse populations of the striatum including GABAergic projection neurons in this animal model. However, this neuroprotective effect does not seem to involve TrkA-specific receptor (Kordower et al., 1999).

The neurotrophic effect of the ciliary neurotrophic factor (CNTF) has recently been demonstrated on a wide range of neurons including GABAergic, cholinergic and dopaminergic neurons. Thus, BHK fibroblasts were genetically modified to secrete human CNTF and then encapsulated in polymer membranes. Then, they were transplanted into the lateral ventricle or into the denervated striatum by quinolinic acid of rats or primates. BHK-CNTF grafts were able to preserve GABAergic and cholinergic neurons within the striatum, and to prevent aberrant motor behavior induced by the lesion. Moreover, BHK-CNTF grafts also protected the normal projection systems for this population of neurons, and prevented degenerative changes secondary to striatal degeneration in the cerebral cortex which are responsible for nonmotor symptoms observed in HD (Emerich et al., 1997; Kordower et al., 1999).

Direct gene therapy approach has been tested by Bemelmans et al. (1999) in an excitotoxic rat model by quinolinic acid. An intrastriatal injection of an adenovirus encoding BDNF was able to prevent degeneration of striatal GABAergic projection neurons induced by the lesion.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that is characterized by selective degeneration of motoneurons in the brainstem and spinal cord. This disorder leads to weakness and muscle atrophy, paralysis and death within three to five years.

The neuropathological features of motoneurons include the hyperaccumulation of phosphorylated neurofilaments (NF), intracellular inclusions of *ubiquitin*, and intracytoplasmic inclusions resembling Lewy bodies (Price et al., 1998; Tran and Miller, 1999).

About 10% of ALS cases are inherited (familial ALS) with mainly an autosomal dominant pattern. Approximately 15-20% of patients with FALS have missense mutations in the gene encoding cytosolic Cu/Zn superoxide dismutase 1 (SOD1). There is clear evidence of an allelic heterogeneity with associated phenotypes (Wong et al., 1998). A variety of chromosomal loci have been associated to forms of ALS. Thus, an autosomal-dominant juvenile ALS has been linked to loci in the 9q34 region. In patients with sporadic ALS, deletion mutations in the carboxy-terminal in the NF-H tail domain have been reported (Wong et al., 1998).

There are various animal models that can in some way mimic an aspect of motoneuron degeneration characteristic of ALS. These models include neonatal axotomy-induced retrograde degeneration or spontaneously occurring murine models such as progressive motor neuronopathy (pmn), murine motoneuron degeneration (mnd), wobbler, neuromuscular degeneration (nmd), paralysé, and muscle deficient (mdf) (Price et al., 1994; Wong et al., 1998; Elliott, 1999). Moreover, the identification of genetic factors in the aetiology of this disorder have allowed to generate transgenic mice that overexpress normal or mutated NF genes, transgenic mice expressing SOD1 mutations or gene knockout mice by gene-targeting strategies.

Knockout mice with targeted deletions of both SOD1 alleles do not develop spontaneous motoneuron degeneration. In contrast, transgenic mice overexpressing mutant SOD1 exhibit spontaneous motoneuron degeneration with progressive clinical weakness demonstrating a toxic gain-of-function for the mutant SOD1 protein in FALS (Gurney et al., 1994). Although the precise mechanisms underlying mutant SOD1 toxicity are unclear, these transgenic mice overexpressing mutant SOD1 provide an excellent animal model of human FALS. Different lines of these transgenic SOD1 mice have been generated.

Because neurotrophic factors exhibit survival-promoting effects on developing motoneurons, they have been readily considered as potential neuroprotective therapeutic molecules for motoneuron degenerative diseases. Thus, gene therapy approaches use neuroprotective strategies and all animal models cited above.

Neonatal axotomy has been used to test an original strategy of selective transfection of motoneurons which is based on the retrograde axonal transport of an adenoviral vector encoding CNTF, BDNF or GDNF. Precise intramuscular injection of the vector prior to axotomy results in transgene expression in motoneurons afferent to the injected muscle, preventing massive degeneration induced by axotomy (Baumgartner and Shine, 1997; Giménez y Ribotta et al., 1997; Gravel et al., 1997). The efficacy of this strategy based on the retrograde axonal transport has also been demonstrated in the *pmn* mouse. An adenovirus carrying neurotrophin-3 (NT-3) gene was injected into three muscles of neonatal *pmn* mice, before onset of symptoms of motoneuron disease. NT-3-treated animals survived longer than control nontreated animals and showed reduced loss of motor axons, and improved motoneuron function as assessed by electromyography (Haase et al., 1997). Co-injection of adenovirus encoding NT-3 and CNTF into skeletal muscles resulted in an synergetic effect (Haase et al., 1997).

An interesting strategy consists to test whether the overexpression of human Bcl-2 proto-oncogene protein or of CNTF can protect in an animal model of ALS.

Thus, transgenic animals overexpressing Bcl-2 were generated and then crossed with SOD1 transgenic, *pmn* or *wobbler* mice. Overexpression of the Bcl-2 in SOD1 transgenic mice delayed the onset of the disease, prolonged the survival and attenuated spinal cord motoneuron degeneration (Kostic et al., 1997). In a hybrid animal carrying both human Bcl-2 transgene and the wobbler mutation, the pathological motoneuron death was not altered (Coulpier et al., 1996). Overexpression of Bcl-2 in *pmn* mutant mice prevented motoneuron loss but did not prevent degeneration of myelinated axons, and it did not increase the life span of the animals (Sagot et al., 1995). In contrast to the beneficial effects of CNTF in preventing motoneuron degeneration in other paradigms, the overexpression of CNTF in *mnd* mice increased the rate of onset of motor disease symptoms (Winter et al., 1996).

An ex vivo gene therapy approach has been developed using encapsulated BHK cells which were previously transfected with CNTF or GDNF gene. Capsules were implanted subcutaneously into the back of *pmn* mice as soon as the disease was detected. CNTF expression delayed the disease progression by increasing the survival time and by improving motor function. However, GDNF did not increase the life span of *pmn* mice, but significantly reduced the loss of motoneurons (Sagot et al., 1995, 1996).

Finally, a phase I clinical study in which ALS patients were implanted with polymer capsules containing genetically engineered BHK cells releasing human CNTF (0.5 μg/day) has been reported. Implants were placed within the lumbar intrathecal space. Levels of CNTF measured within the cerebrospinal fluid demonstrated a continuous delivery of CNTF by an *ex vivo* gene therapy approach (Aebischer et al., 1996).

Retinal pathologies

A group of inherited retinal diseases, collectively termed retinitis pigmentosa (RP) is characterized by the progressive and specific loss of photoreceptors, the light-transducing neurons of the retina. Factors involved in this degeneration can be genetic as well as environmental factors.

Photoreceptor-specific genes directly involved in phototransduction have been considered primary candidates for RP-inducing genes. Indeed, specific mutations in the gene for the beta-subunit of cyclic GMP phosphodiesterase (ß-PDE) induce dystrophies in man and in mice. Mutations in the gene for the structural protein peripherin have also been identified in mice. These mutant animals are useful models for investigation of new therapeutic strategies of gene therapy (Petersen Jones, 1998).

The *rd* mouse is an autosomal-recessive mutant in which the rod photoreceptor-specific ß-PDE gene is altered (Cayouette et al., 1999). This mutation leads to a rapid and massive death of photoreceptors in homozygous animals by 6 weeks of age. The null mutation in *peripherin* gene in the *rds* mouse causes a protracted apoptotic loss of photoreceptors. This mutation has also been detected in patients affected by dominant forms of retinal degeneration.

However, survival and homeostasis of photoreceptors are also dependent on interactions with adjacent cells of retinal pigment epithelium. Thus, the inherited photoreceptor degeneration seen in the Royal College of Surgeons (RCS) rat has been attributed to a defect in retinal pigment epithelium (Cayouette et al., 1999).

Thus, *rd, rds* mice and the RCS rat constitute the current animal models to test substitutive or neuroprotective gene therapy strategies.

A substitutive gene therapy strategy has been achieved with a vector (adenovirus, AAV or lentivirus) containing the gene encoding ß-PDE which was directly injected into subretinal spaces of newborn rd mouse eyes. Expression of ß-PDE was confirmed by two-color confocal immunofluorescence analysis and allowed the rescue of rod photoreceptor cells. Treated eyes showed a two-fold increase in sensitivity to light as measured by *in vitro* electroretinography (Bennett et al., 1996; Jomary et al., 1997; Takahashi et al., 1999). Moreover, when an encapsidated adenovirus mini-chromosome (from which all of the viral genes have been deleted) containing the gene encoding ß-PDE was used, a prolonged transgene expression and a rescue of rod photoreceptor cells was found (Kumar-Singh and Farber, 1998).

A neuroprotective gene therapy strategy has also been performed. In order to prevent photoreceptor degeneration, adenovirus encoding CNTF, basic fibroblastic growth factor (bFGF) or Bcl-2 proto-oncogene protein was injected into the vitreous of rd or rds mice or RCS rats. Expression of the transgene revealed a significant neuroprotective effect which reduced photoreceptor loss and increased the retinal content of the photopigment rhodopsin. These effects were accompanied by a significant increase in the amplitude of the reponse of the scotopic electroretinogram (Bennett et al., 1998; Cayouette et al., 1998; Akimoto et al., 1999).

An *ex vivo* gene therapy approach has been performed in RCS rats. Fibroblasts genetically engineered to secrete hFGF-2 and encapsulated in a biocompatible polymer were transferred into the vitrous cavity of animals. Secretion of hFGF-2 induced a local delay of photoreceptor cell degeneration (Uteza et al., 1999).

Recently, a substitution in the rhodopsin gene (codon P23H) has been detected in some patients with autosomal dominant RP. Ribozymes can discriminate and catalyze the *in vitro* destruction of P23H mutant mRNAs from a transgenic rat model. Intracellular production of ribozymes in photoreceptors was achieved by direct transduccion with a recombinant AAV incorporating a rod opsin promoter. Ribozyme-targeted destruction of P23H mutant mRNA considerably slows the rate of photoreceptor degeneration with functional preservation of the retina evaluated by electroretinography (Lewin et al., 1998).

Other retinal pathologies are characterized by retrograde degeneration of retinal ganglion cells as a consequence of an optic nerve lesion. This retrograde degeneration has been studied in a model of axotomy of the optic nerve and can be prevented by administration of neurotrophic factors. Thus, direct injection in the vitreous of an adenovirus encoding BDNF or GDNF was able to prevent retinal ganglion degeneration induced by axotomy (Klocker et al., 1997; Di Polo et al., 1998). Apoptotic cell death of retinal ganglion cells induced by optic nerve axotomy can be inhibited by an adenovirus encoding antiapoptotic protein p35 administrated at the optic nerve stump (Kugler et al., 1999).

Auditory system pathologies

Hearing impairment is a serious handicap which appears to be due to damage to the peripheral auditory system, consisting of auditory receptors, hair cells in the Corti organ and spiral ganglion neurons in the cochlea. Therapeutic drugs including salicylates, aminoglycosides and chemotherapeutic agents are a major cause of these pathologies.

Organotypic cultures of cochlear explants have been used to explore the mechanisms of action of ototoxins (Zheng and Gao, 1996). Using this assay the target of three classes of ototoxic therapeutic drugs were evidenced. Thus, hair cells are the the primary target of aminoglycosides, which can secondarily induce degeneration of cochlear neurons. Damage to hair cells was also seen after administration of chemotherapeutic agents, and in the case of salicylates the authors demonstrated that this drug was able to produce a selective neuronal degeneration without hair-cell loss (Zheng and Gao, 1996).

The implication of neurotrophins and their associated receptors for the normal development of afferent innervation of inner ear, has allowed the development of new therapeutic strategies using neurotrophins to treat hearing loss (Fritzsch et al., 1997).

Analysis of mice lacking either BDNF or its associated receptor, TrκB have shown a reduced innervation of outer hair cells of the cochlea. Mice lacking either NT-3 or its associated receptor, TrκC lose many ganglion cells of the cochlea. In mice lacking both BDNF and NT-3 or both TrκB and TrκC there is a complete loss of innervation to the inner ear (Fritzsch et al., 1997).

The prevention of neuronal degeneration by neurotrophins has been demonstrated by using the model of organotypic culture of cochlea. Thus, expression of NT3/4 or BDNF was able to protect ganglion neurons from degeneration induced by aminoglycosides (Ernfors et al., 1996; Geschwind et al., 1996).

Direct *in vivo* experiments using adenovirus, AAV or liposomes have demonstrated successful gene transfer into multiple types of cochlear cells (Lalwani et al., 1997), and efficacy of this approach has been demonstrated in guinea pig cochlea. An adenovirus encoding the human GDNF gene was inoculated via the round window membrane prior to injection of aminoglycosides. Expression of GDNF protects hair cell from aminoglycoside ototoxicity (Yagi et al., 1999). Spiral ganglion degeneration after hair cell loss by aminoglycosides was prevented with a HSV1 vector encoding BDNF (Staecker et al., 1998).

Conclusion

Gene therapy studies using experimental models of neurodegenerative diseases have shown in many cases successful and efficient gene transfer in *ex vivo* as well as *in vivo* approaches. However, there are still many issues to resolve before such gene transfer will be applicable to human diseases. Phase I clinical trials of gene therapy are still exceptional and analyze the requirements for an adequate feasibility and security rather than analyzing efficacy. The critical point is the vector. The technology is now available to create designer vectors that can be optimized incorporating features of viral and nonviral vectors for each application. The big challenge for gene therapy in neurodegenerative diseases is to increase the knowledge of the aetiopathogenesis of these disorders and to apply accordingly the most safe, efficient and appropiate targeted strategies.

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