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PERSPECTIVE

Gene therapy in Parkinson's disease: targeting the endplasmic reticulum proteostasis network

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting 1% of the population over 60 years of age. The progressive degeneration of dopaminergic neurons at the *substantia nigra pars compacta* (SNpc) results in a severe and gradual depletion of dopamine content in the striatum, a phenomena that is responsible for the characteristic motor symptoms of this disease. There is no cure for PD and available treatments only aim to restore dopamine deficits. Administration of the dopamine precursor Levodopa (L-DOPA) is the main temporal palliative treatment that increases overall dopamine levels. However, its chronic use limits its effectiveness and generates a number of adverse effects such as debilitating dyskinesia.

The increasing need for more effective therapies is widely recognized. Because PD is a slowly progressing disease, and the brain nuclei affected are anatomically restricted, gene therapy is emerging as an alternative to improve neuronal function and survival. Such a strategy is predicted to reduce the adverse side effects associated to existing palliative treatments. In the last decade, several clinical trials for PD have been initiated using gene therapy (Coune et al., 2012; Bartus et al., 2014) (**Figure 1**). Importantly, delivery of therapeutic genes to the nigrostriatal pathway requires small viral titers and vector volumes. Moreover, several phase I and II clinical trials in PD patients uncovered outstanding safety profiles of delivering lentiviral and adeno-associated virus (AAV) particles into the brain. Importantly, recombinant AAVs are not immunogenic, they have specific tropisms for neurons, they can be produced at a large-scale level and do not integrate into the host genome, reducing the possibility of insertional mutagenesis (Bartus et al., 2014).

Most of the gene therapies tested so far have been designed to provide symptomatic relief (reviewed in Coune et al., 2012; Bartus et al., 2014). Four of these strategies aim to compensate the decrease in dopamine levels using an enzyme replacement strategy to improve the efficiency of its metabolic pathway. One of these approaches involves a tri-cistronic lentiviral vector coding for the rate-limiting enzymes required for dopamine biosynthesis, aromatic-l-amino-acid decarboxylase (AADC), tyrosine hydroxylase (TH) and cyclohydrolase 1 (GCH1) (ProSavin®, Oxford BioMedica, Oxford, UK). An alternative strategy tested is the delivery of AAVs coding for TH and GCH1 to enhance the ectopic production of L-DOPA, or the delivery of AADC to reinforce L-DOPA conversion. Finally, AAV-mediated expression of glutamic acid decarboxylase (GAD) in the subthalamic nucleus (STN) was used as an approach to correct their hyper-metabolism and reduce its negative regulation on the nigrostriatal circuit.

Two gene therapy strategies are currently being tested with the aim of modifying the course of the disease and hopefully restore dopaminergic neuron function. The local delivery of tropic factors, such as the glial cell line-derived neutrophic factor (GDNF) or the close GDNF homolog neurturin (CERE-120), was enforced using AAVs to improve dopaminergic neuron survival. Despite the positive safety results and some positive outcomes reported in the clinical scores, in general all clinical trials reported to date have not revealed clear advantages compared with current pharmacological or electrophysiological therapies. Importantly, the main conclusion of the available trials indicated that gene therapy in PD is remarkably safe and tolerable, with no evidence of risk after administration of the viral vectors (Bartus et al., 2014). Today, one of the main challenges in the field is to identify a potent neuroprotective factor that can be delivered using gene therapy into the brain of PD patients. Thus, understanding the mechanisms involved in the selective neuronal vulnerability of dopaminergic neurons in PD is a key step toward developing an effective disease modifying treatment. These advances should be also accompanied with the development of better PD preclinical models that recapitulate (i) the slowly, selective and progressive nature of the disease, (ii) the contribution of aging, and (iii) the establishment of the complex molecular and behavioral features of PD.

The central pathological hallmark of PD is the formation of cytoplasmic inclusions of insoluble proteins called Lewy bodies, which contain aggregates of misfolded α-synuclein and ubiquitin. Many cellular pathways have been suggested to contribute to the disease process, including oxidative stress, proteasomal, and mitochondrial dysfunction, and more recently, impairment of protein homeostasis (proteostasis). In the last years, alterations to the function of the secretory pathway, and more importantly the endoplasmic reticulum (ER), are emerging as major pathological features driving dopaminergic neuron degeneration (Mercado et al., 2013). Similarly, ER stress has been linked to the occurrence of a variety of protein misfolding disorders including Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease (Hetz and Mollereau, 2014).

ER stress activates a dynamic signaling network known as the unfolded protein response (UPR), which aims to restore proteostasis, or eventually trigger cell death under chronic stress conditions. Three main ER stress sensors initiate the UPR, including PERK, ATF6, and IRE1α. PERK phosphorylates the eukaryotic initiation factor-2 (eIF2α), resulting in the general attenuation of protein translation and the selective expression of the transcription factor ATF4, which controls the expression of UPR target genes involved in redox metabolism and folding, but also apoptosis. IRE1α is an ER-located kinase and endoribonuclease that upon activation initiates the unconventional splicing of the mRNA encoding XBP1. This processing event leads to the expression of a potent transcription factor, termed XBP1s, that

Gene therapy approaches in clinical studies			
Symptomatic approaches			
	Transgene(s)	Viral vector	Outcomes
Dopamine synthesis	TH + GCH1 + AADC	Lentiviral vector	Program suspended; additional work to optimize vector ongoing
L-DOPA conversion	AADC	AAV2	Revised Phase 1 recently announced
L-DOPA synthesis	TH + GCH1	AAV5	N/A
GABA synthesis is STN	GAD	AAV2	Phase 2 mixed results; program suspended
Disease modifying approaches			
Neutrophic factor	GDNF	Lentiviral vector	N/A
Neutrophic factor	Neurturin	AAV2	Phase 1 mixed results; Phase 2 program suspended
Gene therapy approaches in pre-clinical studies targeting UPR components			
Active UPR transcription factor	XBP1s	Adenoviral vector	Decrease dopaminergic neuron loss
UPR chaperone	BiP/Grp78	AAV5	Decrease dopaminergic neuron loss; improved motor performance
Active UPR transcription factor	XBP1s	AAV2	Decrease dopaminergic neuron loss and striatal denervation
* Figure modified from: Coune et al., 2012 and Bartus et al., 2014			

Figure 1 Summary of gene therapy approaches in Parkinson's disease (PD) including current status of clinical trial programs and pre-clinical studies targeting components of the unfolded protein response (UPR).

regulates genes involved in folding, ERAD, and quality control.

Signs of ER stress are observed in most cellular and animal models of PD, in addition to brain tissue from PD patients (Mercado et al., 2013), observing a strong correlation between the appearance of UPR activation markers and the progression of PD histopathological and behavioral features. Since the UPR has dual roles in cell fate under ER stress, the relative impact of the pathway to the disease process may depend on the specific UPR branch affected, and the cell type and the nature of the stress/pathological stimuli involved (Hetz and Mollereau, 2014). Recent validation studies have demonstrated a functional contribution of ER stress to PD. For example, genetic manipulation of ATF6, XBP1, or the ER stress-apoptosis factor CHOP, indicated that the UPR has a relevant impact in adaptation to the stress (cell survival phase), and the neurodegenerative process during late disease stages (apoptosis phase) in mouse models of PD (Mercado et al., 2013). We have also recently reported that the developmental ablation of XBP1 in the nervous system protects dopaminergic neurons against a PD-inducing neurotoxin through an ER-hormesis compensatory mechanism (Valdes et al., 2014). This survival effect was due to a preconditioning condition that resulted from the induction of an adaptive ER stress response. In agreement with this, silencing XBP1 in adult animals triggered chronic ER stress and dopaminergic neuron degeneration. Pharmacological enhancement of eIF2α phosphorylation has also relevant protective effects on PD models (Colla et al., 2012). Thus, ER stress is emerging as a relevant target to alleviate PD-induced neurodegeneration.

Recent advances in the field led to test the consequences of manipulating the ER proteostasis network in PD using gene therapy. Delivery of BiP, a major ER chaperone, into the SNpc using AAV5 demonstrated remarkable neuroprotection against α Synuclein over-expression, improving motor control (Gorbatyuk et al., 2012). We recently tested a gene therapy to deliver active XBP1s into the SNpc using AAVs. This strategy provided neuroprotection and reduced striatal denervation in animals injected with a PD-inducing neurotoxin (Valdes et al., 2014). This approach was also shown to have positive effects in models of Huntington's disease, retinal degeneration, and spinal cord injury (Hetz and Mollereau, 2014). Similarly, adenovirus-mediated overexpression of XBP1s protected animals against the treatment with the PD-inducing neurotoxin MPTP (Sado et al., 2009). One of the major targets of α Synuclein is Rab1, a key regulator of ER to Golgi trafficking. A gene therapy strategy was also developed to revert the pathological effects of α Synuclein by enforcing the expression of Rab1 at the SNpc (Coune et al., 2011). Overall, these findings revealed a fundamental role of ER stress in PD pathogenesis.

We predict that more exciting studies will further validate in the near future the contribution of ER stress to PD in additional animal models. The UPR is a complex signaling network that offers a large spectrum of molecular targets that control specific nodes of the proteostasis network. A growing number of small molecules and gene therapy strategies have been generated in the last years to alleviate ER stress on a number of diseases (Hetz et al., 2013), providing interesting tools that could also be tested in PD models. Thanks to the positive safety outcomes of the available clinical trials in PD, at this stage of development gene therapy vectors and their delivery routes have all the necessary requirements to move forward and enable the testing of novel targets and concepts. It is essential to perform more studies using ER proteostasis-modifying agents in PD models, in addition to define the possible side effects of manipulating the UPR network in the long term. Aging is the major risk factor to develop PD, a process that involves a progressive decline in the proteostatic capacity of the brain. Recent findings indicate that the UPR, and more specifically XBP1s, has a relevant activity in preventing the negative effects of aging over the health of the proteome, which unexpectedly operates through cell-nonautonomous mechanisms (Hetz and Mollereau, 2014). This is why we envision that XBP1s-mediated gene therapy may not only reduce ER stress levels in PD neurons, but also may ameliorate the deleterious consequences of aging, propagating its protective signals to non-transduced neighbor neurons. In summary, the recent advances implicating ER proteostasis impairment in PD have opened new alternatives for the development of disease modifying agents.

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