Gene therapy and the aging nervous system

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Abstract

In recent years, the first attempts have been made to apply gene transfer technology to protect neurons from death following neurological insults. There has been sufficient progress in this area that it becomes plausible to consider similar gene therapy approaches meant to delay aspects of aging of the nervous system. In this review, we briefly consider such progress and how it might be applied to the realm of the aging brain. Specifically, we consider: (a) the means of delivery of such therapeutic genes; (b) the choice of such genes; and (c) technical elaborations in gene delivery systems which can more tightly regulate the magnitude and duration of transgene protection. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

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Gerontology has taken a long and tortuous path to its present state of relative credibility. In its early decades, one challenge was to establish that there was a biology of aging which was independent of the pathologies of aging. Once accomplished, the next challenge was to establish that such biology, rather than being a process of random entropy, was sufficiently patterned to actually be worth studying. With sufficient descriptive studies, it became clear that there were indeed some consistent themes in cellular aging — the accumulation of oxidative damage, energetic impairment, faulty DNA repair, and so on. Finally, with this knowledge...
came the possibilities of intervening in some of these processes and thus, perhaps, of delaying the aging process.

Such interventions have taken a number of forms — altering the caloric intake, antioxidant capacity, exposure to glucocorticoids, and so on. The emergence of molecular medicine has allowed for the possibility of the therapeutic transfer of genes. Such ‘gene therapy’ has prompted tremendous excitement (along with, admittedly, only moderate success). One area of progress has been in the use of gene therapy to protect neurons from insults. In this review, we summarize such progress, and consider how readily the current state of this field can lead towards gene therapy against neuron loss during normative aging.

1. Some principles of gene therapy

   The premise in gene therapy, of course, is the transfer of a transgene(s) in order to prevent a disease from occurring, prevent an adverse consequence of a disease, or facilitate recovery from the consequence. An additional premise related to aging would be the repair and maintenance of cells.

2. Vectors

   Half of gene therapy revolves around the means used to deliver a transgene. Within the nervous system, gene transfer is greatly constrained by the post-mitotic nature of neurons; as such, only a small number of neurotropic viruses, such as herpes simplex virus 1, adenovirus, adeno-associated virus, and a number of lentiviruses can be used as vectors (Kay et al., 2001). These differ as to the amount of DNA which can be transferred, the speed, magnitude and duration with which they express, the extent to which they spread from their site of delivery (by, typically stereotaxic injection into the parenchyma or intracerebroventricular infusion), and the extent of inflammation that they cause. Ongoing work involves: (a) construction of hybrid vectors which incorporate elements of a number of different viruses, in order to optimize a number of features (Zheng et al., 2000; Kay et al., 2001); (b) development of inducible vectors allowing for control over the timing of expression (Clackson, 2000; Ozawa et al., 2000); (c) cre-lox system, or cell-type specific promoters allowing for control over the site of expression (Gorman and Bullock, 2000; Lelievre et al., 2000; Modlich et al., 2000; Shibata et al., 2000).

3. Target selection (genes)

   The other half of the field concerns what genes are transferred — is there sufficient understanding of the mechanisms underlying cell death and dysfunction to identify the Achilles heels most appropriately targeted? The types of efficacious transgenes broadly fall into three categories.
First, a transgene may replace something salutary which fails to occur, or occurs insufficiently in an organism. Under this rubric would fall replacing an enzyme missing in, for example, a lipid storage disease. This would also include identifying protective responses of an organism to an insult (e.g. heat shock protein induction, upregulation of antioxidant enzymes, enhanced energy uptake) and facilitating these defenses further with overexpression of hsp's, antioxidants, and glucose transporters (e.g. Yenari et al., 1998; Kindy et al., 1996; Lawrence et al., 1995, 1996).

A second class of interventions would blunt some damaging process that occurs. Under this category would be, for example, delivery of an antisense vector that blocks the actions of a pro-oxidant protein (Parmentier-Batteur et al., 2001).

Finally, gene therapy may be used to introduce a novel, and hopefully protective, biological process. As a fairly speculative example, cortical neurons in some reptiles are markedly resistant to anoxia (Wilson and Kriegstein, 1991), and an understanding of the molecular basis of this could pave the way for a novel gene therapy. Less speculatively, virally derived anti-apoptotic genes, lacking any known mammalian homologs, can be neuroprotective (Roy et al., 2001).

These general approaches have been applied with some success in the last decade to the challenge of protecting neurologically endangered neurons.

4. Gene therapy against necrotic and degenerative insults in the nervous system

The cascade of cellular dysfunction that mediates neuron death following necrotic insults (such as hypoxia-ischemia or seizure) is well understood. This involves excessive accumulation of synaptic glutamate and of post-synaptic free cytosolic calcium. This leads to oxygen radical formation, cytoskeletal degeneration, energetic collapse and, in a subset of neurons, apoptosis. This understanding provides a number of plausible targets for gene therapy. Sparing of neurons and/or function post-insult has been reported with the overexpression of a glucose transporter (Ho et al., 1995; Lawrence et al., 1995, 1996), potassium channels (to blunt excitotoxicity) (Lee and Sapolsky, 2001), calcium binding proteins (Kindy et al., 1996; Meier et al., 1997, 1998), heat shock proteins (Fink et al., 1997; Yenari et al., 1998), antioxidant enzymes (Kindy et al., 1996), anti-inflammatory proteins (Betz et al., 1995) and anti-apoptotic proteins (Linnik et al., 1995; Lawrence et al., 1996; Phillips et al., 2000; Dumas et al., 2000; McLaughlin et al., 2000).

Progress has also been made in targeting neuron death during neurodegenerative insults. The best studied has been models of the nigrostriatal degeneration of Parkinson's Disease involving delivery of dopaminergic toxins such as 6-OHDA or MPTP. A number of studies have shown a sparing of neuron number, an enhancement of dopaminergic transmission, and/or maintenance of motoric function with overexpression of neurotrophins such as GDNF, of an anti-apoptotic protein, or of tyrosine hydroxylase (reviewed in Costantini et al., 1999; Simonato et al., 2000).
5. Gene therapy against normative neuronal aging

How might this progress be harnessed to design gene therapies to protect the aging nervous system? Four types of intervention might be visualized.

In the most straightforward case (Fig. 1A), the goal would be to overexpress a transgene for the rest of the life of an organism. This approach has been used in a number of studies. For example, an adenoviral vector was used to overexpress the D2R dopamine receptor, whose numbers in the nigrostriatal system normally decline during aging; this improved performance on a motor task (Ingram et al., 1998). Another example involved transplanting fibroblasts engineered ex situ to overexpress nerve growth factor, to reverse the decline of subcortical cholinergic markers in aging rhesus monkeys (Smith et al., 1999). A third study targeted the loss of dopaminergic neurons in the nigrostriatal system of the aging rhesus. The authors introduced a lentiviral vector expressing glial-derived neurotrophic factor into the substantia nigra or the caudate/putamen, demonstrating salutary effects on dopaminergic neurotransmission and motor function (Kordower et al., 2000).

In the second pattern (Fig. 1B), the biological rationale for overexpressing a particular transgene becomes more pronounced with age. No study using this design has been published, to our knowledge. As an example of how this approach might be applied, an excess of glucocorticoids, the adrenal steroids released during stress, can have adverse effects in the brain (Sapolsky, 1999), and basal glucocorticoid levels rise progressively with age in some rat strains (Sapolsky, 1992). Thus by including a glucocorticoid response element (GRE) in the promoter, one can take advantage of these increasing levels to drive a therapeutic gene. This would work only if the increasing age-related signal (i.e. the higher glucocorticoid levels, in this example) cause increasing amounts of transgene expression; this has been shown (Ozawa et al., 2000). As an example of an application for such a system, we are currently testing the efficacy of a vector which, driven by increasing circulating glucocorticoid levels via a GRE promoter, overexpresses 11beta-hydroxysteroid dehydrogenase, an enzyme which degrades glucocorticoids.

As another possibility (Fig. 1C), gene therapy would not target an ongoing deleterious process in the organism, but instead a discretely damaging event with a high likelihood of occurring during senescence. In this scenario, the transgene would be introduced and be transcriptionally quiescent, with expression not occurring until transiently triggered by the insult. As an example of where this approach might be helpful, aging increases the likelihood of episodes of hypoxia-ischemia, with the resulting generation of oxygen radicals. Thus, one might wish to overexpress an antioxidant enzyme. In this instance, expression would be made insult-inducible by inclusion of a hypoxia responsive element (HRE) in the promoter (Gleadle and Ratcliffe, 1998). Furthermore, greatly enhanced expression during the hypoxic period could be brought about by inclusion of a feed-forward element in the construct. This would involve inclusion of the gene for hypoxia-inducible factor-1 (hif-1) which, in response to hypoxia, acts as a transcriptional activator at an HRE (Forsythe et al., 1996; Jiang et al., 1996). Another example of such a regulated system is the treatment of an animal model of diabetes with overexpres-
Fig. 1. Schematic representation of gene expression from different expression cassettes used in gene transfer. (A) Left, gene expression over time from a constitutive cassette. Arrow indicates time of gene transfer. Right, constitutive promoter (ConPro) driving a neuroprotective gene. (B) Left, gene expression over age with expression induced by increasing glucocorticoid levels. Right, simple inducible cassette; glucocorticoid response element (GRE) driving neuroprotective gene. Dashed line corresponds to glucocorticoid levels. (C) Left, gene expression over time from an inducible cassette. Arrow indicates initiation of hypoxia. Hatched box refers to duration of hypoxic event. Right, inducible cassette with amplification loop; HRE driving the expression of two genes, the hypoxia-inducible factor-1 (hif-1) followed by a neuroprotective gene being driven by an internal ribosomal entry site (IRES). (D) Left, expression over time of a transgene from a multi-gene unidirectional expression network. Arrow indicates initiation of hypoxia. Hatched box refers to duration of hypoxic event. Right; Line 1. HRE driving expression of a synthetic transactivator (SynTransAct). Line 2. Synthetic response element (SynRE) driving expression of a neuroprotective gene. Line 3. SynRE driving expression of the SynTransAct with a repressor element (Rep) present in the promoter. Line 4. Constitutive promoter driving expression of a synthetic transcriptional repressor (SynTransRep). SynTransRep is only active in the presence of the appropriate ligand.
tion of a single chain insulin analog. Critically, the promoter in this construct, derived from the L-type pyruvate kinase, is induced by hyperglycemia (Lee et al., 2000). It should be noted that while this study was conceptually similar to what is outlined in Fig. 1C, it lacked an equivalent of the amplifying element (i.e. the hif1 gene).

As a final elaboration (Fig. 1D), expression would be triggered by the damaging event, with sustained (or even increasing) expression thereafter. As one scenario where this might be applied, the hypoxia-inducible vector just discussed might, in this case, trigger permanent overexpression of a growth factor. This requires the creation and introduction of a regulatory network, a technically feasible approach (Desjarlais and Berg, 1993; Choo and Klug, 1994; Rebar and Pabo, 1994; Wu et al., 1995; Greisman and Pabo, 1997; Kang and Kim, 2000). The expression of this network, once activated is exclusively unidirectional. The triggering event (e.g. reduced oxygen tension) would cause expression of a synthetic transcriptional activator (Fig. 1D, line 1) which would specifically recognize a synthetic response element (SynRE) unique within the cell and specific for the gene(s) in this small network (Fig. 1D, lines 2 and 3). The genes driven by this transactivator would be a neuroprotective gene and the transactivator itself. This would set up a positive feedback loop were the transactivator drives its own expression and the continuous expression of the neuroprotective gene. As a potential problem, overexpression could be sufficient to cause toxic side effects, requiring some sort of regulation. This would be done by designing specific promoter strength and by specifically designing the 3'UTR of the mRNA to regulate the mRNA half-life. Another potentially critical level of control would be the utilization of a synthetic transcriptional repressor (Fig. 1D, line 4) (Choo et al., 1994), which would be expressed from a constitutive promoter that is only active in the presence of an activating ligand (Wang et al., 1994; No et al., 1996; Rollins et al., 2000). This allows gene expression to be modulated or shut off.

The last three scenarios require vectors in which: (a) expression can be induced permanently (i.e. integration and stabilization of the transgene(s) within the host chromosomes or their maintenance as a stable episome); (b) expression will increase and/or be maintained at a constant therapeutic level over time; (c) expression is induced by a discrete insult; and (d) the ability to deliver several genes to a single cell.

This paper has introduced strategies for the delivery of genes into neurons with the goal of protecting them from injury and maintaining function. We have not included a discussion of restoration and compensation in instances where neurons are lost. While beyond the scope of this short review, these elements could be covered in the future with the use of neuronal stem cells. The ability of these cells to migrate to areas of injury suggest that in the not to distant future these cell can be used for the repair of damaged areas of the brain (Kempermann and Gage, 2000). Overall the advances in genetic engineering, gene therapy and stem cell research suggest an optimistic future where individuals who live to their maximal lifespan will maintain maximal neurologic function.
References


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