

Gene Therapy Approaches to Neurodegenerative Disease

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The promises of gene therapy approaches to human disease seem to have resided “in the near future” for the last ten years. Despite the increasing knowledge and technical innovations in gene delivery vectors, for example, gene therapy is not yet working as well as one would like. However, we are getting closer as evident at the 1999 Promega Neurosciences symposium, “Gene Therapy Approaches to Neurodegenerative Disease,” held in Miami Beach as a satellite symposium to the Society for Neuroscience Annual meeting. This review briefly discusses the recent findings presented by speakers Evan Y. Snyder, Monique Dubois-Dalcq, Beverly L. Davidson, Andrew I. Brooks, William W. Hauswirth and the keynote lecturer, Richard C. Mulligan. The symposium was moderated by Howard J. Federoff of the University of Rochester Medical Center.

Introduction

Neurodegenerative diseases are a heterologous group of disorders, which vary from early onset, slowly progressive lysosomal storage diseases to acute ischemia, such as in ischemic stroke. The knowledge of the molecular pathophysiology behind each condition is a prerequisite for any kind of specific therapy. In some diseases, like retinitis pigmentosa, this pathophysiology is fairly well known, but much less is known in many other cases, and a lot of work needs to be done. On the other hand, many diseases possess characteristics in common and the targeted prevention of apoptosis, for example, could be useful in therapy even if the causes for it were unknown. The heterology of the neurodegenerative diseases, however, does lead into a broad spectrum of gene therapy approaches, and the advantages and disadvantages of some major choices were discussed in many of the sessions, as well as by the keynote speaker, Richard C. Mulligan of Children’s Hospital, Harvard University.

The decision of what approach to take, between the alternatives of in vivo or ex vivo gene delivery, divides the gene therapy strategies in two (Figure 1). In the first approach, the therapeutic genes are administered directly to the patient (or experimental animal), usually by injecting the gene-carrying vector into the target organ. The

main limitations of this technique in treating neurological disorders have been the rapid immunological silencing of therapeutic gene expression, as well as adequate delivery of therapeutic genes to the target cells, which can be anatomically difficult to reach. Advances in the development of viral vectors seem to have eased the problem of immune response against the treatment, and the newer lentiviral and adeno-associated virus vectors have been reported to continue the expression of the inserted genes for extended periods of time.

The fields of neuronal and other stem cell research have been extremely successful in the last few years, and ex vivo gene therapy research has come to the forefront. The idea is to culture multipotent cells that can be manipulated both genetically and environmentally to a certain phenotype and then administered to the patient, where the cells integrate into the existing nervous system and reverse the deficiency causing the disease.

Neural Stem Cells as Gene Delivery Vehicles May Provide New Strategies Against Neurodegenerative Disease

Evan Y. Snyder and colleagues at Harvard Medical School have been working on the neural stem cells (NSC), and in his talk he described the various properties of NSC biology that make them applicable for treating human neurodegenerative diseases. The NSCs are easily transduced in vitro by common gene transfer methods, and they express the genes of interest rapidly and for long periods of time. NSCs can be manipulated to carry multiple copies or several transgenes that are driven by specific promoters, for example. After ex vivo engineering, they can be relatively easily injected into the germinal zones of the central nervous system, where they are able to reintegrate into the host cytoarchitecture. The engrafted NSCs can even migrate to more distantly affected areas and adjust to different environments due to their high plasticity, which was shown convincingly in Dr. Snyder’s lecture.

Generation of Myelin-Forming Cells from Neuronal Precursors in Rodent and Man

The major advantage of using NSCs is that they can differentiate along both neural and glial lineages. This could be useful in disorders that destroy the whole anatomical region, like stroke, or conditions that are due to the loss of glial function, such as demyelination in multiple sclerosis. Monique Dubois-Dalcq from Institut Pasteur described her laboratory’s findings in myelin repair strategies using NSCs. Her results show that the NSCs, which

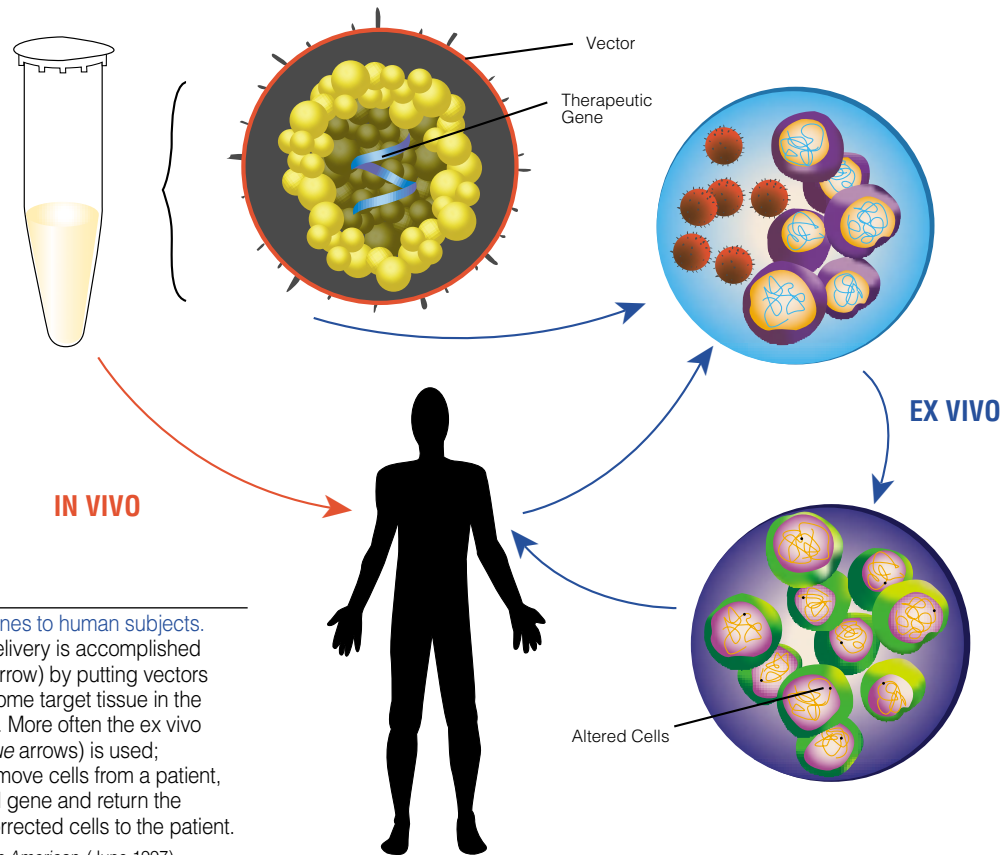


Figure 1. ▶
 Delivery of genes to human subjects. Sometimes delivery is accomplished directly (*red arrow*) by putting vectors straight into some target tissue in the body (*in vivo*). More often the *ex vivo* approach (*blue arrows*) is used; physicians remove cells from a patient, add a desired gene and return the genetically corrected cells to the patient.
 Source: *Scientific American* (June 1997).

in vitro give rise mostly to oligodendrocytes, can differentiate *in vivo* also into myelin-forming Schwann cells of the peripheral nervous system. After transplantation the cells are functional and able to integrate into the nervous system and remyelinate rat spinal cord demyelinated lesions (1). Most of the biology of the NSCs has been studied in animals, but there seems to be little difference across mammalian species, since human fetal NSCs are able to integrate (2) and ameliorate rodent disease model neuronal phenotypes (3). However, the use of fetal human cells in therapy raises several ethical questions, which may be avoided in the future by using autologous adult NSCs, which have been identified recently (4).

Lentivirus Vectors for Correction of CNS Storage

The advances in the *in vivo* gene therapy approach have been steady but perhaps slower than the recent explosion of the stem cell-related, *ex vivo* gene therapy approaches. In neurodegenerative diseases, the main interest of researchers seems to be in the development of newer and better viral as well as other gene-delivery vectors that would allow sufficient, targeted and long-lasting expression of the therapeutic gene. The high titers and efficiency of adenoviral vectors are useful in short-term applications

where one does not have to worry about immune responses, but the treatments for most of the neurological diseases would have to be by an alternative method. The retroviruses are not able to infect non-dividing cells, such as neurons, and most of the research is now done on adeno-associated-, herpes- or lentivirus-based vectors. Beverly L. Davidson, University of Iowa, described her laboratory's work on the β -glucuronidase-deficient mouse, a model for lysosomal storage diseases. Using the feline immunodeficiency virus-based lentiviral vector to correct the enzyme deficiency, she showed that they can reverse not only the cellular inclusions but also the behavioral phenotype of the mice. Her data also suggest that successful gene therapy of severe neurodegenerative diseases is possible and beneficial by lentiviral vectors even after pathological changes in the brain and behavior.

Exploiting Recombination in the CNS to Study Neurologic Disorders

One of the disadvantages in gene therapy research is the limitation of animal models. It is difficult if not impossible to compare the pathological behavior of mice to that of man, even if the cellular and biochemical changes are

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similar. For example, one of the more common neurodegenerative diseases, Alzheimer's or AD, is often noticed and diagnosed because of the patient's cognitive decline, which is usually caused by the malfunction of the brain areas responsible for memory acquisition. But how to study the genes that are involved in maintaining or improving the memory? **Andrew I. Brooks**, University of Rochester Medical School, has addressed the question by using different mazes and quantitative tests after gene therapy-based trophic support to the hippocampal cells. This is done in an elegant combination of in vivo gene delivery and transgenic technology in mice that are engineered to overexpress nerve growth factor (NGF) locally

but only after a viral delivery of the gene for cre recombinase (5). Although the transgenic technology is not applicable to humans, his results show that selective gene transfer can improve brain functions like memory and learning.

Gene Therapy for Autosomal Dominant Retinal Disease

Of the gene therapy approaches presented during the symposium, closest to the clinical use is perhaps the adeno-associated virus-based strategy for treating retinitis pigmentosa that was described by **William W. Hauswirth**, University of Florida. His group is trying to reverse this

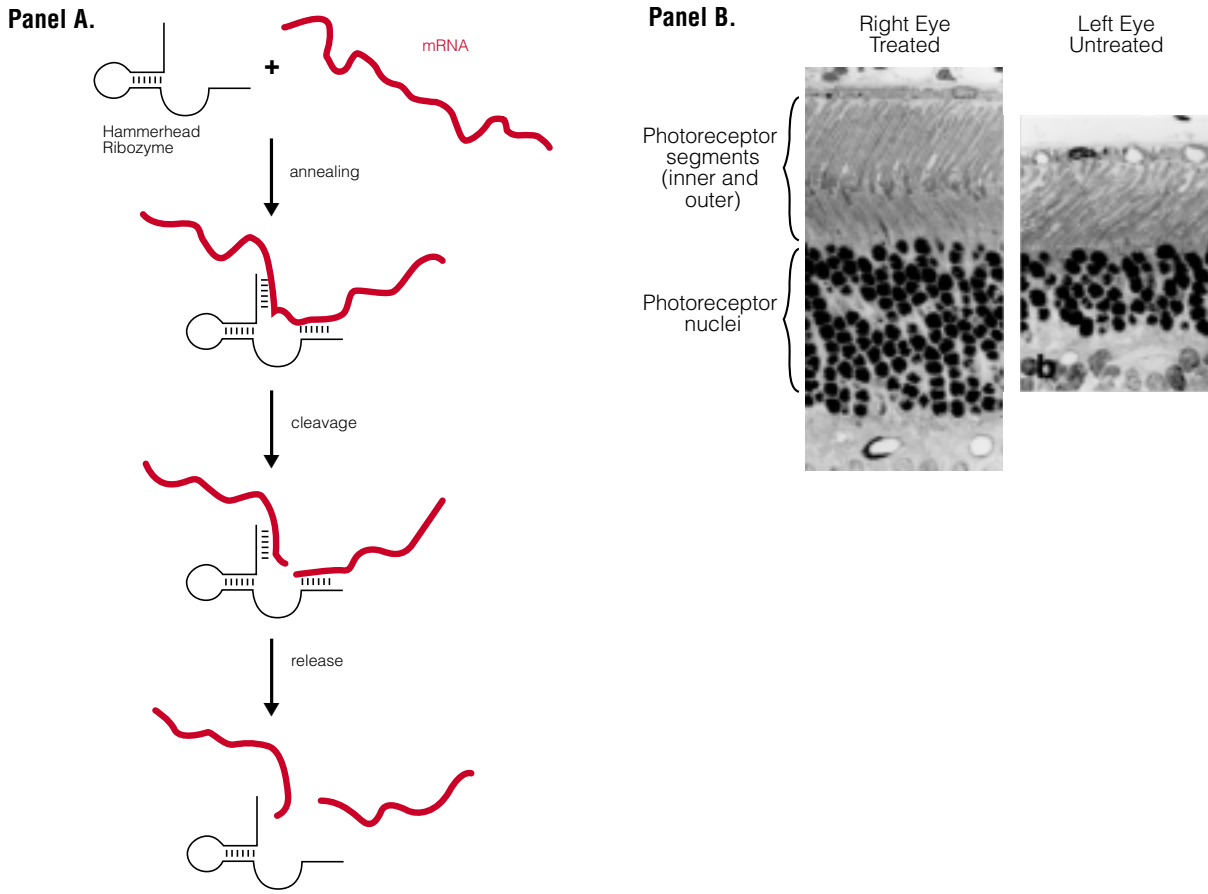


Figure 2. Schematic representation and demonstration of mRNA cleavage for a hammerhead ribozyme. **Panel A:** The two single-stranded arms of the ribozyme provide specificity for annealing to the targeted mRNA. Target cleavage and ribozyme release from the products allow multiple mRNAs to be degraded by a single ribozyme. **Panel B:** Transverse retinal sections of treated and control eyes from a P23H transgenic rat at 75 days following subretinal inoculation with a recombinant AAV vector containing a ribozyme gene that recognizes the P23H mutation. Rats were 89


days old at the time of analysis. The right eye received the vector + specific ribozyme gene; the left eye was untreated. Note the approximate two-fold improvement in the number of darkly staining photoreceptor nuclei. Also apparent are longer inner and outer photoreceptor segments, lightly staining rod-like compartments extending upward from photoreceptor nuclei, in the treated retina. Image adapted from slides presented by Dr. William Hauswirth, University of Florida, at the 1999 Promega Symposium.

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degenerative disease of the retina, which is caused by progressive rod photoreceptor death. The autosomal dominant form of retinitis pigmentosa is often caused by missense or nonsense mutations in the rod opsin gene that is then translated into nonfunctional and toxic protein. Treating this kind of disease is somewhat different than treating a “simple” protein deficiency disease, because the reconstitution of a functional protein is not enough. One needs to rid the eye of the mutant and harmful protein as well. Therefore, Hauswirth’s group uses ribozymes, RNA enzymes that can cleave a target RNA with high specificity. In their approach (6), the adeno-associated virus codes for a ribozyme that is able to distinguish between the normal and the mutant rod opsin mRNA, which is then cleaved and is not able to be translated (Figure 2). The virus is injected under the retina and the ribozyme is transcribed under the control of the rod opsin promoter, which increases the specificity of the treatment. So far the results are quite good: in one pig and several rat models, a single injection has restored up to 80% of the rod photoreceptors compared to the

contralateral (control) eye. The effect has not decreased in over two years, which has been explained by a small amount of viral DNA incorporation to the recipient cells. This AAV-ribozyme-based strategy holds great promise for the therapy of retinitis pigmentosa, as well as other dominantly inherited diseases.

Summary

In summary, the investigators that spoke at the Promega Neurosciences symposium were impressive, and the results of their work spoke for themselves. The enjoyable and educational day was closed with a view on the future of new strategies and technologies by the keynote address lecturer, **Richard C. Mulligan**. His talk abolished the last doubts, if any, of the possibilities of gene therapy for neurodegenerative diseases. 

References


1. Keirstead, H.S. *et al.* (1999) *J. Neurosci.* **19**, 7529.
2. Brustle, O. *et al.* (1998) *Nat. Biotech.* **16**, 1040.
3. Flax, J.D. *et al.* (1998) *Nat. Biotech.* **16**, 1033.
4. Johansson, C.B. *et al.* (1999) *Cell* **96**, 25.
5. Brooks, A.I. *et al.* (1997) *Nat. Biotech.* **15**, 57.
6. Lewin, A.S. *et al.* (1998) *Nat. Med.* **4**, 1033.

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