AMERICAN ACADEMY™ OF OPHTHALMOLOGY

Gene Therapy and Stem Cell Transplantation in Retinal Disease: The New Frontier

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Gene and cell therapies have the potential to prevent, halt, or reverse diseases of the retina in patients with currently incurable blinding conditions. Over the past 2 decades, major advances in our understanding of the pathobiologic basis of retinal diseases, coupled with growth of gene transfer and cell transplantation biotechnologies, have created optimism that previously blinding retinal conditions may be treatable. It is now possible to deliver cloned genes safely and stably to specific retinal cell types in humans. Preliminary results testing gene augmentation strategies in human recessive diseases suggest promising safety and efficacy profiles, including improved visual function outcomes over extended periods. Additional gene-based strategies under development include approaches to autosomal dominant disease ("gain of function"), attempts to deliver genes encoding therapeutic proteins with proven mechanisms of action interfering with specific disease pathways, and approaches that could be used to render retinal cells other than atrophied photoreceptors light sensitive. In the programs that are the furthest along—pivotal regulatory safety and efficacy trials studying individuals with retinal degeneration resulting from *RPE65* mutations—initial results reveal a robust safety profile and clinically significant improvements in visual function, thereby making this program a frontrunner for the first approved gene therapy product in the United States. Similar to gene therapy, progress in regenerative or stem cell–based transplantation strategies has been substantial. It is now possible to deliver safely stem cell–derived, terminally differentiated, biologically and genetically defined retinal pigment epithelium (RPE) to the diseased human eye. Although demonstration of clinical efficacy is still well behind the gene therapy field, multiple programs investigating regenerative strategies in RPE disease are beginning to enroll subjects, and initial results suggest possible signs of efficacy. Stem cells capable of becoming other retinal cell types, such as photoreceptors, are on the cusp of clinical trials. Stem cell–derived transplants can be delivered to precise target locations in the eye, and their ability to ameliorate, reverse, regenerate, or neuroprotect against disease processes can be assessed. Results from these studies will provide foundational knowledge that may lead to clinically significant therapies for currently untreatable retinal disease. *Ophthalmology 2016;123:S98-S106 © 2016 by the American Academy of Ophthalmology.*

Photoreceptors are specialized neuronal cells that convert light energy into electrical signals. This activity requires a complex interaction of enzymes and substrates, nutrients, and energy sources, many of which are provided by retinal pigment epithelium (RPE) cells. Further activities occur in a highly oxygenated environment. Consequently, photoreceptors seem to be particularly susceptible to metabolic, environmental, or genetic alterations within the retina. Not surprisingly, therefore, photoreceptor compromise and loss is the most common end-stage cause of irreversible blindness in the developed world. This article briefly reviews current strategies under development using gene- and cell-based therapies aiming to treat diseases affecting photoreceptors, either by genetic correction, by introduction of modifier genes, or by cell augmentation or replacement.

Before examination of the scientific concepts, it is useful first to see how gene and cell therapy may be applied in different diseases, because there is considerable overlap in the 2 approaches. In age-related macular degeneration (AMD), for instance, it may be possible to alter the microenvironment using gene therapy either to block angiogenesis or to inhibit the alternative complement pathway. Similarly, early improvement of RPE function with stem cell–sourced transplantation may modify the microenvironment sufficiently to the point of altering disease activity. As soon as the outer retina has undergone degeneration, cell replacement likely would be needed to restore function. In the end stages of AMD, it may be necessary to replace or regenerate not just RPE cell line, but also the underlying choriocapillaris and overlying photoreceptors, because all 3 tissues are involved pathologically in the disease process. In contrast to multifactorial conditions like AMD, retinitis pigmentosa (RP) presents a relatively straightforward paradigm early in the course of disease because single gene replacement theoretically can prevent or stall retinal degeneration, and typically only 1 cell type, the photoreceptor, is affected, particularly in recessive diseases.[1] In a subset of inherited retinal degenerations that includes certain forms of RP, the

Statement of Potential Conflict of Interest and Funding/Support: See page S106.

http://dx.doi.org/10.1016/j.ophtha.2016.06.041
ISSN 0161-6420/16

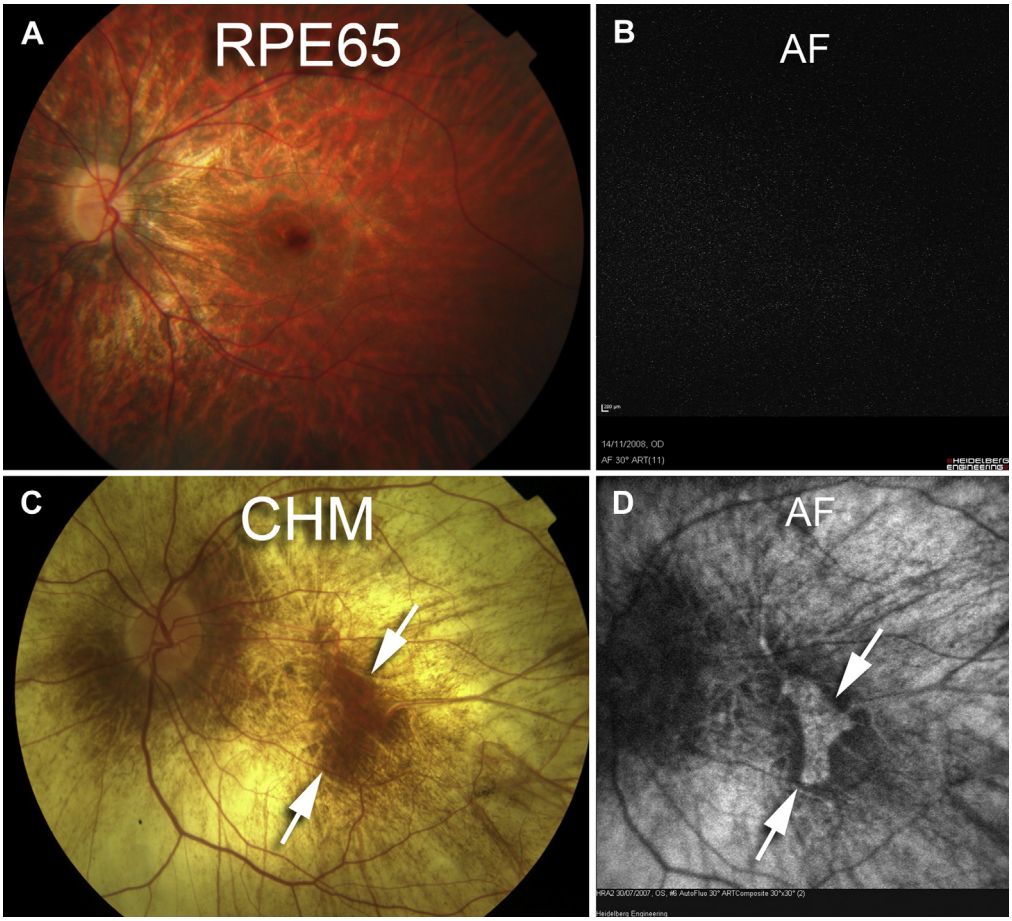

Figure 1. Different gene therapy strategies and imaging parameters in Leber congenital amaurosis resulting from *RPE65* mutations and choroideremia. **A**, Fundus photograph showing a young patient with recessive *RPE65* disease who has had poor vision since birth. **B**, Autofluorescence (AF) image showing a lack of results, which is a hallmark of the disease and is presumed to the be result of the slowing of the visual cycle. **C**, Fundus photograph showing the typical appearance in choroideremia (CHM) with atrophy of the choroid peripherally and only a small area of central retina remaining (*white arrows*) in a patient who had excellent visual acuity earlier in life. **D**, Autofluorescence (AF) image in CHM delineating the corresponding central viable zone of retinal pigment epithelium (*white arrows*). In *RPE65* disease, there is often peripheral retina remaining that has poor function; the visual acuity is limited by cortical amblyopia. Hence, successful gene therapy may be manifest as improved visual field, but not necessarily improved visual acuity. In CHM, anatomic degeneration means that the peripheral field cannot be restored because there is no retina remaining. However, gene therapy may result in improved function on the fluorescent island, which includes visual acuity in cases where it has been compromised.

deficient gene may be in the underlying RPE, such as occurs in some forms of Leber congenital amaurosis and in choroideremia (Fig 1). In these diseases, gene replacement to the RPE before the onset of photoreceptor loss may be the ideal approach. Given the interdependent nature of the photoreceptor, RPE, and choriocapillaris complex, virtually any late-stage retinal disease process, whether monogenic, cell specific, or multifactorial, ultimately leads to loss of all 3 tissues. Thus, cell replacement or regeneration may be necessary for patients in whom end-stage retinal degeneration has occurred.[2] Hence, gene and cell therapies should be considered as overlapping approaches that may or may not correlate to the early and late stages of the disease, respectively. Note also that approaches combining gene and cell therapies, such as ex vivo strategies, make sense in certain circumstances and already are being studied for a number of nonocular conditions.[3] These also may be applicable to retinal disease as expertise evolves.

Recent transformative breakthroughs have yielded the capacity to modify viral vectors and transgene promoters such that gene therapies selectively target specific retinal cell types, such as rods, cones, RPE, ganglion cells, and others.[4,5] Current studies use a variety of delivery strategies such as submacular delivery of therapeutic material, placing the vector in direct contact with the target cell layers. Future studies may develop next-generation vectors evolved with precise tropism to target specific cell types and the ability to cross the internal limiting membrane and to penetrate the neurosensory retina.[6] Similarly, novel promoters may yield improved expression efficiency in specific cell types. Alternatively, it may be possible to regulate promoter activity externally.

Regenerative or cellular therapies face similar delivery challenges as well as additional hurdles to success. Stem cells or stem cell–derived differentiated tissue must survive surgical transplantation and achieve biologically viable anatomic characteristics to replace diseased tissue