

Cell-specific mechanism-based gene therapy approach to treat retinitis pigmentosa

In a paper published on page 1211 of this issue of *Experimental Biology and Medicine* (EBM), a team of researchers at Columbia University Medical Center led by Stephen Tsang, MD, PhD have achieved temporary functional preservation of photoreceptors in a mouse model for retinitis pigmentosa (RP) using novel bipartite gene therapy.

RP is a heterogeneous disorder characterized by progressive degeneration of rod photoreceptors (which mediate night vision) causing night blindness and eventually total blindness. About 36,000 cases of simplex and familial RP worldwide are caused by mutant phosphodiesterase (PDE6). Says Dr Tsang, 'Victor McKusick's Mendelian Inheritance in Man catalog has approximately 4,000 genetic disease entries, but only 4 of these are already curable by gene-therapy approaches. This bipartite vector, which allows cell-specific delivery of both the normal gene and shRNA to knockdown other genes in the pathway, represents a strategy that may help cure many more retinal degenerations. Degeneration of rod photoreceptors also affects approximately nine million Americans with age-related macular degeneration. Strategies to save photoreceptors in mouse models of RP may be applicable to that disease as well.'

In spite of this high prevalence, the interplay between defective PDE metabolism and RP pathogenesis remains poorly understood. In several mouse models for RP, defects in the PDE6B enzyme result in increased levels of the signaling molecules cyclic guanosine monophosphate (cGMP; mediated by GUCY2E cyclase) and Ca^{2+} (mediated by the CNGA1 channel).

Several aspects of the EBM report are innovative. It not only tackles the lack of functional PDE6 β (by augmenting function with conventional gene replacement), but simultaneously counteracts the central biochemical impact of that lost function by decreasing abnormally accumulated cGMP and Ca^{2+} . Furthermore, they used a tissue-specific promoter to achieve cell-specific expression of the transduced genes, which is unusual for shRNA delivery.

The researchers at Columbia developed three different lentiviral vectors, each of them designed to deliver

wild-type *Pde6b* cDNA and one of two shRNAs. One vector delivered the cDNA and GUCY2E shRNA to reduce cGMP levels; another vector delivered the wild-type cDNA and CNGA1 shRNA to reduce Ca^{2+} levels; the third delivered the cDNA and the *Gucy2e* shRNA with a tissue-specific promoter. The bipartite approach was conceived as a way to improve therapeutic efficacy over that of a single-therapy approach, which was tested in an earlier project from Dr Tsang's lab. While the current project did not show improvement over their previous work, the researchers are optimistic that bipartite delivery may represent an important resource in future explorations of gene therapy in the eye and other tissues types.

Typical existing shRNA vector systems use the RNA polymerase III promoter, which is expressed in essentially all cell types, to drive expression of the transduced gene. However, expression of shRNA targeting GUCY2E in cone photoreceptors, which mediates color vision, would have unwanted and deleterious effects on vision. To achieve rod-specific expression of the shRNA, the researchers in this work embedded *Gucy2e* shRNA into the *Pol II* transcription unit of pre-miR34, which is expressed solely in rods. Similar methods can be used to target different tissues, so this method may have wide applicability.

The testing of shRNA therapeutic approaches in *Pde6b*^{H1620Q} mice should facilitate preclinical therapeutic evaluation of RP. Lessons learned from the proposed studies will eventually be translated into strategies for treatment trials in larger animal models of PDE6 β deficiency, such as Irish Setter dogs, before seeing application in human therapeutics.

Dr Steve Goodman, Editor-in-Chief of EBM said 'Researchers at Columbia University Medical Center led by Stephen Tsang, MD, PhD have used a unique gene therapy approach to preserve photoreceptors and prolong functional vision in a mouse model of retinitis pigmentosa (RP). Their work offers the promise of a future gene therapy cure for RP.'

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