Tech-Trends

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In Vivo Retinal Electroporation

phiC31 Integrase Confers Genomic Integration and Long-Term Transgene Expression in Rat Retina

PURPOSE. Gene therapy has shown promise in animal models of retinal disease, with the most success achieved to date with viral vectors used for gene delivery. Viral vectors, however, have side effects and limitations and are difficult to manufacture. The present study was conducted in an attempt to develop a novel system for long-term gene transfer in rat retinal pigment epithelium (RPE), by using nonviral transfection methods for gene transfer and the integrase from the bacteriophage C31 to confer long-term gene expression by means of genomic integration.

METHODS. Efficient nonviral delivery of plasmid DNA to rat RPE in vivo was achieved by using subretinal injection of plasmid DNA, followed by in situ electroporation. Gene delivery was evaluated by analyzing enhanced green fluorescent protein (eGFP) expression in frozen sections. In subsequent experiments, a plasmid expressing luciferase, with or without a plasmid encoding the C31 integrase, was delivered to rat RPE. Luciferase expression was followed over time by using in vivo luciferase imaging.

RESULTS. Subretinal injection followed by electroporation yielded abundant transgene expression in the rat RPE. Expression was strongest 48 hours after delivery. In the absence of C31 integrase, transgene expression declined to near-background levels within 3 to 4 weeks after treatment. By contrast, coinjection of the integrase plasmid led to long-term stable transgene expression throughout the 4.5-month test period. Eyes injected with C31 integrase showed 85-fold higher long-term transgene expression in the retina than eyes without integrase.

CONCLUSIONS. Subretinal injection of DNA followed by electroporation affords abundant transfer of plasmid DNA in rat RPE. C31 integrase confers robust long-term transgene expression by mediating genomic integration of the transgene. Thesefindings suggest that C31 integrase may be a simple and effective tool for nonviral long-term gene transfer in the eye.

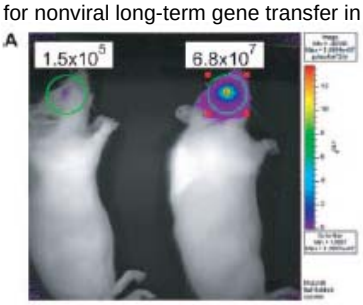

FIGURE 3. Electroporation enhances nonviral gene transfer to rat RPE cells in vivo. (**A**) After intraperitoneal administration of luciferin substrate, luciferase activity was quantified by a cooled bioluminescence camera in vivo. Bioluminescence data was obtained from 1- to 5-minute exposures, and these images were overlaid on photographic reference images. Shown are typical animals after subretinal injection of DNA alone (*left*) or subretinal injection of DNA followed by electroporation in situ (*right*).

ECM® 830* In Vivo Electroporation Protocol

Tissue preparation:

Perform subretinal injections in the superior hemisphere of the animal. Soak BTX Tweezertrodes in PBS and apply to each cornea with negative electrode on the injected eye with 14mm between electrodes. (Figure 2 below)

Electroporation Settings:

Voltage:	140V
Pulse length:	100 msec
Number of pulses:	5
Interval:	950 ms
Field Strength:	100 V/cm

Reference;

(1) Chalberg, T., Genise, H,, Vollrath, D., Calos, M., 2005. phiC31 integrase confers genomic integration and long-term transgene expression in rat retina.Invest Ophthalmos. Cis. Sci. Jun; 46 (6):2140-6

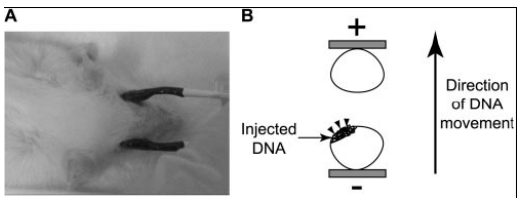

FIGURE 2. Arrangement of electrodes for in vivo electroporation for RPE transfection. (**A**) Tweezer-type electrodes were placed on the corneal surface of either eye of a 1-month-old Sprague-Dawley rat. (**B**) The current was applied with the positive electrode contralateral to the injected eye. After prior injection of plasmid DNA into the subretinal space of the right eye, this arrangement electrophoresed the negatively-charged DNA toward the RPE layer (*arrowheads*).

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