

Non-viral Phi C31 Integrase Mediated In Vivo Gene Delivery to the Adult Murine Kidney.

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Objectives/Background: Degeneration of primary cilia has been implicated in many diseases, including retinal degeneration and polycystic kidney disease. Gene targeting to retinal photoreceptor outer segments (cilia) has been widely demonstrated. This study aims to apply gene transfer principles and demonstrate gene transfer to the adult murine ciliated collecting tubules, in vivo, facilitated by phi C31 integrase.

Methods: Several cohorts of adult (6-10 week old) C57Bl6 mice were used. After adequate inhaled isoflurane anesthesia, mice were prepped and draped for abdominal surgery. A midline laparotomy was performed, and the left kidney was isolated. Connective tissue was bluntly dissected away from the ureter and renal pelvis. A 10 ul retrograde intra-ureteral microinjection was performed utilizing a pulled glass capillary tube, filled with two plasmids, one containing the marker gene, enhanced green fluorescent protein with the attachment site B sequence (pCMV.eGFP.attB) and the other encoding for the integrase enzyme (pCMV.Int). Muscle and skin incisions were closed separately with a running 7-0 silk suture. Animal was provided appropriate post-surgical care. Injected mice were euthanized 72 hours post injection, kidneys collected, placed in 4% paraformaldehyde, cryoprotected and sectioned. Samples were evaluated under fluorescence microscopy.

Results: Green fluorescence protein expression was detected in collecting tubules, renal papilla and transitional epithelium. No immediate gross inflammation was appreciated.

Conclusion: Phi C integrase can facilitate gene transfer to the adult murine kidney in vivo. The study of renal ciliated cells may assist in understanding the role of cilia in other forms of ciliopathy. Further optimization will be needed to increase efficiency of transduction, document stability of transgene expression and evaluate potential toxicities.

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