Delivering Gene Therapy for Kidney Disease

Molecular characterization of the genetic defects associated with a number of renal disorders has stimulated research into using gene therapy for these diseases. Therapeutic genetic interventions in these diseases may involve either functional restoration of a mutated gene or inhibition of acquired pathologic processes. These approaches offer the potential to alleviate the natural course of several progressive renal disorders.

Furthermore, the anatomic accessibility of the kidneys and their paired phenotype (providing a natural internal control) place the kidneys in a unique position for gene therapy endeavors.

However, several considerations must be addressed as prerequisites for the realization of any gene therapy for renal diseases. These include: (1) prior characterization of the genetic defect in hereditary renal disorders; (2) the route of vector delivery into specific renal compartments; (3) relevant animal models; and (4) characterization of the biological pathways implicated in acquired renal diseases.

Specific genetic defects have been defined for various hereditary renal diseases leading to end-stage renal disease (ESRD). Autosomal dominant polycystic kidney disease, medullary cystic diseases, Fabry's disease, Alport's syndrome, and sickle cell nephropathy are some of the disorders in which the molecular defects have been fully characterized.

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For example, autosomal dominant polycystic kidney disease is one of the most common hereditary diseases, affecting approximately 1:700 Americans, and is the fourth leading cause of ESRD. The *PKD-1* gene has been cloned and encodes the 460-kDa polycystin-1 protein, which is involved in ion transport across renal tubules. However, as the *PKD-1* gene is extremely complicated and large, gene therapy to fully correct the genetic defect of this disease is currently impractical.

Much smaller genes, defects in which are associated with Fabry's disease and Alport's syndrome, have been delivered successfully into rodent and porcine kidneys, respectively. Yet, because prolonged gene expression is currently dependent on integration of the viral vector DNA into the genome, severe side effects, primarily malignant transformation, pose a major obstacle to stable restoration of human genetic function.

Malignancy is a potential adverse effect associated with retroviruses. Because of this potential and because retroviruses do not infect quiescent cells, they are inappropriate vectors for kidney gene therapy.

Other methods of gene delivery include viral or nonviral vectors. Whereas the latter, mainly used in the form of liposomes or polymeric DNA-binding cations, are nontoxic and nonimmunogenic, they are relatively inefficient in vivo, and their gene expression profile is transient and untargeted. In contrast, viral vectors may be engineered to modify their biodistribution and may allow high-efficiency gene expression in some instances.

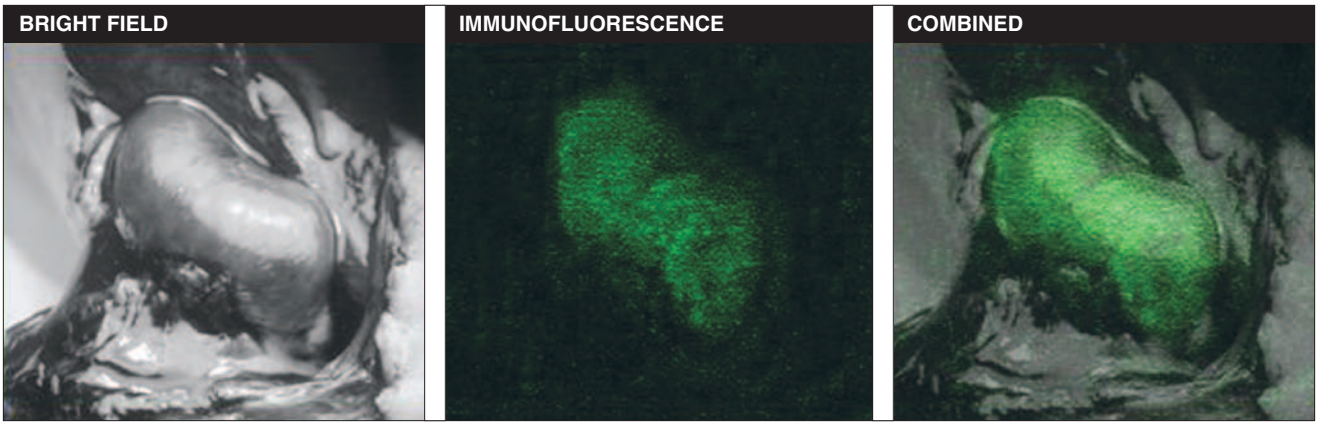
Adenovirus has several attributes that render it a candidate for kidney gene therapy. Adenoviruses have a wide cellular tropism, can infect all types of renal cells including nondividing cells, can be produced in high titers, and can be retargeted to specific tissues.

However, transient gene expression, hepatotropism, immunogenicity, and potential toxicity associated with administration of high viral titers currently limit the systemic administration of these vectors. Yet, because advanced-generation, tropism-modified adenoviral vectors can target specific renal compartments, they eventually may be useful in managing acute renal syndromes.

The relatively large size of the adenovirus appears to preclude glomerular filtration and to limit application of these vectors for diseases of the glomeruli, which are commonly affected early in the course of many renal disorders.

In contrast, adeno-associated viruses (AAV) are much smaller than adenoviruses and appear to be filtered freely through the glomerulus. AAVs do not infect glomerular cells but rather are taken up by epithelial cells of the proximal tubule and collecting duct. Consequently, AAVs may be useful for tubular disorders associated with solute or water transport.

Because of their preferential tubular uptake following antegrade delivery, AAVs may be beneficial for interstitial renal fibrosis, the strongest indicator of imminent ESRD. In this regard, the most urgent therapeutic challenge facing gene therapy for renal diseases is attenuation of the epithelial-to-mesenchymal cellular phenotype transition of the renal tubulo-interstitium. A major setback of AAV is the limited size of their therapeutic payload, potentially compromising their use in many renal diseases.



Live heterologous gene expression of green fluorescent protein (GFP) in kidneys of living mice following direct renal parenchymal injection of an adenoviral vector. Murine kidneys were treated with multiple direct subcapsular injections of an adenovirus encoding GFP (Ad5GFP). After 48 hours, mice were anesthetized, and the kidneys were exposed via a subcostal approach. In the left panel, the renal bed is revealed in a bright field image. Green fluorescence was captured with a confocal inverted epifluorescence microscope equipped with a CCCD camera (*middle*). Merging of the two images (*right*) indicates a renal source of the fluorescent signal. However, in contrast to the gene expression achieved with direct injection, systemic adenoviral injection did not produce significant GFP expression in the kidneys.

Another evolving viral vector approach for kidney gene therapy involves pseudotyped lentiviruses. Lentiviral vectors apparently are able to infect selected types of renal cells, but detailed in vivo characterization of them has yet to be reported.

The hemagglutinating virus of Japan (HVJ)–liposome vector consists of a lipid mixture associated with inactivated Sendai virus. This chimeric vector can efficiently bind the ubiquitously distributed viral receptor, on the one hand, and then fuse with the lipid bilayers of the cell surface and intracellular vesicles, on the other. Because the HVJ complex primarily targets mesangial cells, it may be of significance in a variety of glomerular diseases, and it has been successfully used to block TGF-β-related glomerulosclerosis.

Despite advances in vector-mediated gene delivery into the kidney, none of the currently available vectors employed for kidney gene therapy can convincingly induce prolonged expression of heterologous genes in the renal cortex following systemic administration. Like the choice of vector, the route of vector administration also is a major consideration.

Retrograde vector injection into the ureter results in patchy gene expression, primarily in the papilla and to a lesser extent in the medulla. Retrograde naked DNA injection into the renal vein has been reported to preferentially transduce renal interstitial cells, such as fibroblasts. In contrast, direct parenchymal injection results primarily in gene expression adjacent to the needle track.

These anatomic approaches, unfortunately, can hardly target functional sections of the nephron. Furthermore, with systemic administration, the complex renal architecture blocks filtration of particles above the diameter of 100 nm (or smaller negatively charged particles), whereas the liver rapidly clears many of the currently available gene therapy vectors.

It is unfortunate therefore that although the kidney is highly accessible through the circulation, efficient antegrade transduction of the kidneys by vectors larger than 100 nm in diameter requires physical measures such as clamping of hepatic vessels, complex renal perfusion systems, electroporation of the kidney, or glomerular embolization by large vector constructs.

In view of the central role of glomerular disease in inflammatory and metabolic renal diseases, as well as the glomerular resistance to gene delivery with currently available vectors, another renal targeting approach has been suggested recently. Remote delivery of vectors expressing soluble fusion molecules (based on cytokines or cytokine receptors genetically fused to the Fc portion of IgG), which are subsequently secreted into the circulation for competitive binding of inflammatory mediators, have been used to target the glomeruli in rodents with experimental glomerulonephritis.

For example, an adenoviral vector encoding a soluble TGF-β type II receptor was employed to block TGF-β, a key cytokine mediating fibroproliferative renal disorders. While a vector encoding a truncated, nonsoluble, dominant-negative type II TGF-β receptor did not block renal fibrosis, intramuscular delivery of an adenoviral vector

encoding a soluble type II TGF-β receptor was able to significantly attenuate the progression of renal fibrosis disorders.

Similarly, fusion molecules, containing other soluble receptors for inflammatory mediators, might attenuate the progression of other glomerular diseases.

Thus, an alternate therapeutic paradigm for glomerular renal disease may involve remote delivery and secretion of soluble cytokine antagonists from depot organs, such as muscle or liver. This biologic approach, rather than nonphysiologic glomerular embolization, may serve as a platform for future modulation of glomerular diseases.

Tubular cell targeting also may be attempted using vectors that exit the glomerular vascular network via the efferent arterioles and reach the peritubular capillaries. There, adenoviral vectors (but not polymeric DNA-binding cations) are believed to cross endothelial cells. Because integrins are widely expressed on the surface of renal endothelial cells, capsid modification of adenoviral vectors, incorporating arginine-glycine-aspartate (RGD)-targeting ligands, appears to enhance the capacity of adenoviral vectors to cross peritubular capillaries.

In contrast, nonviral DNA complexes must be filtered through the glomerulus to transduce tubular cells and therefore are limited in diameter to less than 70 to 100 nm. Of note, the combination of cold incubation of the kidney after adenoviral vector delivery and administration of vasoactive drugs may have an opposite effect, redirecting gene expression from the cortex to medullary compartments.

Currently available animal models of human renal diseases are usually more relevant for hereditary than for acquired conditions. For example, knockout mice have been generated for both the autosomal recessive and autosomal dominant forms of polycystic kidney disease. Yet, rodent models of acute glomerulonephritis do not always reflect the true nature of human diseases.

Of note, gene therapy endeavors for diabetic nephropathy are surprisingly scant, not because disease models are lacking but probably because current renal targeting approaches often fail to induce prolonged gene expression in the renal cortex. In contrast, intensive efforts are directed toward transplant nephropathy, a disease model in which ex vivo gene delivery may be inherently employed.

Regardless of the original disease process, because interstitial renal fibrosis and glomerulosclerosis remain the final common pathways in ESRD, these clinicopathologic entities are primary targets for gene therapy. Targeted cytokine antagonism in this context may include TGF-β, platelet-derived growth factor (PDGF), various T_H1-related interleukins, and tumor necrosis factor α (TNF-α), while bone morphogenic protein-7 (BMP-7), vascular endothelial growth factor (VEGF), and various interleukins may be delivered for the purpose of restoring renal function.

Despite the potential benefits of kidney gene therapy, no clinical trials using these approaches have yet been attempted. The combination of rational design of gene therapy vectors, characterization of the biological pathways implicated in ESRD, and identification of relevant animal models is expected to advance the developing field of gene therapy for renal diseases.

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