CELL & GENE THERAPY INSIGHTS

RARE DISEASES – CLINICAL & COMMERCIAL DEVELOPMENTS

SPOTLIGHT

EXPERT INSIGHT

Advances and challenges in developing gene therapies for rare kidney diseases

Valeria Padovano & Michael J Caplan

Gene therapy is an enticing therapeutic option for diseases lacking an effective long-term treatment, including not only monogenic diseases, but also complex disorders such as cancer. A number of clinical trials have shown evidence of improved efficacy and safety, mainly due to advances in vector design and delivery systems. Despite the progress made in the field, one of the main challenges for gene therapy is the ability to target specific organs and even specific cell types within an organ. With its complex architecture and variety of specialized cell types, the kidney epitomizes the difficulties encountered in developing therapeutic gene delivery strategies for highly differentiated tissues. In this review, we give an overview of the anatomical aspects that make the kidney a challenging target, explore different delivery routes and discuss attempts made to deliver exogenous DNA to renal cells and to treat renal diseases using non-viral and viral approaches. Although evidence in animal models indicates that gene therapy for renal diseases is possible, its applicability to human diseases requires further research to optimize targeting and vector delivery.

Submitted: Jun 26 2018 ▶ **Published**: Jan 4 2019

INTRODUCTION TO RENAL ANATOMY & FUNCTION

Each human kidney embodies approximately one million nephrons [1], each of which originates with a glomerulus and empties into a collecting tubule that carries the forming urine to the renal pelvis from which it enters the ureter. Each of these nephrons carries out very similar processes, and thus understanding

the logic that motivates the layout of a single nephron provides insight into the function of the kidney as a whole. In broad outline, the glomerulus generates an ultrafiltrate of the plasma from which all of the blood's cellular elements are excluded, as are most proteins with molecular weights greater than 60,000 Daltons. Each glomerulus produces roughly 40 to 120 nanoliters of this ultrafiltrate per minute [2], contributing to a total glomerular filtration rate of ~120 ml/min, which translates to ~180 liters/day. Roughly 99% of this filtrate is reabsorbed through a series of transport processes mediated along the length of the nephron and by the collecting tubule, resulting in the excretion of ~2 liters of urine per day. This volume and its composition are subject to tight control to ensure that they accommodate dietary fluid and electrolyte intake as well as insensate losses through perspiration, the respiratory tract and the gastrointestinal tract.

The most common causes of end stage renal disease are diabetic nephropathy and nephropathy due to hypertension. It seems unlikely that renal damage associated with these systemic diseases will be susceptible to kidney-targeted gene therapy approaches. The third most common cause of end stage renal disease is due to autosomal dominant polycystic kidney disease (ADPKD). ADPKD is the most common potentially lethal genetic condition, affecting approximately 1 in a 1000 individuals. The kidney is also affected by a large number of other less common genetic conditions. Clearly gene therapy approaches may be relevant to these monogenetic conditions. During the course of this review we will introduce a number of these genetic diseases and discuss in detail those for which gene therapy approaches have been explored.

The glomerulus

The glomerulus is composed of a tuft of capillaries that is surrounded by the squamous epithelial cells of Bowman's capsule, which are continuous with the cuboidal epithelial cells of the proximal end of the renal tubule [3]. Formation of the glomerular ultrafiltrate involves the passage of fluid and dissolved solutes from the capillary lumen into the space delimited by Bowman's capsule, from which it enters the proximal tubule of the nephron. In order to enter Bowman's space, the capillary filtrate must traverse a series of three successive barriers that are presented by the capillary endothelial cells, the glomerular basement membrane and the glomerular epithelial cells, which are also known as podocytes [4]. The capillary endothelium is highly fenestrated and these fenestrae do not possess fenestral diaphragms. Thus, the endothelium retains the cellular elements of the blood, but it can be freely crossed by all of the macromolecular components of the plasma.

Each capillary is completely circumferentially surrounded by the foot process of the glomerular epithelial cells. The cell bodies of each of these podocytes gives rise to numerous processes that interdigitate extensively with those of their neighbors [5]. The foot processes of neighboring podocytes are linked to one another by a complex junctional assembly called a slit diaphragm. These slit diaphragms bridge an intercellular space of ~25 nm, known as the filtration slit. The

DOI: 10.18609/cgti.2018.094

cell membrane of each foot process is endowed with a rich coat of highly anionic glycoproteins and proteoglycans. The negative charge of this polyanion creates an electrostatic repulsive force that holds the foot processes apart from one another and is required for the maintenance of the regular spacing of the filtration slits. Loss of the polyanion, as can occur in both genetic and acquired glomerulonephropathies such as minimal change disease, leads to a collapse of the filtration slits, producing an apparent fusion of neighboring foot processes that is referred to in the term 'foot process effacement' that is used by renal pathologists to describe this morphological transformation [6].

The 'soles' of each glomerular foot process are attached via filaments to the glomerular basement membrane, which resides between the endothelial cells and the podocytes. Like the surfaces of the foot processes, the glomerular basement membrane incorporates highly anionic proteoglycans that impart to it strong net negative charge. Since most plasma proteins are anionic at the pH of the blood, the negative charge of the glomerular basement membrane exerts a repulsive force that helps to prevent any protein larger than 60,000 Daltons from crossing the glomerular basement membrane from the capillary lumen into Bowman's space [7]. Loss of the glomerular basement membrane's strong negative charge will allow larger plasma proteins, including albumin, to be freely filtered and to end up in the urine. This is the source of the profound proteinuria that characterizes nephrotic syndrome, which in turn is a principle feature of minimal change disease. The pathogenic mechanisms that lead to this loss of anion remain largely unknown, although it appears that there can be both immunological and genetic etiologies.

The renal tubule

The glomerular filtrate flows from Bowman's space into the renal tubule, which is lined by cuboidal epithelial cells that carry out the reabsorption of 99% of the filtered fluid and electrolyte. The renal tubule is divided into several segments, including the proximal convoluted tubule, the thin descending limb of the loop of Henle, the thin ascending limb of the loop of Henle, the thick ascending limb of the loop of Henle, the distal convoluted tubule, the connecting tubule and the collecting duct. Each of these tubule segments mediates distinct transport processes that, functioning like a factory assembly line, modify the forming urine both through reabsorption of specific classes of solutes, secretion of waste products and absorption of water. A full description of all of these transport mechanisms, their inter-relationships and their regulation is beyond the scope of the present discussion. There are, however, a few points that are worth noting, as they provide useful background in the context of kidney-related genetic diseases.

Renal epithelial cells are polarized, meaning that their plasma membranes are divided into distinct apical and basolateral domains [8]. The apical domain faces the tubule lumen, whereas the basolateral domain rests on the epithelial basement membrane and communicates with the extracellular fluid compartment. These two membrane domains possess completely different inventories of membrane proteins. The transcellular transport of solutes and fluid

against steep concentration gradients is dependent upon the presence of different subsets of transport proteins embedded in each of these domains [9]. The apical and basolateral domains are separated from one another by tight, or occluding, junctions that prevent the protein constituents of the two membrane domains from intermixing with one another by diffusion. The junctions also control the magnitude and define the selectivity of the paracellular permeability pathway. The vast majority of fluid and electrolyte transport in the nephron is driven by the Na,K-ATPase, which resides in the basolateral plasma membrane of almost every tubule epithelial cell [10]. The ion gradients generated by the Na,K-ATPase are exploited by coupled co-transporters and channels in the apical plasma membranes of tubule epithelial cells to mediate vectorial trans-epithelial transport. Each tubule segment is endowed with a distinct suite of apical transport systems that define its particular physiological function in the absorption and modification of the forming urine. Mutations in genes encoding some of these apical transport systems, including NKCC, ROMK and NCC are associated with genetic diseases of the kidney such as Bartter syndrome and Gitelman syndrome, respectively [11].

The collecting tubule of the kidney contains two morphologically and functionally distinct classes of cells. Principle cells mediate sodium absorption and potassium secretion. This transport activity is stimulated by the mineralocorticoid hormone aldosterone. Mutations in the genes encoding the mineralocorticoid receptor or the epithelial sodium channel (whose expression is regulated by aldosterone) cause pseudohypoaldosteronism, Type 1 [12]. Principle cells also carry out regulated water transport. The loop shape of the nephron is designed to permit the creation of an osmotic gradient within the renal interstitium that drives the absorption of water from the forming urine. The water permeability of the apical membrane of the renal collecting tubule is mediated by the AQP2 water channel, whose residence within the apical membrane is in turn regulated by the Antidiuretic Hormone. Mutations in the genes encoding AQP2 or antidiuretic hormone receptor (AVPR) result in nephrogenic diabetes insipidus [13]. Finally, the proximal tubule of the kidney is the only site within the nephron where glucose, phosphate and amino acids are absorbed. Furthermore, the proximal tubule constitutes the only portion of the nephron that is capable of recapturing the small quantity of protein that is able to traverse the glomerular filtration barrier. This protein is bound by a scavenger receptor called megalin that is expressed at the apical surfaces of proximal tubule cells. Endocytosis of megalin results in the delivery its bound protein cargo to endosomes and lysosomes [14]. Mutations in genes required for this endocytosis lead to Dent disease and the oculo-cerebral renal syndrome of Lowe, and these conditions are characterized by the loss of low molecular weight filtered proteins in the urine [15].

GENETIC DISEASES OF THE KIDNEY AS POTENTIAL TARGETS FOR GENE THERAPY

In recent years, advances in sequencing technology have provided better understanding and diagnosis of renal genetic diseases, thus

DOI: 10.18609/cgti.2018.094

fostering research into the therapeutic potential of gene therapy in the kidney. Genetic renal diseases affect both glomeruli and tubules (for an in depth review of the genetic diseases of the kidney see [16]). Among the monogenic glomerular diseases, Alport syndrome, a basement membrane defect caused by alterations in type IV collagen, is considered a good candidate for gene therapy, as the mutated gene and its function are well known [17]. The majority of cases result from an X-linked mutation in the *COL4A5* gene, while the autosomal recessive form of the disease is caused by mutations in *COL4A3* or *COL4A4*. Interestingly, delivery and deposition in the GBM of type IV collagen α chain protein was successfully achieved in pigs [18] and dogs with X-linked Alport syndrome [19], thus providing proof of principle for effective treatment.

Fabry disease is another genetic disease affecting the kidney that could potentially be treated by gene therapy [20]. Fabry disease is a rare X-linked lysosomal storage disorder caused by mutations in the *GLA* gene that encodes the α-galactosidase A (α-gal A), a lysosomal enzyme involved in the metabolism of glycosphingolipids. Fabry disease affects multiple cell types, including those of the kidney [21]. Successful reduction of globotriaosylceramide (Gb3) accumulation for up to 2 weeks was achieved in the kidney of Fabry disease mice by retrograde injection of naked DNA encoding human α-gal A [22]. Long-term reduction of Gb3 accumulation in the kidney was achieved by neonatal injection of AAV1 vectors [23] and injection of AAV8 vectors carrying sequence encoding the human α-gal A before disease onset in mice [24], suggesting that early gene therapy might be an effective option for the treatment of Fabry disease.

Although gene therapy represents a potentially achievable therapeutic option for some monogenic diseases, its application to other monogenic kidney diseases seems still far away. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human genetic diseases and is caused by mutation in the *PKD1* or *PKD2* genes, coding for polycystin 1 (PC1) and polycystin 2 (PC2), respectively [25], with PKD1 being mutated in the majority of patients [26]. The disease is characterized by the development of large fluid-filled renal cysts that progressively reduce renal function, leading to end stage renal disease (ESRD) in about 50% of patients by the fifth to sixth decade of life [27]. Somatic mutations in the normal *PKD* allele that occur randomly throughout a patient's lifespan are thought to trigger the disease [28]. Several aspects make gene therapy for ADPKD currently not a realistic option. PC1 is a 4,302 amino acid membrane protein that has been implicated in many cellular pathways [29]. Thus, delivery of this very large gene to the renal epithelial cells or correction of the downstream affected pathways would be challenging. Moreover, expression of the transgenic *PKD1* would need to be finely regulated, as evidence shows that overexpression of PC1 leads to a cystic phenotype [30] and gene dosage represents an important modulator of cyst progression [31]. In this section, we have only discussed a few examples of monogenic diseases that represent potentially easy or far more complicated targets for gene therapy. Improvements in the targeting

of gene delivery systems to renal cells and the introduction of gene editing techniques *in vivo* will certainly drive more research into gene therapy for the broader inventory of genetic diseases of the kidney.

CHALLENGES IN GENE THERAPY ACCESS & TARGETING BASED ON RENAL ANATOMY & PHYSIOLOGY

The kidney's remarkable architecture is one of the chief factors that substantially limits its availability as an easy target for gene therapy. Consider, for example, the glomerular epithelial cells, or podocytes. Many forms of steroid-resistant nephrotic syndrome are attributable to mutations in genes that encode proteins that are expressed in podocytes [32], as are genetically caused forms of focal glomerular sclerosis [33]. Furthermore, the effects of these mutations are cell autonomous, so that correcting the mutation or delivering a wild type gene to the podocytes would be expected to correct the disorder. It is important to remember, however, that podocytes reside between the glomerular basement membrane and Bowman's space. Thus, any reagent delivered via the blood stream must cross the glomerular basement membrane in order to reach the podocytes. Since the pore size of the glomerular basement membrane is on the order of 8 nm, no particle with a diameter larger than this value can be expected to deliver any therapeutic benefit to podocytes with a high degree of efficiency. This size restriction essentially dampens enthusiasm for employing any blood-borne gene therapy approach to treat genetic diseases of glomerular epithelial cells. The only route through which large gene therapy reagents such as viral particles or DNA adducts can be delivered in sizeable and reliable quantity to glomerular epithelial cells is to instill these reagents into the bladder or ureters under conditions that favor their retrograde flow through the renal tubules until they reach the podocytes.

The epithelial cells that line the renal tubules and collecting ducts present somewhat less daunting challenges than do the glomerular epithelial cells, but their cell biological and physiological properties are by no means favorable for standard gene therapy approaches. Firstly, under normal circumstances renal epithelial cells are quiescent. They are not proliferative and exhibit extremely low rates of mitosis. Furthermore, and perhaps more importantly, their structural polarity presents a substantial obstacle. As noted above, the apical surfaces of renal epithelial cells face the lumen of the renal tubule, whereas the basolateral plasma membrane domains face the extracellular fluid compartment. Thus, while the basolateral membrane domains of renal epithelial cells can in theory be reached by any blood-borne particles that are able to diffuse through its adjacent vascular endothelia, the apical surfaces of renal epithelial cells are essentially unassailable by blood-borne gene therapy reagents. The only pathway through which the renal epithelial cells apical membranes can be accessed is, once, again, via retrograde delivery through the bladder or ureter. This is a critically important limitation, since the apical membranes of renal proximal tubule epithelial cells appear to be far more active in endocytotic uptake than are their basolateral

DOI: 10.18609/cgti.2018.094

membranes. As noted above, one of the functions of the proximal tubule cells is to scavenge from the forming urine any small amount of protein that managed to traverse the glomerular filtration barrier. This scavenging function is accomplished through a very extensive capacity for endocytosis that is a structural and functional characteristic of the proximal tubule apical membrane. The basolateral membranes of these cells are not similarly endowed and, under normal circumstances, perform far less endocytotic uptake than do the apical membranes. Since endocytotic uptake is a pre-requisite for the cellular entry and thus the efficacy of many gene therapy reagents, it is especially frustrating that the abundant endocytotic capacity of the proximal tubule cell apical membrane is not accessible to such reagents delivered via the blood stream. In addition, it appears that uptake of complexes between DNA and cationic lipids is mediated through binding of these complexes to cell adhesion receptors [34]. Thus, disrupting the integrity of the epithelium is required to stimulate uptake of DNA from either surface of the cell. Since this sort of disruption would be expected to produce severe pathological consequences *in vivo*, it is not practical to consider this approach as a mechanism to enhance gene delivery to renal epithelial cells.

Finally, the sheer number of nephrons constitutes a major challenge to gene therapy approaches. Consider, for example, the most common genetic cause of renal failure, autosomal dominant polycystic kidney disease (ADPKD), which was introduced above. Roughly 10% of patients with end stage renal disease reach this state as a consequence of ADPKD. Over the course of decades, a few hundred to a few thousand of an ADPKD patient's nephrons transform into large fluid-filled cysts whose growth and expansion compress and compromise the surrounding renal parenchyma [35]. It has been suggested that each cell that will participate in cyst formation has undergone a 'second hit' mutagenesis event that disrupts the wild type allele of the relevant ADPKD gene [36]. This transformation need only occur in far less than 1% of a kidney's one million nephrons in order to produce cyst growth sufficient to seriously compromise the function of the remaining vast majority of normal, unaffected renal epithelial cells. As noted above, the most common genetic mutations responsible for ADPKD occur in the *PKD1* gene. Ignoring for a moment the previously discussed challenges to gene therapy associated with the sheer size of the *PKD1* gene product and its associated coding sequence, the preceding discussion suggests that any gene therapy approach that is designed to suppress the effects of a very rare but highly consequential second hit mutagenesis event must be sufficiently efficient to ensure that even a tiny minority of renal epithelial cells will not undergo cystic transformation. Thus, the therapeutic reagent must be able to gain access and deliver its payload to a large fraction of renal epithelial cells. Furthermore, since ADPKD is a disease that progresses slowly over the course of decades, presumably because of the slow rate at which second hit mutations are acquired, any gene therapy must persist in the targeted cells essentially for the lifetime of the patient. Clearly, the architecture of each nephron and multiplicity of nephrons that populate each kidney create serious obstacles to the application of standard gene therapy strategies.

Moreover, to make cell type-specific gene targeting even more challenging, a recent study showed that our knowledge of the different cell types comprising the nephron is still incomplete [37]. Single-cell transcriptomics revealed a new transitional cell type in the collecting duct, thus suggesting the intriguing possibility that at least some renal diseases might originate from as yet unknown cells types. The advances in singe-cell analysis techniques applied to the kidney reveal that we still have a lot to learn about this organ. The new discoveries that these methods will produce may aid in the development of cell-specific gene therapies.

GENE THERAPY APPROACHES

Targeting glomeruli

Different methods have been successfully employed for gene transfer in the glomerulus. In particular, the liposome delivery system based on the hemagglutinating virus of Japan (HVJ) or Sendai virus has proven to be effective in selectively delivery of genes to the glomeruli in rats through a catheter in the renal artery [38]. This modified liposomal gene delivery system takes advantage of fusion proteins present on the HVJ envelope and can be combined with DNA-binding chromosomal proteins, such as high mobility group-1 (HMG-1), to facilitate DNA translocation into the nucleus [39]. Despite the high expression levels achieved with this method, the expression is transient and therefore not ideal for long-term gene therapy. Combination of HVJ liposomes with the Epstein–Barr virus (EBV) replicon vector, however, showed that the delivered DNA is retained in rat glomeruli up to eight weeks after transfection [40].

Adenoviral vectors present several advantages such as transfer and expression of DNA in non-dividing cells, high titers, and wide cell type tropism [41]. Systemic injection of adenoviruses, however, does not represent the ideal administration route for gene therapy in the kidney, as the majority of the injected adenoviruses are sequestered by the liver. To overcome this issue, Heikkila *et al.* successfully achieved glomerular expression of the type IV collagen α chain gene in pigs by kidney perfusion, and the recombinant α5 chain was correctly deposited into the pig glomerular basement membrane (GBM) [18]. Adenoviral vectors have also been successfully targeted to the glomerulus by generating adenoviral-microsphere complexes [42]. The polystyrene microspheres get trapped in the glomerulus [43], thus delivering the adenovirus to the glomerular capillary. Through aortic injection of these complexes, Nahman *et al.* were able to achieve expression of a reporter gene in endothelial and mesangial cells of rat glomeruli for up to 21 days.

In vitro electroporation is widely used for transfer and expression of plasmid DNA in cells and has been effectively used to transfer DNA into mouse muscle [44], mouse skin [45] and rat liver [46]. In order to apply this non-viral based gene transfer method to the kidney, Tsujie *et al.* developed a glomerulus-targeting *in vivo* electroporation, in which the DNA is injected in the renal artery, followed by electroporation of the kidney [47]. This method allowed for expression in mesangial cells of the transgene in 75% of glomeruli. In order to achieve long-term expression of plasmid

DOI: 10.18609/cgti.2018.094

DNA through recombination in the genome, Otani *et al.* performed electroporation after direct injection of plasmids coding for a reporter gene (luciferase) and the *Streptomyces* bacteriophage φC3 integrase in the kidney [48]. The authors detected integration of the plasmid DNA in a hotspot for integration on chromosome 2 and the expression was sustained for up to 60 days. The area of the kidney that can be transfected with this method, however, was not analyzed and, although no toxicity was reported in this study, further investigation is required to assess safety of plasmid DNA integration using φC3 integrase.

Targeting tubular cells

Naked DNA can be employed for gene transfer in the renal tubules. Intravenous infusion of anti-sense oligodeoxynucleotides (AS-ODNs) leads to the accumulation of the ODNs mainly in the proximal tubule for up to 4 days [49] and has been successfully employed to suppress protein expression in rat kidneys [50,51]. Renal interstitial administration of AS-ODNs has also proved successful [52]. Moreover, Hajarnis *et al.* has recently shown that a chemically modified anti-miR oligonucleotide (anti-miR-17) injected subcutaneously can accumulate in the collecting duct and attenuate cyst growth in mouse models of ADPKD without inducing acute liver or kidney toxicity [53], thus supporting the use of naked DNA injection as an effective delivery option for gene therapy of renal genic diseases.

Non-viral targeting of tubular cells has been achieved in rat kidneys using plasmid DNA complexed with the cationic polymer polyethylenimine (PEI) [54]. Injection of DNA-PEI complexes in the renal artery resulted in expression of a reporter gene (β-galactosidase) almost exclusively in proximal tubular cells [54]. Gene expression peaked at 2 days post injection and was undetectable at day 14.

Adenoviral vectors have also been used efficiently to target renal tubules. Moullier *et al.* demonstrated that different administration routes can be used to achieve targeting of different areas of the kidney [55]. Injection of the adenoviruses in the renal artery of adult rats resulted in gene expression in proximal tubular cells, while retrograde infusion into the ureter resulted in gene expression in the papilla and medulla. Moreover, expression in the proximal tubule was detected for up to 4 weeks [55]. Ortiz *et al.* showed that injection into the outer medullary interstitium of adenoviruses encoding green fluorescent protein (GFP) as a reporter gene led to expression in at least 77% of thick ascending limbs (THALs) [56]. The expression, however, was detectable only up to 14 days post-injection, making this technique unlikely to be adaptable for human for gene therapy. In order to target gene expression to specific cells of the tubules, Watanabe *et al.* used the promoters of three genes known to be expressed in different segments of the kidney to drive the expression of green fluorescent protein (GFP) [57]. By using promoters for the sodium-dependent phosphate transporter type 2a (NPT2a), the sodium-potassium-2-chloride cotransporter (NKCC2), and the aquaporin 2 (AQP2) genes, the authors obtained GFP expression in the proximal tubule, in the thick

ascending limb of Henle (TALH) and in the collecting duct, respectively [57].

Adeno-associated virus (AAV) is an attractive gene delivery system, as it can infect dividing and non-dividing cells, is less immunogenic than other viruses, can integrate in the host genome or remain in an episomal form and recombinant adeno-associated viruses (rAAV) contain no viral genes [58]. There are 9 AAV serotypes (AAV1-9), with AAV2 being the first developed into recombinant vectors for transgene delivery [59]. Lipkowitz *et al.* were the first to show that intraparenchymal injection of AVV resulted in a reporter gene expression in tubular epithelial cells for up to 3 months [60], but only in the vicinity of the injection site. On the contrary, intrarenal arterial administration of rAAV2 resulted in expression of a reporter gene in proximal tubules, with the highest expression in the S3 segment, which lasted for up to 6 weeks [61]. Retrograde ureter injection was also tested for AAV delivery and showed efficient gene expression in renal tubular cells [62]. Gene delivery to the kidney using rAAV can also be achieved *ex vivo* during transplantation to reduce the probability of organ rejection. Benigni *et al.* showed that *ex vivo* injection of AAV-CTLA4Ig in the renal artery of the donor kidney resulted in expression of the CTLA4Ig immunosuppressant protein in proximal tubular epithelial cells and was effective in preventing chronic allograft rejection in rats [63].

In a study aimed at evaluating transduction efficiency of AAV serotypes 1-5 using a catheter-based delivery system, only AAV2 showed efficient transduction of proximal tubular cells [64]. AAV2 transduction of the kidney was also reported after intraperitoneal injection, even though the distribution within the organ was not analyzed [65]. Rocca *et al.* tested rAAV serotypes 5,6,8 and 9 through retrograde renal vein injection and found that while rAAV6 and 8 mainly transduced the medulla, rAAV9 transduced both medulla and cortex and the reporter gene expression was found in tubules and glomeruli [66]. The therapeutic potential of rAAV9 was shown in a mouse model of methylmalonic acidemia (MMA), in which retro-orbital re-injection of a rAAV9 vector coding methylmalonyl-CoA mutase induced renal expression of the enzyme and rescued the phenotype [67]. As rAAV8 and 9 can cross the vascular endothelial cell barrier and lead to gene delivery to other organs [66,68], improvement of cell specificity is an important aspect of rAAV vector engineering.

Targeting non-tubular & non-renal cells

In an effort to improve the applicability of gene therapy to kidney diseases and to overcome the challenges of gene delivery to the renal tubules, cells other than tubular cells as well as non-renal cells represent alternative therapeutic targets. In a recent study, Ikeda et al have developed a synthetic AAV called Anc80 that can transduce kidney stroma and mesangial cells with high efficiency [69]. Pericyte-specific ablation of Gli2 or β-catenin in $Gli2^{f/f}$ or $\beta\text{-catenin}^{f/f}$ mice after systemic injections of AAV/Anc80 encoding the CRE recombinase resulted in reduction of renal fibrosis

DOI: 10.18609/cgti.2018.094

after unilateral ureteral obstruction (UUO), thus suggesting that gene therapy of non-epithelial cells can be a useful therapeutic approach to reduce renal fibrosis [69]. As AAV/Anc80 exhibits high-efficiency transduction of other organs such as liver, muscle and retina, improvement of AAV tropism and/or development of cell-specific promoters are required to reduce potential extra-renal toxicity.

Gene transfer into non-renal cells of constructs encoding proteins that might modulate renal disease if secreted into the systemic circulation represents another potential strategy for renal gene therapy. Skeletal muscle is an easy target for gene delivery, as injection of naked DNA leads to protein expression that can last up to 2 months [70]. Nakamura *et al.* showed that delivery of a chimeric platelet-derived growth factor β (PDGF-β) receptor extracellular domain-IgG Fc domain expression vector to the skeletal muscle by electroporation resulted in the secretion of the protein into the serum and improvement of glomerulonephritis in rats [71]. Transduction of a chimeric extracellular domain of the transforming growth factor-β (TGF-β) type II receptor fused to the IgG-Fc domain or decorin, an inhibitor of TGF-β, into skeletal muscle by HVJ liposomes also ameliorated the phenotype of rat models of glomerulonephritis [72,73]. Moreover, plasmids encoding erythropoietin (EPO) have been delivered to skeletal muscle by adenoviral vectors [74] and electroporation to treat anemia associated with renal failure [75,76]. These studies indicate that organs other than the kidney can be suitable targets for gene therapy of renal diseases.

CONCLUSIONS & FUTURE PERSPECTIVES

The kidney is a structurally and functionally complex organ that is susceptible to a large number of clinically important diseases that could, in theory, be addressable by gene therapy approaches. The details of the kidney's architecture, however, ensure that its constituent cells are very challenging targets for gene delivery. In order to be effective, gene therapy of the kidney will require continuing refinement of gene delivery systems and the development of novel delivery strategies that can overcome these challenges and permit the realization of the potential utility of gene therapy in the treatment of renal disease.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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AFFILIATIONS

Valeria Padovano

Department of Cellular and Molecular Physiology, Yale University School of Medicine, P.O. Box 208026, New Haven, CT 06520-8026, USA
valeria.padovano@yale.edu
Tel.: +1 203 785 6833

Michael J Caplan

Department of Cellular and Molecular Physiology, Yale University School of Medicine, P.O. Box 208026, New Haven, CT 06520-8026, USA
michael.caplan@yale.edu
Tel.: +1 203 785 7316

DOI: 10.18609/cgti.2018.094