Gene therapy approach in renal disease in the 21st century

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Abstract
Theoretically, gene therapy has distinct potential to treat renal disease at the most fundamental level. However, the ability to pursue gene therapy has been limited by the availability of an adequate system for gene delivery to the kidney. The present viral vector systems seem to have limitations for clinical use because of uncertainty regarding their toxicity and immunogenicity; however, adenovirus-mediated gene transfer succeeded in gene expression in the kidney. Adeno-associated virus has a potential to be utilized as a vector targeting both kidney and skeletal muscle. Given that the systemic delivery of the functional protein can serve for the therapy of the renal diseases, skeletal muscle targeting gene therapy might be an alternative strategy for treatment of renal disease. Non-viral vectors such as the haemagglutinating virus of Japan (HVJ)-liposome method and cationic liposome are possibilities, but their efficiency needs to be improved. Electric pulse is emerging as a new and less harmful strategy of gene transfer to various tissues, including the kidney. I believe that two plausible strategies exist for the therapeutic use of gene transfer in the near future: skeletal muscle-targeting gene therapy and kidney transplantation. Application of gene therapy to the transplanted kidney may potentially improve graft outcome by reducing acute and chronic rejections. The emerging strategies of gene transfer in kidneys are reviewed and the potential application of gene therapy in renal diseases are discussed.

Keywords: electroporation; HVJ-liposome; skeletal muscle; transplant kidney

Introduction
An explosion of patients with end-stage renal disease (ESRD) has occurred throughout the world. In Japan, the incidence of chronic renal failure has been increasing during the last two decades. Thirty thousand ESRD patients were introduced to chronic dialysis therapy in 1999 and the total number of chronic dialysis patients exceeded 200,000 during 2000 [1]. In the United States in 1996, the prevalence of ESRD was 283,000 and the incidence of ESRD was 73,000 patients per year [2]. In contrast to ESRD, the incidence of mortal cardiovascular diseases such as ischaemic heart disease and stroke has been in decline over the last 15 years. This fact implies that we have no effective strategy to halt the progression of renal diseases. Prevention and successful treatments of progressive renal diseases are highly desirable and the development of innovative therapies to halt renal diseases is crucial for reducing the number of dialysis patients, and in turn the medical expense, which has cost $10 billion in Japan.

One of the most promising therapies is gene therapy. Gene therapy has a distinct potential to treat renal disease at the most fundamental level. By the end of 1998, 3089 patients had been treated with gene therapy according to 367 protocols. However, most of these methods seem to have failed in treating diseases. Some protocols against malignancies may inhibit the growth of certain cancer types, but it cannot be concluded that gene therapy can treat all cancers. It should be pointed out that the application of human gene therapy has principally been limited to incurable diseases, such as life-threatening malignancies and HIV infection. However, the main reasons for the failures result from technical problems such as imperfection of vectors, difficulties in gene delivery, and shutdown of transgene expression after successful gene transfer. Several approaches regarding gene transfer to the kidney exist at the experimental level [3–6]. I review here recent strategies of gene transfer into the kidney and discuss the potential application of gene therapy to renal diseases in the future.

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Strategy of gene transfer to the kidney

The reported approaches of gene transfer to the kidney are listed in Table 1.

In vivo gene transfer to the kidney

1. Viral vectors

Retroviral vector. Retroviruses have been employed in >50% of clinical trials, but only few reports are available on the application of kidney-targeted gene transfer. The major disadvantage of the retrovirus vector is the prerequisite for the replication of the target cells to integrate into the host genome. Since many target cells in the kidneys are non-dividing and terminally differentiated, retroviral vector-based gene transfer systems have restricted their use almost entirely to ex vivo applications to bone marrow derived cells.

The direct retroviral gene transfer to the proximal tubule was reported by Bosch et al. [7]. Rats were pretreated with folic acid to induce proliferation of the proximal tubule, and then retroviral vector was injected into the renal artery. Weak expression was detected in the proximal tubule.

Adenoviral vectors. The adenoviral vector has distinct advantages such as high titres and the highest level of transgene expression among all available vectors. The adenoviral vector has a significant advantage in delivering relatively large genes (up to 10 kb) into quiescent or terminally differentiated cells, since this virus can infect both dividing and non-dividing cells. However, the expression of the transfected gene is limited to weeks or months because the adenovirus does not integrate into the host cell genome. A crucial problem of adenoviral gene transfer is that it elicits immunological responses. The host response consists of an initial non-specific inflammation followed by specific cellular and humoral immune responses directed at the cytotoxic T lymphocyte (CTL)-mediated clearance of the recipient cells [8]. In fact, the death of a patient treated by adenoviral vector, from which the viral genes related to cytotoxicity and immune response are supposed to be eliminated, invokes serious difficulties in the clinical application of gene therapy [9]. This toxicological feature of the adenovirus must be improved. Some reports exist regarding adenovirus-mediated in vivo gene transfer to the kidney. Moullier et al. observed very weak and patchy expression of the reporter gene in the proximal tubule when the adenoviral vector was injected into the renal artery. The expression lasted for 2 weeks. The retrograde approach from renal pelvis caused intense expression of the reporter gene in the papilla and medulla. In contrast, Heikila et al. [10] demonstrated intense and diffuse gene expression in glomeruli. They perfused the solution containing the adenoviral vector for 2 h. Four days later, the expression of reporter gene was observed in 75% of the glomeruli and lasted for 4 weeks. Interestingly, they did not find reporter gene expression in tubular cells and glomerular parietal epithelial cells. The adenovirus perfusion system allows selective gene transfer, targeting the glomerulus with high efficacy. Information concerning the immunoreaction to the adenovirus vector is lacking in these reports.

Adeno-associated virus (AAV) vector. Recombinant AAV has emerged as an attractive alternative to

Table 1. Strategies for gene transfer into the kidney

<table>
<thead>
<tr>
<th>Species</th>
<th>Targeted region/cell</th>
<th>Vector</th>
<th>Access/approach</th>
<th>Duration of expression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vivo gene transfer into the kidney</td>
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<tr>
<td>Rat</td>
<td>glomerulus</td>
<td>HVJ-liposome/plasmid</td>
<td>renal artery</td>
<td>7 days</td>
<td>[7,8]</td>
</tr>
<tr>
<td>Pig</td>
<td>glomerulus</td>
<td>adenovirus</td>
<td>renal perfusion</td>
<td>3 weeks</td>
<td>[9]</td>
</tr>
<tr>
<td>Rat</td>
<td>glomerulus</td>
<td>plasmid;electroporation</td>
<td>renal artery</td>
<td>8 weeks</td>
<td>[10]</td>
</tr>
<tr>
<td>Rat</td>
<td>proximal tubule</td>
<td>native ODN</td>
<td>peripheral vein</td>
<td>ND</td>
<td>[11,12]</td>
</tr>
<tr>
<td>Rat</td>
<td>proximal tubule</td>
<td>DOTMA:DOPE/plasmid</td>
<td>renal artery</td>
<td>3 weeks</td>
<td>[13]</td>
</tr>
<tr>
<td>Rat</td>
<td>proximal tubule</td>
<td>adenovirus</td>
<td>renal artery</td>
<td>1–2 weeks</td>
<td>[14]</td>
</tr>
<tr>
<td>Rat</td>
<td>proximal tubule after folate injury</td>
<td>retrovirus</td>
<td>renal artery</td>
<td>3 weeks</td>
<td>[15]</td>
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<tr>
<td>Rat</td>
<td>proximal tubule</td>
<td>AAV</td>
<td>intraparenchymal</td>
<td>3 months</td>
<td>[16]</td>
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<tr>
<td>Mouse</td>
<td>proximal tubule</td>
<td>DOTMA:DOPE/plasmid</td>
<td>renal pelvis</td>
<td>20 days</td>
<td>[17]</td>
</tr>
<tr>
<td>Rat</td>
<td>outer medulla cold/vasodilator</td>
<td>adenovirus</td>
<td>renal artery</td>
<td>4–8 weeks</td>
<td>[18]</td>
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<tr>
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<td>IMCD</td>
<td>adenovirus</td>
<td>renal pelvis</td>
<td>1–2 weeks</td>
<td>[14]</td>
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<tr>
<td>Rat</td>
<td>interstitial fibroblast</td>
<td>HVJ-liposome</td>
<td>ureter</td>
<td>2 weeks</td>
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<td>Ex vivo gene transfer to the kidney</td>
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<td>Rat</td>
<td>glomerulus</td>
<td>mesangial cell</td>
<td>renal arterial</td>
<td>4–8 weeks</td>
<td>[20]</td>
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<tr>
<td>Rat</td>
<td>glomerulus</td>
<td>macrophage</td>
<td>renal arterial</td>
<td>ND</td>
<td>[21]</td>
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<tr>
<td>Mouse</td>
<td>inflamed glomerulus</td>
<td>macrophage</td>
<td>intraperitoneum</td>
<td>8 days</td>
<td>[22]</td>
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<td>Mouse</td>
<td>interstitium subcapsular</td>
<td>proximal tubule cell</td>
<td>renal subcapsular</td>
<td>4 weeks</td>
<td>[23]</td>
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<td>Gene transfer to the transplanted kidney</td>
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<tr>
<td>Pig</td>
<td>glomerulus</td>
<td>adenovirus</td>
<td>renal perfusion</td>
<td>2–3 weeks</td>
<td>[9]</td>
</tr>
<tr>
<td>Human</td>
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<td>adenovirus/polylsine-Ab</td>
<td>perfusion</td>
<td>ND</td>
<td>[24]</td>
</tr>
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</table>
retroviral vectors. AAV, a parvovirus, is a single-stranded DNA virus that has the ability to integrate site-specifically into the human chromosome 19 [11]. This property is, however, lost in recombinant viral vector devoid of the AAV gene [12]. AAV can infect both non-dividing cells and dividing cells. The infection of the AAV has not yet been associated with disease, and replication of wild-type AAV requires a helper virus such as adenovirus. However, the generation of a recombinant virus is generally intricate and results in low viral titres. Baudard [13] reported a new method, which uses liposome as a vehicle for in vivo delivery of AAV vectors. The human multidrug resistance protein was successfully expressed in the spleen, liver and kidney by single intravenous administration of a liposome–AAV complex. Recently, Lipkowitz et al. [14] reported that the AAV delivered in vivo by intraparenchymal injection resulted in at least 3 months of reporter gene expression in tubular epithelial cells but not in glomeruli or vascular cells.

2. Non-viral vectors

Direct intravenous injection. Intravenously injected oligonucleotides (ODN) accumulate in the proximal tubule by phagocytosis. Rappaport et al. [15] studied the fate of 32P-labelled ODN after intravenous injection. The labelled ODN were localized predominantly in the proximal tubule of kidney and liver within 30 min of injection. Oberbauer et al. [16] also reported that intravenously injected ODN did not merely accumulate in the brush border or lysosomal compartment in proximal tubular cells, implying that they were not totally destroyed after being phagocytosed by the proximal tubule cells. These results suggest that the renal proximal tubule is a good target of antisense therapy.

Liposome-mediated gene transfer. Lien and Lan [17] reported that the DOTMA:DOPE–DNA complex is successfully transferred into the tubular cells by intra-renal artery injection as well as by renal pelvic injection in mice. They observed tubular expression of the reporter gene mainly in the outer medulla for 6 weeks after intra-renal pelvic injection. However, the expression in the inner medulla is weak and there was no expression in the glomerular, vascular or interstitial components.

Haemagglutinating virus of Japan (HVJ)-liposome method. HVJ-liposome mediated gene transfer utilizes the fusogenic character of HVJ, which belongs to the paramyxovirus family. We have previously reviewed in depth this HVJ-liposome mediated gene transfer method [18]. Tomita et al. [19] first reported gene transfer to glomerular cells by the HVJ-liposome method. Isaka et al. [20] and Arai et al. [21] also succeeded in the overexpression of TGF-β and renin genes, respectively, in the glomerular mesangial cells. These results suggest that the HVJ-liposome method allows selective gene expression in the mesangial cells.

A shortcoming of the HVJ-liposome method is its low transfection efficiency. To overcome this limitation, Saeki and Kaneda generated a new liposome in which the lipid composition mimics the viral envelope, and named this the artificial viral envelope (AVE) [22]. The AVE-type HVJ-liposome provides a 6- to 10-fold higher transfection efficiency than the conventional HVJ-liposome method. Recently, Tsujie et al. [23] demonstrated successful gene transfer into the interstitial fibroblast in the kidney after ureteral injection of AVE-type HVJ-liposome DNA complex. Expression of the Lac Z gene persists for 14 days in tubulo-interstitial fibroblasts in rat kidney. In addition, in order to achieve sustained gene expression, we utilized Epstein–Barr virus (EBV) replicon apparatus [24]. EBV has been analysed thoroughly in terms of its latent infection, which is characterized by autonomous replication and nuclear retention of the EBV genome in host cells [25]. Both the cis-acting oriP (the latent viral DNA origin) sequence and the trans-acting Epstein–Barr virus nuclear antigen-1 (EBNA-1) are required for EBV latent infection. The insertion of the sequences of EBNA-1 and oriP into the plasmid resulted in sustained expression of the reporter gene in the glomeruli for at least 56 days. The two improvements made the HVJ-liposome method a more powerful and promising technique in kidney targeting gene transfer [26].

Electroporation. Electroporation is a method for introducing a foreign gene into cells by making a pore in the membrane by electric pulse. The membrane becomes extremely conductive and the current lines pass through the inside of the cell according to the increase in field strength. Consequently, pore formation by electrical breakdown, electroporation, is accomplished. Pore formation is a very rapid event, occurring within 10 ns [27]. The size of the electric pore was estimated to be <10 nm in diameter [28,29]. With increasing length of the electric field the number of pores increase and they merge. When the pores merge into one large pore, irreversible breakdown occurs. Theoretically, if the molecule is smaller than the pore size, as is the case with oligonucleotides and chemical compounds, they can be transferred into the cytosol of the cells by diffusion. It is suggested that in case of a large section of DNA (>150 kb), the attachment of DNA to the cell membrane is a prerequisite for transfection by electroporation [30].

Electroporation has been applied to gene transfer in vivo since 1991. However, the in vivo molecular mechanism leading to the pore formation following electrical breakdown is unclear. In vivo electroporation can be achieved locally by insertion of electrodes into the tissue or by holding the tissue with plate-type electrodes. A relevant clinical application, electrochemotherapy, is being pursued in oncology, by using cell electroporeabilization to allow the entry of hydrophilic anti-cancer agents into the cells [31]. Short (<100 μs) and high electric pulses (250 V) were
used for electrochemotherapy and this approach resulted in a drastically increased anti-tumour effect of bleomycin. Titomirov *et al.* [32] first transfected DNA to skin by electroporation. Several reports were published to prove the efficacy and reproducibility of *in vivo* electroporation in various tissues. Among them, skeletal muscle targeting gene transfer has been studied extensively [33–36]. Most of the studies were performed by using electric pulses with short (100–300 ms) duration at 100–400 V, and the transgene product was detectable in the serum for 9 months at most [33]. Skin [32,37], liver [38,39], testis [40] and solid tumours [41,42] can be transfected by electroporation with similar electric field conditions.

We studied the possibility of transferring a foreign gene into the kidney by electroporation. First, we selectively infused fluorescein isothiocyanate (FITC)-labelled ODNs into the left renal artery, and clamped the left renal vein immediately after infusion. Thereafter, the left kidney was electroporated between a pair of tweezer-type electrodes. Although the electrodes did not cover the overall surface of the kidney, FITC-labelled ODNs were diffusely present in all glomeruli, including the upper or lower pole in the transfected left kidneys. To confirm the transgene expression by gene electrotransfer, X-gal staining was performed on isolated glomeruli 4 days after transfer of LacZ gene expression vector. β-Galactosidase expression was observed in 75% of the glomeruli [43]. The luciferase activity tended to be increased in proportion to the voltage. There was no histological damage in the glomerular and tubular epithelial cells by electric pulses, even at 100 V. The electroporation-mediated gene transfer technique resulted in a 10-fold higher glomerular luciferase activity than the HVJ liposome method [43]. Thus, *in vivo* gene electrotransfer for targeting kidney is an effective method for gene transfer and can provide a new technique for kidney-targeting gene therapy.

**Ex vivo gene transfer**

The *ex vivo* gene transfer approach has the merit of avoiding contamination of transfection accessories such as viral proteins or chemicals.

1. **Transfusion of genetically modified cells**

Two strategies have been reported on glomerular targeted *ex vivo* gene transfer. Extensive work on *ex vivo* gene transfer using the mesangial cell vector system has been done by Kitamura *et al.* [44,45]. Cells from an established mesangial cell line, continuously expressing the transfected foreign gene, were injected into the renal artery. The mesangial cells were trapped in the capillaries of the glomeruli and the expression of the exogenous gene was observed in the glomeruli for 4 weeks. This mesangial cell vector system provides a strong and long-term expression of the transfected genes *in vivo*. However, the transplanted mesangial cells did not migrate into the original mesangial area, but remained in the endocapillary lumen.

Kitamura and Suto [45] modified monocytes/macrophages by retroviral vector-mediated gene transfer. They injected genetically engineered macrophages into the renal artery of lipopolysaccharide-treated rats. Soon after the injection, they isolated glomeruli and observed accumulation of the macrophages in the glomeruli. Yokoo *et al.* [46] also reported a similar gene delivery system to inflamed tissues. Bone marrow cells of DBA/2 mice were differentiated to mononuclear cells, expressing CD11b and CD18. They then injected the developed mononuclear cells into the intraperitoneal cavity. The cells accumulated in the glomeruli of the mice treated with lipopolysaccharide. They applied the mononuclear cells as a vector of the glucoserebrocidase (GC) gene and injected them intraperitoneally. Consequently, the GC activity increased 3.2-fold in isolated glomeruli, suggesting that site-specific gene delivery in inflamed glomeruli is feasible. Implantation of genetically modified tubular epithelial cells (TECs) into the subcapsular region has been reported [47,48]. The transplanted TECs remained localized under the renal capsule and the transferred gene maintained its expression for at least 4 weeks. The authors applied this modified TEC as a vehicle to deliver a particular cytokine.

2. **Transplanted kidney**

The transplanted kidney could be a good target for gene transfer. Zeigler *et al.* [49] reported the first trial of *ex vivo* gene transfer into the isolated human kidney under the conditions of organ preservation. They used an adenovirus polylysine DNA complex and pulsatile perfusion for 2 h at 4 °C. A significant fraction of the gene expression was localized in the proximal tubular epithelial cells. Heikkila *et al.* perfused isolated pig kidneys with a solution containing adenovirus. The perfusion of the adenovirus vector in the renal artery for 12 h resulted in an intense expression of the reporter gene in 85% of the glomeruli [10].

**Gene therapy for experimental renal diseases**

**In vivo gene therapy of glomerular diseases**

1. **Antisense therapy**

The ability of short, single-stranded ODNs to inhibit individual gene expression in a sequence-specific manner is the basis of antisense therapy [50,51]. Antisense ODNs, which are designed to bind to complementary mRNA, can inhibit gene expression by cleavage of the target mRNA with RNaseH and/or by attenuation of translation. Akagi *et al.* [52] demonstrated that anti-TGF-β antisense ODN inhibited TGF-β expression in Th1 glomerulonephritis using the HVJ-liposome method. Extracellular matrix
(ECM) accumulation of Thy1 was suppressed in TGF-β antisense ODN treated rats in parallel with the reduction of TGF-β expression in the glomeruli.

2. **Transcription factor decoy**

A novel gene therapy strategy using a transcription factor decoy has been reported by Morishita et al. [53]. Double-stranded ODN containing a *cis*-element for a particular transcription factor acts as a ’decoy’ to dissociate competitively the transcription factor from the endogenous *cis*-element. Consequently, the expression of particular genes is inhibited. Decoy strategies have some advantages in comparison to antisense strategy: (i) double stranded DNA is more stable; (ii) a single decoy can suppress multiple genes coding the same *cis*-element in the promoter region; and (iii) a decoy can inhibit the constitutively expressing factor. At the experimental level, two reports have been published on the inhibitory effects of the progression of Thy-1 glomerulonephritis. Both the E2F decoy [54] and the NFκB decoy [55,56] successfully inhibited mesangial cell proliferation and glomerular matrix expansion in experimental glomerulonephritis.

3. **Macrophage vector system**

Yokoo et al. [57] described a new strategy of gene therapy by employing bone marrow-derived monocytes harbouring the IL-1 receptor antagonist (IL-1ra). They injected the monocyte expressing IL-1ra into the intraperitoneal space. The modified monocytes expressing intercellular adhesion molecule 1 (ICAM-1) ligands CD11b and CD18, accumulated in the inflammatory glomeruli of anti-GBM glomerulonephritis in mice. Consequently, renal function was ameliorated and the pathological changes of the experimental glomerulonephritis were reduced by the treatment. They concluded that the lesions of acute glomerulonephritis are effectively suppressed by this prophylactic intervention.

**In vivo gene therapy of tubular disorder**

1. **Antisense ODN**

Intravenous injection of antisense ODN results in accumulation in the proximal tubule and inhibits individual gene expression. Noiri et al. [58] applied antisense ODN against inducible nitric oxide (NO) synthase (iNOS) for the inhibition of iNOS in ischaemic kidneys. A single intravenous injection of iNOS antisense ODN attenuated acute renal failure and improved the morphological changes. Oberbauer et al. [59] applied ODN for the inhibition of the sodium/phosphate cotransporter. A single injection of the antisense ODN against Na/Pi cotransporter inhibited both mRNA and the protein for the Na/Pi cotransporter, and suppressed phosphate uptake into brush border membrane vesicles of the proximal tubule. Antisense ODN has the potential to treat a variety of renal diseases, particularly proximal tubule disorders; however, the short-term effects are a reason for concern.

2. **Cationic liposome (DOTMA:DOPE)/plasmid complex**

Lai et al. [60] tried to manipulate by gene therapy the carbonic anhydrase II (CAII) deficiency mouse with renal tubular acidosis. They treated the CAII-deficient mouse with cationic liposome complex containing human CAII gene by retrograde injection via renal pelvis. CAII expression peaked at day 3 and decreased thereafter, but was still detectable at 1 month. The ability to acidify the urine persisted for 3 weeks. These results illustrate the potential of gene therapy as a novel treatment for hereditary renal tubular acidosis. However, the gene expression of CAII was patchy, suggesting that the effects of gene transfer may be limited.

3. **Adenovirus vector**

Yang et al. [61] injected adenovirus vector encoding aquaporin-1 cDNA into the tail vein of an aquaporin-1-null mouse. The expression of aquaporin-1 was observed in the liver and proximal tubule cells of kidney. The water permeability of the apical membrane was increased and urinary concentration defect was partially corrected in the null mouse, which had gained aquaporin-1.

**Strategies of gene therapy targeting interstitial cells**

Tubulointerstitial inflammation and fibrosis are common features in a variety of renal diseases. This inflammation may predict renal function and long-term prognosis more accurately than glomerular injury [62,63]. Fibrotic structural alterations of the tubulo-interstitial compartment occur in virtually all progressive renal diseases. Interstitial fibrosis is characterized by accumulation of matrix proteins in the renal tubulointerstitial compartment. The mechanisms underlying the progression of interstitial fibrosis are not well understood, but this accumulation of matrix is accompanied by infiltrating macrophages and an increased number of activated fibroblasts in the interstitium [64].

Because the pathogenesis of tubulointerstitial fibrosis shares certain key mechanisms, targeting the interstitial expression of TGF-β1 should be able to prevent progression of interstitial fibrosis [65]. Targeting interstitial cells has proven to be challenging because no effective vehicle has been available to introduce the genetic material into interstitial fibroblasts. We recently developed an interstitial fibroblast targeting gene transfer technique using the AVE-type HVJ-liposome method. LacZ gene expression was
Gene therapy in renal diseases

mainly observed in fibroblasts in the tubulointerstitial space for 2 weeks after the LacZ expression vector was injected in the ureter [23]. We applied the technique for the gene therapy of experimental renal fibrosis to a prophylactic protocol. The antisense ODNs for TGF-β1 by the AVE-type HVJ-liposome method were injected into the ureter in the unilateral ureteral obstruction (UO) rat model [66]. UO induces acute tubulointerstitial fibrosis with macrophage infiltration. However, introduction of antisense ODN for TGF-β1 into the renal interstitial fibroblasts inhibited interstitial TGF-β1 expression and prevented interstitial fibrosis. These findings indicate that the introduction of TGF-β1 antisense ODNs into interstitial fibroblasts may be a potential therapeutic intervention for interstitial fibrosis.

Skeletal muscle-targeting gene therapy in kidney disease

1. Simple naked DNA transfection

Delivery of heterologous genes to skeletal muscle has been attempted since 1990. Wolff et al. first injected plasmids encoding reporter genes for chloramphenicol acetyltransferase, luciferase and β-galactosidase separately into mouse skeletal muscle to estimate the efficiency of gene expression [67]. The expression of the luciferase gene, which was driven by the Rous sarcoma virus promoter, persisted for at least 2 months. Thereafter, similar observations have been published using expression vectors encoding secreted proteins, e.g. erythropoietin [68], vascular epithelial growth factor (VEGF) [69] and endostatin [70]. Generally, a large amount of naked plasmid is required for gene expression in skeletal muscle and expression increases in a manner dependent on the dose of plasmid administered. Regenerating muscle increases the uptake of the plasmid DNA and improves the transfection efficiency. Tokui et al. pretreated the muscle with bupivacaine 2 days before to induce muscle regeneration and then observed enhanced expression of the transgene [71].

2. The HVJ-liposome method

The HVJ-liposome method has been used for skeletal muscle-targeting gene transfer. The HVJ-liposome-mediated gene transfer into skeletal muscle results in 10 times higher gene expression compared with the simple naked plasmid injection. Isaka et al. proved that the reduction of overexpressed TGF-β ameliorates the ECM expansion of anti-Thy1 glomerulonephritis. They used a proteoglycan, decorin [72], and a soluble receptor for TGF-β, which is composed of the extracellular domain of TGF-β type II receptor and IgG-Fc (TGF-RII/Fc) [73]. Decorin and the soluble receptor TGF-RII/Fc bind to active TGF-β, resulting in its inactivation. Decorin was generated in skeletal muscle where a plasmid encoding decorin cDNA was transfected and accumulation of the decorin was seen in glomeruli. The same behaviour was also observed in the transfection of TGF-RII/Fc. Interestingly, the increase of ECM was reduced, comparable to the decrease of TGF-β mRNA. These results suggest that manipulation of overexpressed TGF-β by skeletal muscle-targeting gene therapy may ameliorate the progression of glomerulonephritis.

3. AAV vector

AAV vector was used for skeletal muscle-targeting gene transfer. Skeletal muscle is a good target for the long-term gene expression of AAV. Kessler et al. [74] demonstrated that the sustained expression of the erythropoietin gene lasted for 32 weeks after single intramuscular injection of the AAV vector. During this time period, the serum level of erythropoietin increased up to 700 mU/ml and the haematocrit was sustained over 80%. Gene therapy transferring AAV vectors into skeletal muscle may be promising for the constant provision of individual proteins through circulation.

4. Electroporation

Mesangial cell proliferation and phenotypic alterations occur in an early phase of glomerular injury and precede the increased ECM accumulation. A pivotal growth factor responsible for mesangial proliferation is platelet-derived growth factor (PDGF), which is a potent mitogen. We generated a chimeric cDNA encoding an extracellular domain of the β-PDGFR receptor fused with IgG-Fc, termed PDGFRII/Fc, and examined the feasibility of gene therapy targeting PDGF using PDGFRII/Fc [75]. The chimeric PDGFRII/Fc molecule completely inhibited the tyrosine phosphorylation of β-PDGFR receptors and the cellular proliferation induced by PDGF in vitro. We then introduced the PDGFRII/Fc expression vector into the muscle of nephritic rats by electroporation. The chimeric PDGFRII/Fc concentration in plasma was ~200 ng/ml 4 days after transfection. On day 5, PDGFRII/Fc gene transfer significantly reduced the number of PCNA-positive cells and glomerular cell numbers. Northern blot analysis demonstrated that glomerular mRNA levels of αSMA, TGF-β1 and type I collagen were also suppressed by the PDGFRII/Fc transfection. We also observed a significant reduction in matrix score in the transfected nephritic rats. These results suggest that gene therapy, by the manipulation of PDGF action using electroporation-mediated PDGFRII/Fc gene transfer to the skeletal muscle, might be a useful therapy for mesangioproliferative glomerulonephritis.

A similar approach can be used in an experimental mouse model of lupus nephritis. Lawson et al. reported that muscle targeting gene therapy by plasmid encoding a soluble receptor for IFNγ, IFNγR/Fc, significantly reduced the serum level of IFNγ and...
the disease activity, including lupus nephritis [76]. Gene transfer of the soluble cytokine receptor may be applicable for human gene therapy using skeletal muscle.

Future perspectives and conclusions

Although gene therapy has great potential in the therapy of renal diseases, it is clear that the present technology is still immature for clinical application. The improvement of the technology is indispensable. From a practical point of view, two strategies are promising for the therapy of renal diseases: skeletal muscle-targeting gene therapy and kidney transplantation.

Given that systemic delivery of the functional protein can serve as a therapy for renal diseases, skeletal muscle-targeting gene therapy is a good application. Skeletal muscle-targeting gene therapy is easy to perform, repeatable, and highly efficient in generating the secreting protein. At present, plasmid-based gene transfer by electroporation can generate \(~100\) ng/ml of gene product in the serum [75]. In addition, it is less expensive than administration of the purified recombinant protein. The diverse characteristics of the secreting protein, e.g. inhibitor, activator or soluble receptor, can be realized by simple molecular biology techniques in the laboratory and may be directly applicable for clinical use.

The transplanted kidney is an ideal target for gene therapy, as discussed above. The feasibility of delivering therapeutic genes ex vivo has been shown to reduce the rejection rate of transplanted kidneys in an animal model. Adenoviral vector was used for the transplant kidney approach. Swenson et al. [77] showed that Fas ligand gene transfer to the kidney protected the kidney from acute rejection and improved allograft survival in the rat. Chronic rejection can be a target for gene therapy. Tomasoni et al. [78] reported that donor-specific tolerance to the rat allograft was successfully induced by gene transfer with adenovirus vector coding CTLA4Ig, which is a recombinant protein competitively inhibiting the interaction between CD28 and B7. Rats who received the allograft after CTLA4Ig gene transfection survived up to 100 days, while rats who received allograft without gene transfer died within 1 week. Recently, Azuma et al. [79] reported that the fibrotic changes in the model of chronic kidney rejection can be attenuated by administration of the anti-fibrotic hepatocyte growth factor. These results suggest that the prevention of early fibrosis by gene therapy is applicable in the therapy of chronic rejection. If gene transfer could be achieved by a non-viral vector, which should be relatively non-toxic and non-immunogenic, the feasibility of gene transfer to the transplanted kidney could be improved.

We believe that in the near future, innovative strategies may overcome the actual limitations of gene transfer technology and may lead to the clinical application of gene therapy in human renal diseases.

References

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