

Gene Therapy for Renal Diseases: Its Potential and Limitation

ENYU IMAI

Division of Nephrology, Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Suita Osaka, Japan.

Gene therapy emerged 15 yr ago with great expectations for a marriage between the remarkable advances in molecular biology of the previous decade and clinical medicine. It was originally an innovative treatment for incurable diseases, so-called genetic disorders, in which the disease was caused by mutation, truncation, or complete loss of a single gene. In 1990, the first successful gene therapy was performed on two girls with adenosine deaminase (ADA) deficiency, which causes severe immunodeficiency (1). The number of peripheral lymphocytes increased by repeated injection of lymphocytes carrying the exogenous ADA gene, and the girls' health stabilized to the point that they were able to attend school. Soon after, however, the investigators realized that the success of the gene therapy for ADA deficiency is a rare exception because most of the monogenic disorders cannot be treated simply by unlimited overexpression of the deficient gene. In addition, some of the monogenic diseases cannot be treated with available vectors because the genes are much larger than the size of the gene cassette of the vectors.

Meanwhile, gene therapy began to be applied directly in cancer therapy (2). The treatment of cancer by gene therapy represented a paradigm shift from the application of gene therapy for the correction of a gene defect. The rationale for cancer gene therapy is to kill cancer cells rather than attempting to make them normal. One strategy was to strengthen immunity against cancer cells or viruses; the other was to kill the cancer cells by transfection of a suicide gene, which is turned on by Ganciclovir. Both of these strategies seemed to be effective in experimental animals. Unfortunately, the clinical trials of gene therapy for cancer were strictly limited to advanced cancer because the safety of therapy could not be guaranteed. Positive outcomes from clinical trials of gene therapy for cancer have not been realized to date, although more than 50% of human gene therapy has been attempted in cancer patients. In the last 5 yr, the applications of gene therapy have expanded from rare neurological diseases to common diseases, including cardiovascular diseases. Gene therapy for ischemic heart diseases (3) and critical limb ischemia (4) particularly have found favorable results in phase II trials. In contrast, gene therapy for

kidney disease lags behind because of the low efficiency of gene transfer and the difficulty in targeting specific cells in the kidney.

Skeletal muscle-targeted gene therapy is another option in therapy for renal diseases by systemic delivery of peptides and artificial proteins (5). Skeletal muscle cells can accept genes by simple injection, but manipulations such as HVJ-liposome-mediated gene delivery (6) and regenerating muscle by bupivacaine followed by electroporation (7) improve the efficiency significantly. Nakamura *et al.* (8) demonstrated that gene therapy with a chimeric soluble receptor composed of the extracellular domain of the PDGF- β receptor and IgGfc reduced mesangial proliferation and matrix accumulation in experimental glomerulonephritis. In this issue of *JASN*, Furuichi *et al.* (9) clearly establish the potential of skeletal muscle-targeted gene therapy in the kidney by using cDNA for truncated monocyte chemoattractant protein-1 in a model of ischemia-reperfusion injury. The dominant negative type truncated MCP-1 inhibited MCP-1/CCR2 signaling in the kidney and preserved renal histology. Erythropoietin gene therapy has also been successfully carried out in uremic rats and improved anemia (10). Skeletal muscle-targeted gene therapy has a potential advantage over recombinant protein therapy because the gene product can be constantly generated and delivered for a relatively long time, and the cost can be less than that for repeated injection of recombinant protein.

Clearly, the ideal gene therapy for renal disease should be targeted to the kidney and, if possible, limited to specific cells because localized expression of the therapeutic molecule reduces the risk of systemic delivery and increases the local concentration in the kidney. To date, however, the efficiency of gene transfer to the kidney seems to be 10- to 100-fold less compared with skeletal muscle. The complex structure of the kidney, in which podocytes and tubular cells are sequestered from vessels by basement membrane, reduces the chance for successful gene transfer to the targeted cells. As of this point in time, many approaches have been developed to deal with these limitations. Among viral vectors, adenovirus vector-mediated gene transfer has been most commonly used to introduce genes into the kidney. However, simple injection of the vector into the renal artery or ureter cannot achieve efficient gene transfer. Some maneuvers to improve the efficiency of transfer have been attempted, such as incubation of the kidney with adenoviral vector for 18 h with cooling (11) and perfusion of the kidney with a solution containing vector for hours (12). Nonviral vectors also offer efficient gene transfer into the kidney. Electric pulse-mediated gene transfer is promising (13),

Correspondence to Dr. Enyu Imai, Division of Nephrology, Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita Osaka 565-0871, Japan. Phone: 81-6-6879-3632.; Fax: 81-6-6879-3639; E-mail: imai@medone.med.osaka-u.ac.jp/

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particularly for the introduction of small nucleotides, anti-sense, and decoy oligonucleotides and double-stranded RNAs. The types of cells to be transduced are, however, limited; only mesangial cells, proximal tubules, and interstitial fibroblasts have so far been reported to be effectively transfected. In another very promising report in this issue of *JASN*, Chen *et al.* (14) document the efficacy of a new adeno-associated viral vector system in specifically targeting the S3 portion of the proximal tubule and intercalated cells in the collecting duct and delivering the transfected protein for over 6 wk. Their results are vividly depicted on the cover of this issue.

In contrast to the enthusiasm for the potential positive outcome of gene therapy, the safety of gene therapy must be seriously taken into account. Two cases of death have been reported in human gene therapy using adenoviral (15) and retroviral vectors (16). Viral vectors elicit immune reactions: acute toxicity from the infusion of the foreign materials, humoral immune responses against the gene products, and cellular immune responses against the transduced cells. Some of the integrating viral vectors still have a potential risk of insertional mutagenesis. In gene therapy for experimental renal diseases, few reports document adequately the harmful effects of gene transfer itself and the expression of exogenous genes in the kidney and other organs. Most publications show only the beneficial effects of gene therapy in the diseased kidney. Subtle changes of renal tissue caused by gene transfer might be overlooked by the superimposed pathologic changes caused by the experimental condition itself.

Although gene therapy has great potential in the treatment of renal diseases, present technology is currently too preliminary for clinical application. Targeted and regulatable gene expression must be developed. Novel vectors are under development. The efficacy in renal cells of lentiviral vector (17) and a hybrid vector with adenovirus/adeno-associated virus (18), which can transduce into quiescent and terminally differentiated cells, may be worth testing.

Tryggvason *et al.* (19) have progressed step by step toward gene therapy of Alport syndrome caused by mutations in the X-chromosomal gene encoding the type IV collagen $\alpha 5$ chain. They succeeded in the introduction of the type IV collagen $\alpha 5$ chain gene into pig glomeruli using an adenoviral vector and showed the generation of the triple helix of the type IV collagen in glomerular basement membrane with collagen $\alpha 3$ and $\alpha 4$ chains. Now they are attempting gene therapy in dogs with Alport syndrome.

Transplantation-based gene therapy using stem cells is also promising. Yokoo *et al.* (20) generated a bone marrow-derived cell transduced with an IL-1 receptor antagonist (IL-1ra) gene. The cells expressing CD11b and CD18, which are markers of macrophages, migrated to inflamed glomeruli in experimental glomerulonephritis. Moreover, the prophylactically injected cells expressing IL-1ra suppressed inflammation, preserved renal function, and mitigated morphologic changes in anti-GBM glomerulonephritis for 2 wk. The combination of regenerative medicine using stem cells and gene manipulation techniques now seems possible and promising.

Transplanted kidneys provide an ideal setting for gene therapy because we can manipulate the kidney *ex vivo* and wash out the remaining genetic materials from renal vessels before transplantation. Two preclinical reports have been published for inhibition of acute rejection. Swenson *et al.* (21) and Tomasoni *et al.* (22) demonstrated improvement of allograft survival in a rat acute rejection model by Fas ligand gene transfer and by CALA4Ig gene therapy, respectively. Currently, in a preclinical trial, we are studying prevention of chronic allograft nephropathy by gene therapy using hepatocyte growth factor in transplanted pig kidneys. Interstitial fibrosis of the transplant kidney has been significantly reduced by hepatocyte growth factor gene therapy (23, unpublished data).

Gene therapy is a creative scientific approach to therapy of human diseases invented by molecular biologists. Theoretically, all of the proteins, actual or artificial, coded by DNA can be generated in human cells, suggesting infinite potential. For the clinical scientist dreaming of an innovative approach to renal diseases, the technology remains primitive but is rapidly advancing, and the future for human gene therapy in nephrology is both exciting and promising.

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See related article, “Gene Therapy Expressing Amino-Terminal Truncated Monocyte Chemoattractant Protein-1 Prevents Renal Ischemia-Reperfusion Injury,” on pages 1066–1071.