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Application of Gene Therapy in Common Renal Diseases

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Abstract: *Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein. It is a technique that treats disease by modifying genetic defects or regulating gene expression. It is also a potential new way to cure drug-refractory diseases. Kidney disease is an important factor that threatens the health of human beings. Existing techniques and drugs are ineffective in treatment of a variety of kidney diseases, including metastatic renal cell carcinoma, renal allograft side effects, and polycystic kidney disease. With the development of molecular biology technology and the elucidation of the related mechanisms of kidney disease, gene therapy has become a new choice for the precision treatment of kidney diseases.*

Keywords: *Gene therapy, kidney disease, diabetic nephropathy, renal failure, kidney transplantation, polycystic kidney disease*

1. INTRODUCTION

For a long time, gene therapy has drawn widespread attention from scientists and clinicians. The concept of gene therapy was first proposed for some single-gene genetic diseases. The scientists realized that the introduction of the normal gene for the mutant gene could cure the disease (1). With the development of science and technology, the existing gene therapy not only treats the casual genes, but also focus on the key pathways of gene expression. Specific methods include: 1) The replacement of a mutant gene with a normal gene; 2) Inactivation of mutant genes that express aberrant functions; 3) Repair of abnormal genes by point-directed mutagenesis; 4) Regulation of the extent to which abnormal genes or their upstream and downstream pathway loci on or off; 5) Introducing new genes to help cure the disease (2).

In 1990, the research result about first clinical trial of retroviral treatment in melanoma was published on the New England Journal (3). This result confirmed the feasibility of gene therapy. By February 2016, a total of 2409 gene therapy trials have been approved worldwide. More than half of the clinical trials are still in Phase I. Tumor gene therapy trials accounted for 64.5%, followed by a single genetic disease 'infectious diseases and cardiovascular diseases.

Normal kidney function is crucial to maintaining life. Kidneys are involved in the maintenance of systemic homeostasis which plays important role in regulation of the acid-base balance, electrolyte concentration, extracellular fluid volume and blood pressure. According to the occurrence and duration of kidney disease, kidney disease improving prognosis organization (KDIGO) classified pathological changes of reduced kidney function as acute kidney injury (AKI) and chronic kidney disease (CKD). Reduced renal function is a common outcome for many kidney diseases, including kidney cancer, infections, kidney stones, hereditary nephropathy and secondary nephropathy. Most chronic kidney disease eventually develops into uremia. At this stage, dialysis or kidney transplantation are the limited methods to sustain their lives, posing a serious psychological and financial burden to patients. In recent years, with the advanced research on kidney genetics and molecular biology, the pathogenesis of kidney disease is revealed on pathogenic genes and signaling pathways level. In kidney disease, gene therapy can directly treat defects that affect renal cell function, target the regulation of toxic metabolites produced by other cells, and improves chronic kidney inflammation affected by immune system. This article summarizes the recent advances in gene therapy in renal diseases.

2. AKI AND CKD

AKI is a serious clinical kidney lesion with a high morbidity and mortality and which

increases the risk of ESRD in patients. In ischemic or toxic AKI lesion, stress pathways are usually activated in tubular epithelial cell. Lesion originates from neutrophil-derived myeloperoxidase and FAS/FASL interactions which mediate renal cell death (4). Infiltration of immune inflammatory cells further aggravates renal tissue lesion.

Molitoris et al exploited the efficacy of siRNA targeted to p53, the pivotal protein in the apoptotic pathway, to prevent kidney injury. They found that naked synthetic siRNA to p53 injected intravenously 4 hours after ischemic injury maximally protected both proximal tubule cells and kidney function. Analysis of renal histology and apoptosis revealed improved injury scores in both cortical and corticomedullary regions. Their data indicates that rapid delivery of siRNA to proximal tubule cells following intravenous administration reduces AKI-induced renal ischemia and the degree of damage to the kidneys tissue. Targeting siRNA to p53 leads to a dose-dependent attenuation of apoptotic signaling, suggesting potential therapeutic benefit for ischemic and nephrotoxic kidney injury (5).

Alidori group got the similar result (6). They generated the ammonium-functionalized carbon nanotube (fCNT) and found that fCNT-mediated transport of siRNA selectively and with high efficiency to renal proximal tubule cells in animal models of AKI. fCNT enhanced siRNA delivery to tubule cells compared to siRNA alone and effectively knocked down the expression of several target genes, including Trp53 and Mep1b. Prophylactic treatment with a combination of fCNT/siMep1b and fCNT/siTrp53 significantly improved progression-free survival compared to controls via a mechanism that required concurrent reduction of meprin-1β and p53 expression. siRNA gene therapy down-regulates potential targets including protein stress mediators, regulates proteins involved in epithelial inflammatory responses or apoptosis mechanisms. All of these are effective ways to reduce AKI-induced kidney lesion.

CKD is a chronic kidney disorder with the diagnostic criteria for a decline of renal function greater than 3 months with or without GFR decrease, which can be divided into five phases based on the levels of GFR and proteinuria (7). The occurrence of CKD is associated with many risk factors, of which diabetes is one of the most important risk factors. Excessively high levels of blood glucose lead to the occurrence of diabetic nephropathy (8).

Hepatocyte growth factor (HGF) is a polypeptide growth factor that promotes the growth and migration of a wide variety of cells. HGF possesses anti-fibrotic and regenerative properties that play a role in the development of kidneys and in the repair of kidney damage (9). Flaquer et al (10) confirmed the role of the HGF gene in slowing the progression of diabetic nephropathy in db/db mouse model. They first generated chimeric db/db mice as a model of diabetes that produce enhanced green fluorescent protein (EGFP) in bone marrow cells and performed treatment with HGF gene therapy either alone or in combination with granulocyte-colony stimulating factor, in order to induce mobilisation of haematopoietic stem cells in these diabetic and chimeric animals. They found that there is no significant change of the blood glucose level in each group before and after treatment, but the progression of proteinuria of experimental group was slower than that in control group, which indicated that HGF gene could delay the damage of kidney caused by diabetes. Pathological analysis showed that the deposition of renal collagen IV and glomerular fibronectin was significantly reduced in the experimental group. Decreased glomerular sclerosis further demonstrates that HGF plays an important role in the db/db mice kidney injury repairment.

CKD can also lead to renal anemia, mainly due to iron deficiency and lack of production of erythropoietin (EPO). Renal anemia is one of the main factors of poor prognosis of CKD (11). CKD patients with anemia have a higher incidence of cardiovascular disease and mortality (12). About 50% of stage 3 and 4 CKD patients are with anemia. The incidence of anemia can be as high as 75% at ESRD stage (13).

Pederson et al found that hydrodynamic gene therapy with Epo can restore haemoglobin levels in anaemic transgenic mice and attenuate the extracellular matrix accumulation in the kidneys. They transferred a plasmid encoding murine Epo in a transgenic mouse model that over expresses TGF-β1 locally in the kidneys. This model develops anaemia due to chronic kidney disease characterized by thickening of the glomerular basement membrane, deposition of mesangial matrix and mild interstitial fibrosis. After a single hydrodynamic

administration of plasmid DNA containing murine EPO gene, sustained high haemoglobin levels are observed in both transgenic and wildtype mice from 7.5 ± 0.6 mmol/L to 9.4 ± 1.2 mmol/L and 10.7 ± 0.3 mmol/L to 15.5 ± 0.5 mmol/L, respectively. This result indicated that Epo treatment in this model of chronic kidney disease normalized haemoglobin levels and played a role in the treatment of renal anemia.

3. Renal Cell Carcinoma

Renal cell carcinoma (RCC) is one of the common malignant tumors of the urinary system, accounting for about 80% to 85% of all kidney tumors (14). The most common subtypes include clear cell carcinoma papillary and chromophobe. 60% to 70% of RCC is focal lesion (stage I or II). Five years Survival rate is higher than 70% after partial nephrectomy or radical nephrectomy. III and IV stages of RCC show metastasis, and are not sensitive to radiotherapy and chemotherapy. Five-year survival rate is low (15). Tumor cells induce the destruction of the internal environment between cells and extracellular components, increase expression of fibronectin (FN), degrade extracellular matrix and promote epithelial-mesenchymal transitions (16).

Endostatin (ES) is an α1 chain fragment of collagen XVIII that possesses anti-angiogenic activity (17). Interaction of ES and the receptor integrin α5β1 of FN plays an inhibitory effect on FN (18). Chaves et al generated the RCC Orthotopic metastatic mouse tumor model AND ES cell line that overexpress NIH/3T3-LendSN-clone3 (19). Balb/C mice bearing Renal cells were treated with NIH/3T3-LXSN cells or NIH/3T3- LendSN cells. At the end of the experiment, the ES serum levels in treated mice were higher than those in the control group ($P<0.05$). ES treatment led to significant decreases at the FN mRNA ($P<0.001$) and protein levels ($P<0.01$). Their result demonstrated the ES antitumor effect that is mediated by down- regulation of FN expression in mRCC.

4. Polycystic Kidney Disease

Polycystic kidney disease (PKD) is an inherited disorder in which the renal tubules become structurally abnormal, resulting in the development and growth of multiple cysts within the kidney (20). These cysts may begin to develop before birth or in infancy, in childhood, or in adulthood (21). Cysts are non- functioning tubules filled with fluid pumped into them, which range in size from microscopic to enormous, crushing adjacent normal tubules and eventually rendering them non-functional also. The two main types of polycystic kidney disease are: autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD).

Autosomal dominant polycystic kidney disease (ADPKD) is the most common of a group of inherited kidney disorders characterized by progressive cyst development and various extrarenal manifestations (22, 23). Cystogenesis in the human kidneys can progressively occupy the normal parenchyma of the kidney and lead to renal failure, which usually occurs in mid-to-late adulthood. This disease is the fourth most common single cause of end-stage renal failure worldwide (24, 25).

ADPKD is caused by mutations in the *PKD1* or *PKD2* genes, which encode the proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Approximately 85% of ADPKD patients have mutations in *PKD1*, and the remaining 15% have mutations in *PKD2* (26, 27). PKD1 interacts with PKD2 through a coiled-coil domain in the C-terminal portion which forms the polycystin-signaling complex that play a role in chemosensory or mechanosensory signal transduction. Disruption of this complex result in cyst formation. Considerable progression has been obtained in our understanding of the disease mechanism, many signaling pathways involve in ADPKD cyst formation such as the mammalian target of rapamycin (mTOR) (28, 29), cyclic adenosine monophosphate (cAMP) (30, 31), Wnt signaling pathway (32, 33), G protein coupled receptor (GPCR) (34) and Signal Transducer and Activator of Transcription (STAT) signaling pathway (35). Although much is known about the molecular genetic mechanisms of ADPKD, no effective treatment for the disease is currently available. There are many drugs that interfere with cell signaling pathways in clinical trials, but some are associated with significant clinical side effects. Our previous work demonstrated the *in vivo* effects of causal gene replacement in orthologous gene models of ADPKD in mice. We cross-mated our human *PKD2* transgenic mouse ($PKD2^{tg}$) with a *Pkd2*-null mouse model, which is embryonically lethal and forms kidney and pancreas cysts. $Pkd2^{-/-}$ mice with human *PKD2* transgene ($Pkd2^{-/-}$; $PKD2^{tg}$) were born in expected Mendelian ratios indicating that the embryonic

lethality of the *Pkd2*$^{-/-}$ mice was rescued. *Pkd2*$^{-/-}$;*PKD2*^{tg} mice survived up to 12 months and exhibited moderate-to-severe cystic phenotypes of the kidney, liver, and pancreas. Moreover, *Pkd2*$^{-/-}$ mice with homozygous *PKD2*^{tg}- transgene alleles (*Pkd2*$^{-/-}$;*PKD2*$^{tg/tg}$) showed significant further amelioration of the cystic severity compared to the *Pkd2*$^{-/-}$ mice with a hemizygous *PKD2*^{tg} allele (*Pkd2*$^{-/-}$;*PKD2*^{tg}), suggesting that the ADPKD phenotype was improved by increasing the transgene dosage. By further analyses, we have also found that cystic improvement mainly results from reduced proliferation, rather apoptosis, of cyst-prone epithelial cells in the mouse model. The finding that the functional restoration of human PC2 significantly rescued ADPKD phenotypes in a dose-dependent manner suggests that increasing PC2 activity may be beneficial in some forms of ADPKD (36).

5. PERSPECTIVE

Most diseases associate with multiple gene interactions or epigenetic regulation. Although gene therapy was first targeted to single-gene inheritance, it is possible to play roles in treating more diseases by regulating the key pathways of the disease. In the past three decades, there is an increasing number of studies on gene therapy in kidney diseases. However, most of the experiment still remain in the preclinical stage due to safety and efficiency of the gene transfer vectors, the complex pathogenesis of kidney disease and social ethics. Less application was performed in clinic. With the renal disease-related pathogenic genes and signal pathways are gradually elucidated as well as the continuous development of gene transfer systems, the future application of gene therapy in kidney diseases will be even broader.

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