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Development of new therapies, including regeneration of the kidney, for chronic kidney diseases

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Abstract

The increasing number of patients on chronic hemodialysis is a great problem in the field of nephrology in Japan and Western countries. Current therapies for chronic kidney diseases (CKDs) can retard the progression of renal failure, but cannot completely stop their progression to endstage renal failure (ESRD). Many researchers are now studying new therapeutic targets for CKDs, by various methods. Furthermore, because organ donation for kidney transplantation is very limited in Japan, research on kidney regeneration is an important issue for the therapy of ESRD. To regenerate the kidney, stem cells and growth factors for the kidney are being extensively studied, although the clinical application of the results of these studies has not yet taken place.

Key words Kidney · Regeneration · Prorenin · Microarray · Stem cell

Introduction

The number of patients on chronic hemodialysis in Japan is continuously increasing, and reached about 250 000 in 2005. Every year in Japan, more than 30 000 patients newly receive hemodialysis. Diabetic nephropathy and chronic glomerulonephritis are the two leading causes of endstage renal failure (ESRD) requiring hemodialysis and the number of the patients on hemodialysis because of diabetic nephropathy is increasing tremendously. Currently, antihypertensive therapy is the main therapy for chronic kidney diseases (CKDs), including diabetic nephropathy and chronic glomerulonephritis, with blockade of the renin-angiotensin-aldosterone system being widely accepted as the therapy of first choice. However, these therapies are not

able to completely prevent the progress of CKDs. On the other hand, the number of organ donors is extremely limited in Japan, and there are fewer than 200 cases per year of kidney transplantation from cadavers, which is thought to be the best renal replacement therapy in patients with ESRD. To solve these problems in patients with CKDs and ESRD, the development of new therapies is required, with kidney regeneration being regarded as perhaps providing a solution to the shortage of organ donors.

Search for new target genes for the treatment of chronic kidney diseases (CKDs)

There are, essentially, two ways to search for target genes for the treatment of diseases. One is research on known genes that are thought to be involved in the pathogenesis of the disease. A good example in kidney disease is angiotensin II, and there is no doubt about the efficacy of the blockade of angiotensin II effects for the treatment of CKD. In addition, many factors, such as transforming growth factor (TGF)- β , endothelin, and monocyte chemoattractant protein (MCP)-1, have been reported as key molecules involved in progressive renal damage.¹ Another way to search for target genes is to establish a new target for the treatment the disease, searching for novel genes by various methods; for example, microarray analysis.

In searching for candidate genes for the therapy of diabetic nephropathy, we have recently reported that the renin/prorenin receptor plays a pivotal role in the development of diabetic nephropathy in rats.² The renin/prorenin receptor was originally reported by Nguyen et al.,³ who reported that the binding of prorenin to the renin/prorenin receptor induced conformational changes in prorenin, resulting in renin activity without changes in the molecular weight of prorenin, with the renin/prorenin receptor itself being activated. This activated renin/prorenin receptor induces the activation of mitogen-activating protein (MAP) kinases. We have developed a decoy protein for the binding site of prorenin to its receptor; continuous infusion of this

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protein with an osmotic minipump completely abrogated diabetic nephropathy in streptozotocin-induced diabetic rats. This effect of the decoy protein did not alter systemic renin-angiotensin levels, while the intrarenal angiotensin content was decreased. We expect that the renin/prorenin receptor will be a good candidate for the development of new drugs for the treatment of diabetic nephropathy.

In searching for new target genes for the therapy of CKD, microarray analysis is a powerful tool to investigate the expression of thousands of genes simultaneously, although there are limitations of sensitivity and specificity. Recently, CK2, a kind of protein kinase, was reported, by Yamada et al.,⁴ to be involved in the pathogenesis of anti-Thy 1.1 antibody-induced nephritis. In that report, the authors compared changes in mRNA expression in the kidneys of rats with anti-Thy 1.1 antibody-induced nephritis with normal control rat kidneys, and several genes were identified, including *CK2*. They focused on *CK2* and confirmed its increased expression in anti-glomerular basement membrane (GBM) glomerulonephritis, too. Furthermore, it was shown that *CK2* expression was increased in IgA and lupus nephritis. We have also tried to find a new target gene for the therapy of CKD, using microarray analysis.⁵ It is known that a single injection of anti-Thy 1.1 antibody induces reversible and self-limiting acute glomerulonephritis in rats, while heminephrectomy prior to the antibody injection induces irreversible glomerulonephritis. We compared the gene expression patterns in these two rat models, using microarray analysis, and found that ten genes were upregulated in the irreversible model. Of these ten genes, we focused on thymosin β -4 and confirmed the increase in its mRNA expression by real-time polymerase chain reaction (PCR). Currently, the role of thymosin β -4 in CKD is under observation.

These genes are examples of new target genes for the treatment of CKD; it is possible that completely new drugs for CKD will be developed in the future, based on these studies.

Regeneration of the kidney

Strategies for kidney regeneration

Because it is not practical to regenerate a whole mature kidney in an in vitro system, many researchers consider that transplantation of a kidney precursor, such as the metanephros, is a possible alternative. In an animal study, human embryonic metanephros was transplanted to an immune-deficient mouse, and the metanephros transplanted into the host kidney developed into a mature kidney.⁶ This transplanted metanephros produced urine-like fluid, although the ureter was not developed. From this report, it appears that if we could develop metanephros in an in vitro system and transplant them, it may be possible to develop small kidneys within the original kidney. But how can we produce metanephros in the in vitro condition? Nigam's group (Steer et al.⁷) reported very interesting results on the

propagation of metanephros. They divided the metanephros into mesenchymal tissue and ureteral buds, and then the mesenchymal tissue and ureteral buds were each cut into one-thirds and each portion was cultured. After 8-day culture, each portion of mesenchymal tissue grew to the original size of the mesenchyme, and they cut each portion of mesenchymal tissue into one-thirds again. After another 8-day culture, each portion had again grown to three times its original size again. A similar method was applied for the ureteral buds, and the ureteral buds propagated. They then placed the mesenchyme and ureteral bud together in one piece and cultured the tissues in vitro; a metanephros-like structure was developed. From these reports regarding the transplantation and propagation of the metanephros, it is reasonable to assume that, if we can develop mesenchymal cells and ureteric buds from stem cells, we will be able to produce a metanephros in an in vitro system.

Stem cells for the development of kidney precursors

We do not know if there are stem cells in the kidney, although it is reasonable to presume that some kind of stem cell contributes to the tissue repair of the kidney after acute renal injury. Several reports have shown that bone marrow stem cells contributed to the tissue repair after acute renal failure in animals with allograft bone marrow transplants.⁸⁻¹⁰ In these reports, bone marrow cells, labelled with genetic markers such as β -galactosidase, were transplanted to irradiated animals, and the labeled cells were found to be present in the proximal tubules after ischemic acute renal failure. Without acute renal failure, these transplanted cells were present in the glomerulus, and these cells were thought to differentiate into mesangium cells. However, two reports have recently cast doubts on the differentiation of bone marrow stem cells into tubular cells.^{11,12} Bonventre's group showed that endogenous β -galactosidase, upregulated by ischemia,¹¹ was stained in the kidney of animals with bone marrow transplants, and that a specific antibody to *Escherichia coli*-derived β -galactosidase, which is expressed in transplanted bone marrow cells, failed to show immunostaining in the kidney, except in interstitial cells. On the other hand, if bone marrow mesenchymal stem cells are placed in the developing kidney, these cells can differentiate into various cells in the nephron. Yokoo et al.¹³ reported a very interesting method to develop renal precursor cells from bone marrow mesenchymal stem cells. Firstly, they removed a whole embryo at 11.5 days and injected mesenchymal stem cells into the place where the metanephros would develop. The whole embryo was then cultured in a special incubator for 2 days and the metanephros was then taken out. This metanephros was subsequently cultured in the incubator, and Yokoo et al.¹³ found that the injected mesenchymal stem cells had differentiated into condensed mesenchymal cells and ureteric buds. By this procedure, it was shown that mesenchymal stem cells could be a source of renal regeneration, if we could collect differentiated renal precursor cells derived from the injected mesenchymal stem cells. An interesting

article was recently published on a method to obtain precursor cells for the development of the metanephros.¹⁴ Osafune et al.¹⁴ reported that a single cell from the metanephric mesenchyme, expressing Sall 1, reconstituted the kidney structure in an organ culture system. If we could collect these cells efficiently, it may be possible to develop a metanephros in an in vitro system.

Another candidate for the source of renal regeneration is embryonic stem (ES) cells. Kobayashi et al.¹⁵ reported that Wnt4-transformed mouse embryonic stem cells injected into an adult kidney developed tubule-like structures and expressed AQP-2 on the luminal membrane. Yamamoto et al.¹⁶ reported that ES cells injected into the kidney developed into a ureteral bud-like structure and expressed many genes that are expressed in developing kidneys. From these results, it is suggested that ES cells are a good candidate for kidney regeneration, although there are still ethical problems, and rejection by the host has to be overcome.

Factors involved in renal development that could be employed for kidney regeneration

It is conceivable that the developing kidney and the kidney in the recovery phase of acute renal failure express developmental and growth factors, and that these factors could also be useful for regeneration of the kidney. Several important factors for renal development have been identified, including Pax-2, GDNF, WT-1, Wnt-4, BMP7, and Pax-8. Among these factors, BMP7 was reported to be a possible candidate for the treatment of chronic kidney diseases.¹⁷ We have recently reported that leukemia inhibitory factor (LIF) and its receptor were involved in the recovery phase of ischemic acute renal failure,¹⁸ although we have not determined whether LIF is actually essential for the tissue repair after ischemic acute renal failure. LIF was found by the screening of known developmental factors in the recovery-phase kidney after ischemic injury. Using differential display, we also found a new developmental factor, metanephros-derived tubulogenic factor 1 (MTF-1).¹⁹ This factor is upregulated by protein kinase C activation, and we found that recombinant MTF-1 facilitated the development of the ureteric bud. Many growth and developmental factors have been identified in previous studies, including our studies, although no clinical application of these factors has been done so far.

As mentioned above, research on kidney regeneration is now advancing very rapidly. However, the application of these studies to clinical practice still seems far away. We definitely need more extensive research in this field.

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