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REVIEW ARTICLE

Stem cells and kidney regeneration

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Kidney disease is an escalating burden all over the world. In addition to preventing kidney injury, regenerating damaged renal tissue is as important as to retard the progression of chronic kidney disease to end stage renal disease. Although the kidney is a delicate organ and has only limited regenerative capacity compared to the other organs, an increasing understanding of renal development and renal reprogramming has kindled the prospects of regenerative options for kidney disease. Here, we will review the advances in the kidney regeneration including the manipulation of renal tubular cells, fibroblasts, endothelial cells, and macrophages in renal disease. Several types of stem cells, such as bone marrow-derived cells, adipocyte-derived mesenchymal stem cells, embryonic stem cells, and induced pluripotent stem cells are also applied for renal regeneration. Endogenous or lineage reprogrammed renal progenitor cells represent an attractive possibility for differentiation into multiple renal cell types. Angiogenesis can ameliorate hypoxia and renal fibrosis. Based on these studies and knowledge, we hope to innovate more reliable pharmacological or biotechnical methods for kidney regeneration medicine.

Introduction

Kidney disease and its related complications are an important issue of public health worldwide.[1–6] The incidence and prevalence of end stage renal disease (ESRD) in Taiwan are among the highest in the world.[5,6] In Taiwan, dialysis is a heavy financial burden that consumed about 7% of Taiwan's annual budget for national health insurance in 2011.[5] Effective strategies are urgently needed to restore the renal function by kidney regeneration as well as to prevent acute kidney injury (AKI) and chronic kidney disease (CKD) progression.[7–13]

Broadly defined, kidney regeneration includes both renal repair and regrowth of partial or whole nephron in kidney disease. Neonephrogenesis, the process to regenerate every component of nephron, is a distinctive feature of

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lower branches of the animal kingdom but does not occur in the mammalian kidney.[14] The reconstruction of the human kidney is more difficult than the regeneration of any other organ because of its complicated anatomical structure and no neonephrogenic zone of renal tissue to form new nephrons.[15] However, many studies in kidney regeneration, primarily from animal models, have identified methods of renal cell modulation pharmacologically or genetically to promote kidney regeneration.[16] Stem cell or progenitor cell therapy is also viewed as a promising strategy in regenerative medicine. Recent research has also identified that restoring the renal microvasculature might be effective in repairing the structure of diseased kidney.[17] In this review, we will describe the mechanisms of kidney regeneration and recent progress in different strategies of regenerative nephrology (Fig. 1).

Mechanisms of kidney regeneration

The nephron is the functional unit of kidney and there are almost one million nephrons in each adult kidney. The essential components of the nephron include the glomerulus, proximal tubule, loop of Henle, distal tubule, and collecting duct. The nephron is also encircled by abundant blood vessels.[18] A variety of kidney diseases result in injury of different cell types including podocytes, tubular epithelial cells, mesangial cells, or endothelial cells. Although the sublethal injury impairs renal function at various degrees, it also activates the mechanisms responsible for the regeneration of injured kidney tissues.[18]

According to current studies, there are four key processes of kidney regeneration, including reprogramming of endogenous renal cell, migration of bone marrow-derived cell (BMDC) and macrophage into kidney, renal progenitor cell differentiation, and neoangiogenesis. Growing evidence has shown that the regeneration process is similar to renal development through cell dedifferentiation. The genes vital during nephrogenesis may regulate cellular regeneration and tissue repair following injury in adult kidney.[19,20] In kidney following ischemia—reperfusion injury (IRI), the surviving tubular epithelial cells recapitulate an immature mesenchymal phenotype with re-expression of vimentin and Pax2, a process of dedifferentiation also termed as reprogramming.[21—23] The dedifferentiated cells regain the ability to proliferate and repopulate the denuded area. In addition, BMDCs migrate to the kidney after kidney injury and can inhibit renal cells apoptosis by an anti-inflammation effect and enhance renal cell proliferation.[24,25] Macrophages may scavenge the dead tissues in acute phase and promote regeneration of tubular epithelial cells during repair.[24—26] Different renal progenitor cells, either from local residence or recruited from circulation, have the potential to differentiate into target cells and promote surviving renal cell proliferation and kidney repair after injury.[16] Neoangiogenesis is stimulated through vascular growth factors and endothelial progenitor cells (EPCs), and can ameliorate oxidative stress and reduce nephron loss.[19]

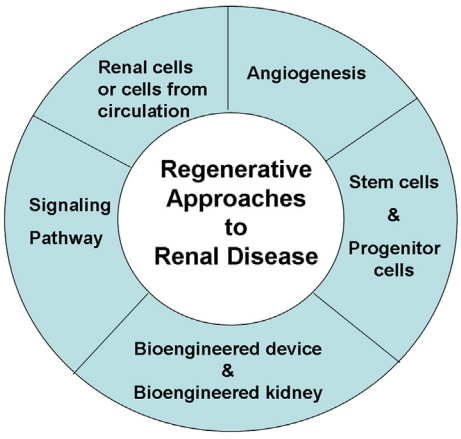

Figure 1 Regenerative approaches to renal disease.

Cells involved in kidney regeneration

Many cells are involved in kidney regeneration. First, injured proximal tubular epithelial cells can dedifferentiate and proliferate.[21] Using genetic fate-mapping techniques, Humphreys et al[18] indicated that the intrinsic, surviving tubular epithelial cells is the predominant source of new cells in repair of the postischemic nephron. Second, distal tubular cells can release growth factors such as epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), and these reparative growth factors then act on receptors in the proximal tubular epithelial cells to promote regeneration via paracrine effect.[27] Third, wound-healing or proreparative macrophages can produce a variety of growth factors including Wnt7b to promote tubular epithelial cell proliferation, angiogenesis, and kidney repair.[24—26]

Moreover, the integrity of the renal vasculature can have a profound impact on kidney regeneration following injury. Recent study identified that a novel developmental gene and protein, SCUBE1, is expressed in endothelial cells. *In vitro*, suppression of SCUBE1 can inhibit the proliferation of tubular epithelial cells.[28] Normal kidney pericytes can maintain the stability of microcirculation.[29] Although sustained activation of pericytes/perivascular fibroblasts promotes kidney fibrosis, transient activation of pericytes surrounding damaged tubules might be a normal repair process and beneficial to functional recovery after AKI.[8,30] Renal fibroblasts produce cytokines such as fibroblast growth factors-1 and -7 to stimulate proliferation of renal tubular epithelial cells, supporting the beneficial role of activated pericytes during kidney repair after AKI.[31] In addition, replacement of renal tubular epithelial cells cannot occur unless the reconstitution and stabilization of the tissue structure because surviving tubular epithelial cells need collagen framework to support their proliferation and migration to repopulate the denuded area.[31]

Reprogramming the kidney: a novel strategy for kidney regeneration

Cell reprogramming is defined as a switch in gene expression of one kind of cell to that of another cell type.[32]

Reprogramming describes not only dedifferentiation of a cell to a prior differentiative and pluripotent state, but also conversion between two unrelated differentiated cell types that does not involve a pluripotent intermediate state (termed lineage reprogramming or transdifferentiation).[33] This concept was introduced by Conrad Waddington 50 years ago and has been widely used in stem cell and renal progenitor cell therapy in kidney regeneration.[33] Recently, studies have found more factors to modulate cell reprogramming. Takahashi and Yamanaka[34] demonstrated that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only four factors, Oct3/4, Sox2, c-Myc, and Klf4. This technique has been applied to many organ regeneration as well as kidney disease. For example, recent research succeeded in generation of induced pluripotent stem cells from human kidney mesangial cells and from a renal epithelial cells shed into the urine.[35,36] Nevertheless, how to manipulate these stem cells or progenitor cell to regenerate the renal cells is still a big challenge (Fig. 2).

Stem cell-based therapy

Stem cells are multipotent and can divide and differentiate into diverse specialized cell types. They can also self-renew to produce more stem cells. The research of stem cell therapy involves induction of repair using exogenous or endogenous stem cells or the reprogramming of the organ to reinitiate development. Here, we discuss the research of stem cells including BMDCs, autologous adipose-derived mesenchymal stem cells (ADMSCs), embryonic stem (ES) cells, induced pluripotent stem (iPS) cells, and renal stem/progenitor cells for the repair of damaged renal tissue.

BMDCs

The bone marrow contains two major populations of stem cells, hematopoietic stem cells (HSCs), and mesenchymal stromal cells, which provide stromal support for HSCs. Stem cells from bone marrow have long been known for the repair of other organs.[37] Several studies have shown that BMDCs can engraft into the kidney and participate in normal tubular epithelial cell turnover and repair after AKI.[38] The evidence is based on the presence of Y-chromosome-positive renal tubular epithelial cells in kidneys of male recipients who received a renal transplant from a female donor, and accounted for $< 1\%$ of the tubular epithelial cells.[39,40] Other investigators have demonstrated elegantly that there is no evidence of differentiation of BMDCs into tubular epithelial cells of the kidney, even though postischemic functional renal impairment was reduced by intravenous injection of bone marrow mesenchymal stromal cells.[40] Although intravenous injection of HSCs could be recruited to the injured kidney and expressed markers consistent with endothelial progenitors at an extremely low level, repair of the kidney microvasculature, tubular epithelial cells, and functional recovery after IRI could be enhanced by paracrine mechanisms rather than replacement of vasculature or tubular epithelial cells.[8] Based on this evidence, we can suggest that rather than replacement of the injured epithelial and endothelial cells, the major contribution of BMDCs for renal repair is by paracrine mechanism. Taking

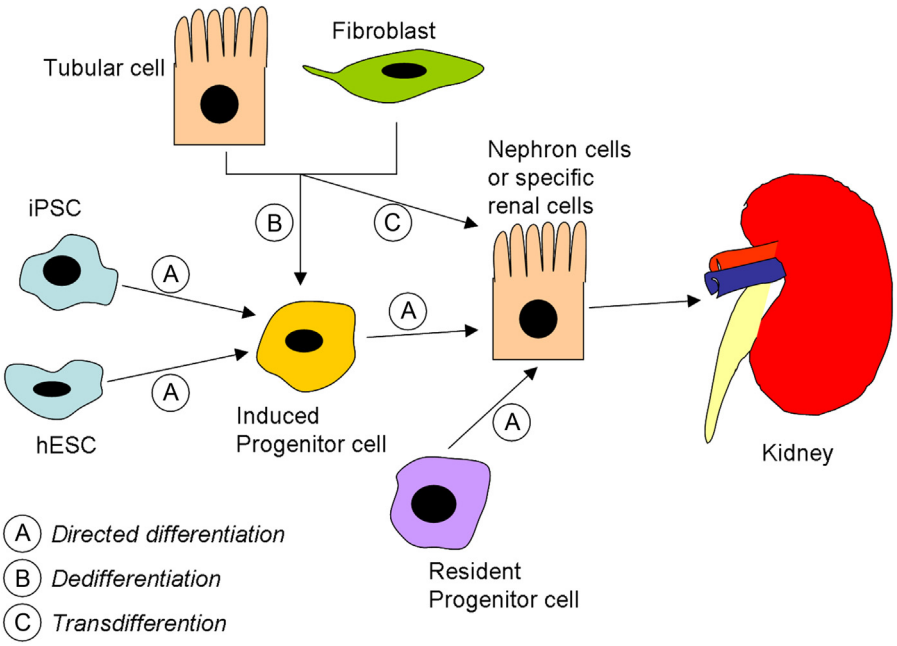

Figure 2 The application of reprogramming to the kidney. (A) Reprogramming involves the stepwise differentiation of induced pluripotent stem cells (iPS cells)/embryonic stem cells (ES cells) to a renal lineage. The iPS cells may be derived from any other adult cell type. (B) Lineage reprogramming induces dedifferentiation of differentiated cells to specific renal progenitor cells. (C) Adult kidney cells or any other available adult cell type may transdifferentiate to desired cell type without involvement of a pluripotent intermediate state.[34]

ischemic renal injury for example, BMDCs can initially ameliorate the injury either by directly inhibiting cell apoptosis and preventing inflammatory cell influx. During the repair phase, BMDCs secrete factors that promote tubular epithelial cell dedifferentiation and proliferation.

BMDCs also contribute to glomerular regeneration. A small number of donor-bone marrow-derived mesangial and endothelial cells were identified in an anti-Thy-1.1-glomerulonephritis model.[41—43] BMDCs can also differentiate into podocytes and mesangial cells for the turnover of glomeruli and ameliorate glomerular defects in Alport syndrome model.[39]

ADMSCs

ADMSCs are an attractive source of stem cells with regenerative properties that are similar to those of BMDCs. ADMSCs have the capacity to differentiate into other cell types such as adipocytes, myocytes, osteoblasts, and neurons.[44] ADMSCs have the advantages of minimal invasiveness of harvesting and plentiful supply from culturing. These cells have no ethical problem regarding the source and less concern about safety of allo- and xenografting. Most importantly, ADMSCs have more potent anti-inflammatory and immunomodulating functions than BDMCs.[44] Some studies have demonstrated that ADMSCs can reduce the severity of IRI and prevent progression of renal fibrosis after injury through suppressing oxidative stress and inflammatory response.[44,45]

ES cells

ES cells were initially derived from the inner cell mass of the blastocyst of mouse embryos.[43] These cells have the ability to differentiate into several cell types of the mesodermal, endodermal, and ectodermal lineages. Therefore, they have the potential to be used as an effective tool for kidney regenerative therapy. It has been shown that mouse Wnt4 transfected-ES cells can differentiate into tubule-like structures that express aquaporin-2 both *in vitro* and *in vivo*, and the expression of aquaporin-2 was enhanced in the presence of HGF and activin A.[46] Steenhard et al[47] injected ES cells into Day 12—13 embryonic metanephroi and then placed these in Transwell organ culture. These ES cells differentiated into renal epithelial structures that resembled tubules with an efficiency approaching 50%. In addition, Kim and Dressler[48] also demonstrated that ES cells injected into a developing metanephros can be induced to differentiate to tubular epithelia with almost 100% efficiency by using a combination of retinoic acid, activin-A, and bone morphogenetic protein-7. However, a concern about this technique is that in addition to producing renal cells, ES cells can develop teratoma 14 days and 28 days after transplantation into mouse, as demonstrated by Yamamoto et al.[49] There are also many legal and ethical issues associated with ES cell use. Overall ES cells are a valuable cellular source for investigating the mechanism of kidney regeneration, but there are still many limitations for clinical applied regeneration therapy.

iPS cells

iPS cells, able to develop into all types of cells in the body, were first discovered by Takahashi and Yamanaka[34] in 2006 who reprogrammed human fibroblasts to become pluripotent stem cells by introducing four genes. These groundbreaking discoveries have completely changed our view of the development and cellular specialization. The Nobel Prize in Physiology or Medicine 2012 was awarded to Dr Yamanaka for this outstanding achievement. The following studies also generated pluripotent stem cells from adult mouse liver and stomach cells.[50] Nevertheless, not all adult cells can be similarly reprogrammed, suggesting that critical factors for reprogramming are cell-dependent. In addition to established Oct4, Sox2, Klf4, and c-Myc factors, reprogramming of mature B cells from adult spleen to iPS cells required an additional factor, C/EBPα.[51] There are some advantages of iPS cells in kidney regeneration medicine such as no ethical issues and no immune rejection when compared to ES cells, but the risk associated with iPS cells concerns us because Klf4 and c-Myc are oncogenic factors. In fact, recent investigation showed that oncogenic risk associated with iPS cells, generated from human renal proximal tubular cells can be decreased by expression only two transcription factors: Oct4 and Sox2.[36] However, there are still many disadvantages for iPS cells-based therapy such as no established differentiation protocols for moving from pluripotent state to functional kidney cell, undefined optimal final culture conditions for target cell, and multiple steps each requiring different factors to induce a stepwise differentiation.[23] Moreover, some cells differentiated from iPS cells can express abnormal gene and induce T-cell-dependent immune response in syngeneic recipients.[52] Therefore, the immunogenicity of therapeutically valuable cells derived from patient-specific iPS cells should be evaluated prior to when they can be used in a clinical application.

Renal progenitor cells

Multipotent adult stem cells that are important for the turnover of the skin, bone marrow, stomach, intestine, and cornea have been known for a long time.[53] However, no definitive evidence to date establishes the existence of a pluripotent, self-renewing cell population in the adult kidney. During kidney development, condensed mesenchyme around the tips of the branching ureteric bud contains self-renewing cells capable of generating all other elements of the nephrons, interstitium, and vasculature via an initial mesenchyme—epithelial transition event.[54] The cells of condensed mesenchyme are regarded as the renal stem cell population. Endowment of new nephron in humans is complete by Week 36 of gestation,[55] whereas it continues for 1—2 weeks after birth in the mouse and the rat. Hartman et al[56] reported that stem cells of condensed mesenchyme ceased asymmetric division and self-renewal and then exhibited spontaneous commitment to mesenchyme—epithelial transition. These cells were exhausted prior to the perinatal stage.[56] This suggests that complete regeneration involving a complete replacement of the nephron lost does not occur in the mammalian kidney.

Nevertheless, ever more studies have discovered the stem cell-like pluripotent cells in adult kidney, which are called renal progenitor cells.[57] In contrast to the stem cells, renal progenitor cells can only differentiate to some particular cellular lineage and display no or only limited self-renewal potential.[58]

Renal progenitors are identified by cell marker CD133 and CD24. CD133 is a marker of several types of adult tissue stem cells.[59] CD24 is a surface molecule that is expressed in human metanephric mesenchyme.[60] In the adult mammalian nephron, renal progenitor cells gather at the urinary pole and disperse over the Bowman capsule, the proximal tubule, the thick ascending lim, b and the distal convoluted tubule at the point of connection with the ureter.[57,61] Renal progenitor cells at the urinary pole can differentiate into glomerular as well as tubular epithelial cells. Tubular progenitor cells represent 2—6% of all tubular epithelial cells in healthy adult kidneys and express CD133 and CD24, as well vimentin, cytokeratin 7 and 19, Pax2, and nestin that are not expressed by differentiated tubular epithelial cells.[51] Glomerular progenitor cells localize within the Bowman capsule and can differentiate toward the podocyte phenotype. Tubular progenitor cells cannot express CD106, whereas glomerular progenitor cells can express this surface marker.[51] The elegant study by Lee et al[57] isolated mouse kidney progenitor cells from the interstitium of medulla and papilla which can differentiate to endothelial cell and tubular epithelial cell. Treatment with mouse kidney progenitor cells can reduce the mortality in mice after ischemic injury. Regarding glomerular injury, Ronconi et al[62] reported that CD133+ CD24+ cells can replace podocytes and improve chronic glomerular damage in adriamycin-induced nephropathy.

Renal progenitor cells have higher resistance to injury in comparison to all other differentiated cells of the kidney. When injected in severe combined immunodeficient mice affected by rhabdomyolysis-induced AKI, both of these populations displayed the capacity to integrate into the tubules, generate novel tubular epithelial cells, and improve renal function.[63] The perivascular site has also been a reserve of progenitor cells. These cells express markers of both pericytes and mesenchymal stem cells that are able to proliferate in response to focal injury and promote tissue repair.[64] It is an attractive strategy that if renal progenitor cells can also be induced via lineage-instructive reprogramming or differentiated from extrarenal stem cells such as mesenchymal stem cells, ES cells, and iPS cells. However, achieving this exact state of differentiation *in vitro* has not been proven successfully.[65] These exogenous renal progenitor cells may enhance renal repair in addition to a small population of endogenous renal progenitor cells.

Because stem cells cannot be manipulated easily to differentiate to desired renal cells, less potency but reliable differentiation process makes renal progenitor cells more applicable in kidney regeneration medicine. Compared to stem cells, renal progenitor cells have advantages, such as that knowledge of intermediate cell culture conditions is not required and renal progenitor cells can transit directly from one phenotype into another. However, a reliable method of inducing extra renal stem cells to differentiate into renal progenitor cells has not been established at this time.[23]

Signaling pathways in mediating the regenerative process

Convergent evidence has shown that many cells are involved in kidney regeneration, including endogenous renal tubular epithelial cells, macrophages, fibroblasts, BMDCs, and renal progenitor cells. How these cells interact with each other and what factors influence the cells to involve in the regenerative process after renal injury remains uncertain. We also need more additional research to identify the factors in specific signaling pathways involved in kidney regeneration and try to find novel therapeutic interventions for this serious disease.

PI3K/AKT/mTOR pathway

It has long been observed that growth factors such as EGF, HGF, and IGF-1 can accelerate recovery of renal function after AKI.[66] These growth factors activate a lipid kinase (phosphotidyl-inositol-3-kinase, PI3K) that phosphorylates phosphotidylinositol-4,5-bisphosphate to yield phosphotidylinositol-3,4,5-trisphosphate. The latter phosphorylates and activates Akt. Once activated, Akt stimulates mammalian target of rapamycin (mTOR) by regulating the activity of intermediary kinases. The activation of mTOR leads to phosphorylation of downstream substrates and then induced cell regeneration. Lieberthal et al[67] demonstrated that inhibition of mTOR by rapamycin substantially delays recovery of renal function. Akt may inactivate some proapoptotic factors such as Bcl-2-associated death promoter, procaspase-9 and forkhead family transcription factors. Akt also activates antiapoptotic genes.[68] Deletion of the EGF receptor in renal proximal tubular epithelial cells impairs phosphotidyl-inositol-3-kinase/Akt signaling and delay recovery from AKI.[69]

MAPK/ERK pathway

Mitogen activated protein kinases (MAPKs) is a family of kinases that have been commonly studied on the kidney disease. There are four different MAPK pathways in mammalian cells: extracellular signal-regulated kinase-1 and -2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38MAPK, and extracellular signal-regulated kinase-5 (ERK5).[70,71] ERK is mainly activated by mitogenic stimuli such as growth factors and ERK1/2 pathway has been widely investigated in kidney regeneration. *In vitro*, ERK pathway activation could enhance renal epithelial cell survival during oxidative injury.[72] Activation of the signal transducer and activator of transcription-3 (STAT3) during oxidative stress can attenuate EGF receptor-mediated ERK activation and renal tubular cell survival.[73] *In vivo*, inhibition of ERK pathway reduces kidney regeneration in rats with myoglobinuric AKI.[74]

JAK/STAT pathway

When a growth factor such as EGF binds to the EGF receptor, Janus-activated kinase (JAK) is activated and phosphorylates the intracellular domain of the receptor

and allows recruitment and phosphorylation of a STAT. Through the activation of JAK/STAT pathway, erythropoiesis-stimulating proteins suppress renal tubular cell apoptosis *in vitro* and enhance renal recovery in cisplatin-induced AKI.[75] Contrary to the positive role in the aforementioned study, inhibition of the JAK/STAT pathway can decrease tubular epithelial cell apoptosis and kidney inflammation in murine AKI.[76] Therefore more studies are required to clarify the effect of JAK/STAT pathway on renal repair.

Wnt-GSK3-β-catenin pathway

The Wnts are a family of secreted and glycosylated protein ligands. Wnt signals can inhibit glycogen synthase kinase 3 (GSK3) by phosphorylation. When GSK3 is inhibited, β-catenin is stabilized and translocates into the nucleus to act as a transcriptional coactivator of the T-cell factor/lymphoid enhancer-binding factor family of transcription factors, and drive the expression of its target genes.[77] This pathway is involved in the regulation of cell fate, protein synthesis, glycogen metabolism, cell mobility, proliferation, and survival.[78] Wnt pathway responses are induced in the kidney following acute injury.[24] Genetic inactivation of Wnt signaling has been shown to impair kidney regeneration and renal function recovery. Among the increased Wnt ligands in the kidney after injury, macrophage-derived Wnt7b has been shown to promote tubular epithelial cell regeneration and kidney repair.[24] In the downstream of Wnt signaling, GSK3 is normally inhibited by Wnt ligands, as well as by many other proliferative, prosurvival signals that increase serine9 phosphorylation, such as IGF, EGF, and fibroblast growth factors 16,19, and 23.[79] In AKI, GSK3 can promote the systemic inflammatory response and participates in a number of apoptotic signaling pathways by phosphorylating transcription factors that regulate apoptosis.[80] TDZD-8, a GSK3β inhibitor, can inactivate ischemia-induced activation of GSK3, Bax, and caspase 3; ameliorate tubular epithelial cell apoptosis; and significantly protect renal function.[81] Expression of β-catenin can be induced in AKI and renal tubule-specific knockout of endogenous β-catenin aggravates AKI in mice.[82] Another study reported that inhibition of GSK3β can ameliorate nonsteroidal anti-inflammatory drug-induced AKI.[83] Taken together, activation of the Wnt-GSK3-β-catenin pathway is beneficial for many kidney disease and GSK3 inhibitor can be a target of therapeutic agents in the future.

Angiogenesis and kidney regeneration

Vasculature integrity is essential for kidney regeneration and attenuation of renal dysfunction, especially in CKD. No matter what etiologies induce CKD, the common pathway is progressive loss of the renal microvasculature, which leads to tissue hypoxia and inflammation, further fibrotic change, and nephron loss. Tissue fibrosis resulted in further rarefaction and this vicious cycle cause irretrievable renal function deterioration.[8,84,85] In a normal kidney, an equilibrium exists between proangiogenic and antiangiogenic molecules, but an imbalance of angiogenesis-related factors is noted in the progression of CKD.[29,85–87] Vascular endothelial growth factor, a proangiogenetic factor, can reduce fibrosis and stabilize renal function in the remnant kidney model of progressive renal failure.[88] However, dysangiogenic isoform of vascular endothelial growth factor became dominant in fibrotic kidneys induced by unilateral ureteral obstruction or IRI might play a role in microvascular rarefaction.[85] Angiopoietin-1 gene therapy using adenoviral vector resulted in the reduction in albuminuria, suppression of mesangial expansion and podocyte injuries accompanied by reduced macrophage infiltration and attenuation of chemokines and adhesion molecules in diabetic nephropathy model.[89] By contrast, elevated plasma levels of angiopoietin-2 are associated with cardiovascular disease in CKD patients and might also play a role in CKD progression.[90,91] How these angiogenic growth factors are involved in the kidney regeneration needs further study. The EPCs have been shown to participate in reconstructing the microvessels of the interstitium and glomeruli.[92] In mice with adriamycin-induced nephropathy, systemically injected EPCs homed to areas of injury and inflammation in the kidney, a maneuver that improved renal function, reduced proteinuria, improved vascular density, and reduced apoptosis.[93] The restoration and extension of microvasculature is a key potential target for therapies to heal the diseased kidney.

Bioengineered kidney

Because there are numerous ESRD patients under dialysis and a shortage of kidney donors, recent studies have investigated the possibility of bioengineered kidney. Previous study designed hemofiltration devices equipped with bioengineered renal tubules that can replace renal function in uremic dogs and temporarily improved renal function in patients with acute renal failure.[94,95] Further research attempts to invent a bioengineered kidney that have the kidney's architecture and function and permit perfusion, filtration, secretion, absorption, and drainage of urine.[96] They decellularized rat cadaveric kidney by detergent perfusion and yielded acellular scaffolds with vascular, cortical, and medullary architecture, a collecting system, and ureters. Then they seeded rat kidney scaffolds with epithelial and endothelial cells. After several days in organ culture, regenerated kidney constructs produced urine *in vitro*. When transplanted in an orthotopic position in rat, the grafts were perfused by the recipient's circulation and produced urine *in vivo*. Although regenerated kidney can only replace partial renal function, this technique is still a landmark of kidney regeneration medicine.

Future challenges and perspectives

Although there are many exciting approaches for kidney regeneration medicine, many hurdles remain. In the future, we should characterize gene expression profiles of regeneration associated cells and elucidate corresponding signaling molecules more clearly. Based on the knowledge of renal development, a more reliable method is needed for the manipulation of stem cell and progenitor cell therapy. Enhanced understanding of the mechanism of current available drugs with the capacity of renoprotection may

also help us to find the novel pathway for kidney regeneration and more specific pharmacologic or gene therapy should be discovered for different renal disease. Of note, bioengineered kidney is an important research field because it offers resolution for the ESRD patient awaiting a donor kidney.

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