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Novel regenerative therapy for acute kidney injury

Takafumi Toyohara[1] and Kenji Osafune[2*]

Abstract

Acute kidney injury (AKI) is a renal disease that is associated with high mortality. Current treatments mostly rely on supportive therapies and do not directly target the disease. Regenerative medicine, however, offers potentially direct AKI therapy through two strategies: cell transplantation and kidney reconstruction. Regarding cell transplantation, several cell types are potential sources, including hematopoietic stem cells, mesenchymal stem cells, and renal stem/progenitor cells within the adult kidney tissue or derived from human-induced pluripotent stem cells (iPSCs). On the other hand, kidney reconstruction could provide a curative treatment for severe AKI and consequent chronic kidney disease (CKD). Many methods have been proposed for the kidney reconstruction, including self-organization, blastocyst complementation, decellularization, and bioartificial kidneys. However, there are still a number of obstacles to overcome before reconstructed kidneys reach clinical use. In this review, we summarize the recent progresses in cell transplantation and kidney reconstruction as strategies to treating AKI.

Background

Acute kidney injury (AKI) is a frequent renal disease in hospitalized patients, especially those in intensive care unit, and is associated with high mortality [1, 2]. Although mild AKI normally recovers, in many cases, severe ischemia leads to chronic inflammation and fibrosis [3]. Indeed, AKI is a cause of chronic kidney disease (CKD) and also a risk factor for cardiovascular diseases [4]. Although effective treatments for AKI are needed, only supportive therapies are available [5].

Recently, Yamanaka et al. and Thomson et al. simultaneously reported the generation of human induced pluripotent stem cells (iPSCs) [6, 7]. These cells can self-renew and have the potential to differentiate into any cell type of the adult body, including renal cells. iPSCs also eliminate the concern of immune rejection when transplanted into a patient. These reasons have made them the basis for cell therapies against various diseases [8]. Although regenerative medicine for kidney diseases still remains unavailable because of the complexity of the organ structure and the incomprehensibility of the pathophysiology, iPSC technology has allowed for substantial progress in this field.

* Correspondence: osafu@cira.kyoto-u.ac.jp
[2]Center for iPS Cell Research and Application (CiRA), Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
Full list of author information is available at the end of the article

Several types of stem and progenitor cells have been examined as cell sources for regenerative medicine against kidney diseases. In general, progenitor cells differentiate from stem cells. These progenitor cells are still immature and can differentiate into multiple cell lineages but differ from stem cells in that they lack pluripotency. It is sometimes difficult to distinguish progenitor cells from stem cells, and thus, the two cell types are often equated. In this review, therefore, we describe the cells that can specifically differentiate into kidney lineage cells as "renal stem/progenitor cells" or "renal progenitors."

When we consider the clinical application of regenerative medicine for AKI, we can classify the strategies mainly into two groups: (1) cell transplantation and (2) kidney reconstruction. Regarding cell transplantation, transplanted renal stem/progenitor cells contribute to the host kidney tissues or secrete paracrine factors that help recovery from the kidney injury. Several cell types have been reported as the source for cell therapy, including hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and renal stem/progenitor cells within the adult kidney tissue or those derived from iPSCs. On the other hand, kidney reconstruction might be a curative treatment for severe AKI and consequent CKD by compensating for the function of the diseased kidney. Although there are a number of obstacles to overcome before functional reconstructed kidney tissues are established, innovative strategies for

their generation, such as self-organization, blastocyst complementation, decellularized kidney, and bioartificial kidney, are being developed. In this review, we summarize the recent progress in these regenerative medicines for treatment against AKI.

Cell transplantation

Acute tubular necrosis (ATN) is mainly observed in AKI as a consequence of acute injury induced by multiple causes, such as ischemia or nephrotoxic agents. After ATN, renal tubule cells show a tendency to spontaneously regenerate, but complete repair is not achieved in severe cases. Further, if ATN is observed in a large area of the kidney, interstitial renal fibrosis occurs [9]. It remains controversial whether stem cell populations exist within the adult kidney and whether they contribute to the regeneration of renal tubule cells after ATN. Recent reports have described dedifferentiated cells from renal tubule epithelia as mainly responsible for the repair of the renal tubule in AKI [9, 10].

Cell transplantation could help during the repair phase after ATN. For example, transplanted stem/progenitor cells could provide additional cell materials for the kidney regeneration and/or secrete paracrine factors that help the renal tubule regenerate. A paracrine effect is more likely if the epithelial dedifferentiation of renal tubule cells is the main mechanism for recovery from AKI. However, the replacement of injured renal tubule cells is also a likely and intriguing mechanism. Several types of stem/progenitor cells have been reported as candidate cell sources (Table 1). Below, we outline how each can be used for treatment against AKI.

HSCs

HSCs are somatic stem cells in the bone marrow that can self-renew and differentiate specifically into blood cells, such as erythrocytes, thrombocytes, and leukocytes. Several cell surface markers have been identified to isolate HSCs in mouse and human [11, 12]. HSCs have been reported to have a therapeutic potential against AKI. For example, mouse $Lin^{-}Sca\text{-}1^{+}$ HSCs are able to differentiate into renal epithelial tubule cells [13, 14], and human $CD34^{+}$ HSCs were shown to promote the proliferation of both endothelial and epithelial cells after ischemia/reperfusion (I/R) injury [15]. However, there remains dispute about whether HSCs truly contribute to recovery from ATN in adult animals, as cell fusion obscures whether HSCs can become renal stem/progenitor cells [16]. There is also evidence indicating HSCs can exasperate AKI, as it was reported that an increase in the number of circulating HSCs induced by pharmacological mobilization from the bone marrow was associated with increased severity and mortality [17]. Furthermore, the mechanisms by which HSCs protect renal tubules against AKI remain to be clarified. It might be that HSCs repair the kidney injury by producing protective and regenerative factors rather than by producing renal stem/progenitor cells.

MSCs

MSCs are a heterogeneous stem cell population derived from the bone marrow, adipose tissue, and other organs but are the easiest to acquire from adipose tissue [18]. MSCs show a fibroblast-like cell shape and can differentiate into cells constituting mesenchymal tissues, which include cartilage, bone, muscle and fat. MSCs were also thought to have differentiation potential into kidney lineage cells and thus could replace destroyed renal cells [19, 20]. At the same time, it has been reported that MSCs only remain temporarily in injured kidneys and show renoprotective function by secreting paracrine factors that act as anti-apoptotic, mitogenic, anti-inflammatory, and immune-modulating molecules [21, 22]. MSCs secrete multiple renotrophic factors, including hepatocyte growth factor (HGF), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), and angiopoietin 1 (ANG-1) [22]. In addition, extracellular vesicles/microvesicles, which contribute to cell-to-cell interactions, are also candidate therapeutic factors secreted by MSCs [23]. MSCs have shown therapeutic effects in various types of animal AKI models, such as those induced by

Table 1 Characteristics of candidate renal stem/progenitor cells for cell therapy against AKI

	Source	Accessibility	Supply	Therapeutic effects	Tumorigenicity	Ethical issue	Immune matching	Clinical application/ clinical trial	Obstacles to overcome
Mesenchymal stem cells	Bone marrow, adipose tissue, other tissues	Moderate	Limited	Paracrine effects	Possible	None	Yes	Phase II trial	Variable therapeutic effects
Adult renal stem/ progenitor cells	Various locations in adult kidney	Difficult	Limited	Replacement of renal tissues or cells/paracrine effects	Unknown	None	It is up to their stable supplies	None	Unknown origin/ unpredictable and limited supply
Renal progenitors derived from iPSCs	Skin, blood, other cell type	Easy	Infinite	Replacement of renal tissues or cells/paracrine effects	Possible	None	Yes	None	Unestablished differentiation protocol

cisplatin or gentamicin administration, by the intramuscular injection of glycerol and by I/R injury [24–27]. Recently, adipose tissue-derived MSCs were shown to have therapeutic benefits against AKI [28–31], which could secure supply of MSCs and promote the clinical applications. Several studies have shown the multiple therapeutic effects of MSCs in vivo, and phase 1 and phase 2 clinical trials using MSCs have been done for AKI following cardiac surgery (#NCT00733876 and #NCT01602328) [32]. However, further optimization of the therapeutic protocols, such as the route of delivery, the volume of the MSC infusion, and the timing of the administration, is needed [33]. In addition, despite its promise, it has not yet been reported that MSCs have protective effects against AKI or other kidney diseases in human. Additionally, MSCs derived from different adult and neonatal tissues exhibit heterogeneity and variation in their surface marker expression and function [18, 34], making it difficult to establish clonal MSC populations that have stable therapeutic effects against AKI.

Adult renal stem/progenitor cells

While recent reports have demonstrated that renal stem/progenitor populations derived from dedifferentiated renal tubule cells in the adult kidney are mainly responsible for the regeneration of renal tubules [9], other stem/progenitor cell types have been isolated from various locations in the adult kidney [35–39]. Stem cell populations proliferate very slowly and show specific markers. Taking advantage of these features, scientists have isolated renal stem/progenitor cells using several methods, such as label-retaining assays [35, 36], clonogenic cell assays [37], side population (SP) assays [39], and flow cytometry [38].

Label-retaining cells (LRCs) are identified by the injection of the thymidine analog 5-bromo-2-deoxyuridine (BrdU) into mice. BrdU is retained in slow cycling tissue-specific stem cells. Maeshima et al. used this strategy to find LRCs in renal epithelial tubular cells, which contribute to tubular regeneration after I/R injury, in the rat kidney [36].

Kitamura et al. used clonogenic cell assays to establish clonal cells derived from the S3 segment of rat proximal renal tubules. These cells showed potent proliferative capacity and expressed Sca-1, c-kit, and Pax2, markers of stem cells, and embryonic renal progenitors [37]. Transplantation of the clonal cells significantly improved the renal function in both cisplatin-induced and I/R AKI models [40]. Although these cells could differentiate into tubular epithelial cells, it was suggested that their therapeutic effects mainly come from paracrine factors they produced.

SP assays have been used to purify cells that can efflux the DNA-binding dye Hoechst 33342 and exhibit stem cell-like characteristics. These cells are accordingly referred to as SP cells. Hishikawa et al. clarified the gene expression profile of kidney SP cells in both renal failure models and healthy controls and showed that these cells express musculin/MyoR [39]. Musculin/MyoR might play crucial roles in the regenerative processes of the adult kidney. The injection of kidney SP cells improved renal function in drug-induced nephropathy models, even though the SP cells located in the interstitial spaces of the kidney and seemed to exert their therapeutic effects by producing paracrine growth factors [39].

Regarding stem cells isolated by cell surface markers, CD133(+)CD24(+) cells have been reported as renal stem/progenitor cells that localize at the urinary pole of the Bowman's capsule in adult human, mouse, and rat kidneys. When the cells were intravenously injected into severe combined immunodeficiency (SCID) mice with AKI induced by rhabdomyolysis, they integrated into the host renal tissues, differentiated into glomerular podocytes and renal tubular epithelium, and replaced the injured renal cells [38].

Among the renal stem/progenitor populations described above, only LRCs and CD133(+)CD24(+) cells produce their therapeutic benefits against AKI by integrating into the adult renal tubules as the main mechanism. The other stem/progenitor cell populations can improve AKI but do so mainly by secreting paracrine factors. LRCs and CD133(+)CD24(+) cells too likely secrete paracrine factors. In general, however, the considered stem/progenitor cells have not been fully characterized in terms of their origins or the molecular nature of their paracrine factors. Further, it is difficult to obtain a sufficient number of these cells from adult kidneys for treatment. In addition, there is a risk of immunorejection. Further studies are needed before these cells have clinical use.

Renal stem/progenitor cells differentiated from pluripotent stem cells

Recently, two reports including one from our group have shown the therapeutic effects of renal progenitor cells differentiated from human iPSCs against AKI [41, 42]. Because of the potential to infinitely proliferate and bypass immunorejection issues, iPSCs are considered a more stable and suitable cell source for renal stem/progenitor cells. We thus aimed to establish a method by which iPSCs can be differentiated into embryonic renal progenitor cells that have the capacity to further differentiate into nephron-forming glomerular and renal tubular cells.

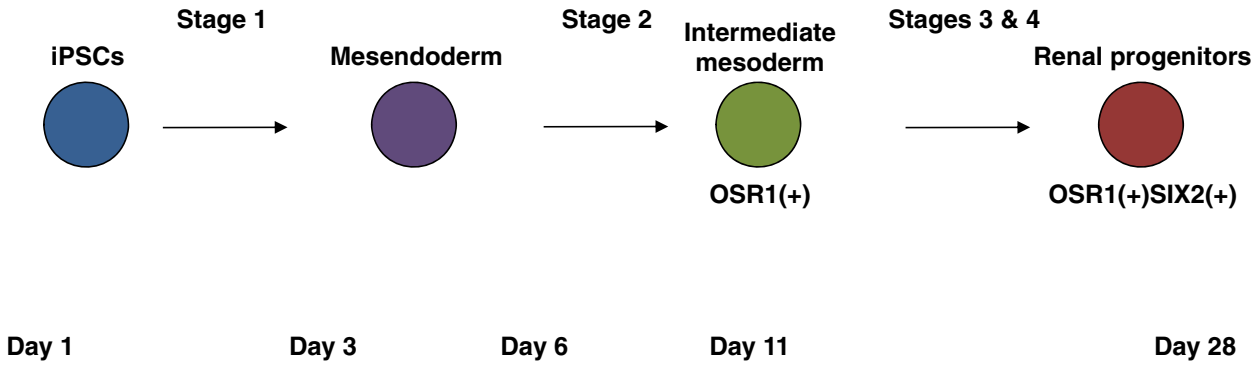
In the developmental process, renal progenitors exist in metanephric mesenchyme (MM), which is derived from intermediate mesoderm (IM) [43]. Lineage-tracing analyses showed that *Odd-skipped related 1* (*Osr1*) is one of the earliest markers of IM [44]. We generated OSR1-GFP knockin human iPSC lines and established efficient methods to induce OSR1$^+$ IM cells using a combination treatment with growth factors and small molecules [45, 46]. *Osr1* is expressed in renal progenitors

in IM and MM, although it is also expressed in the lateral plate mesoderm of early-stage mouse, chick, and fish embryos [44, 47, 48]. On the other hand, renal progenitors in MM express the homeodomain transcriptional regulator *Six2* in addition to *Osr1*, although *Six2* is also expressed in other fetal organs, such as the skeletal muscle, limbs, heart, eyes, and middle ears [49, 50]. Additionally, *Osr1* and *Six2* interact synergistically to maintain nephron progenitor cells in MM [51]. We thus defined $OSR1^+SIX2^+$ cells as renal progenitors.

In order to establish a multistep differentiation protocol from human iPSCs into $OSR1^+SIX2^+$ renal progenitors, we derived OSR1-GFP/SIX2-tdTomato double knockin human iPSC lines [41]. We differentiated the human iPSC line into $OSR1^+$ IM cells using our robust differentiation protocol and then screened for growth factors and compounds that can further differentiate $OSR1^+$ IM cells into $OSR1^+SIX2^+$ renal progenitors. As a result, we found that the combination of transforming growth factor (TGF)β1 isoforms and bone morphogenetic protein (BMP) signal inhibitors, such as DMH1, induced $OSR1^+SIX2^+$ renal progenitors (Fig. 1). We confirmed that the induced $OSR1^+SIX2^+$ cells expressed other renal progenitor markers and that these cells reconstituted three-dimensional (3D) renal structures in vitro and in vivo, although they could mainly differentiate into renal tubule cells.

Assuming that $OSR1^+SIX2^+$ renal progenitors could replace and regenerate renal tubule cells in AKI, we injected them into the kidney parenchyma of mouse AKI models induced by I/R injury. However, only a small number of $OSR1^+SIX2^+$ renal progenitors were integrated into the host renal tubules, and no therapeutic benefit was observed. Then, we transplanted $OSR1^+SIX2^+$ renal progenitors into the renal subcapsular space, expecting that paracrine factors secreted from the renal progenitors might exert therapeutic effects. Indeed, AKI in mice induced by I/R injury was ameliorated, as indicated by the significant suppression of elevated blood urea nitrogen (BUN) and serum creatinine (Cre) levels (Fig. 2a) and attenuation of histopathological damages, such as urinary cast formation and tubular necrosis. Notably, the treatment improved interstitial fibrosis at day 12 after I/R, which suggests the possibility of preventing the progression to chronic disease (Fig. 2b). We then examined the molecular identity of the paracrine factors secreted from the $OSR1^+SIX2^+$ renal progenitors by microarray and multiplex protein detection analyses, identifying them, such as ANG-1, VEGF, and HGF. Our results demonstrate that the transplantation of renal progenitors derived from human iPSCs has therapeutic benefits against AKI, mainly by producing trophic effects [41].

Imberti et al. have also reported that the transplantation of renal progenitors differentiated from human iPSCs improves AKI [42]. The group differentiated renal progenitors using numerous growth factors and compounds, including activin A, fibroblast growth factor (FGF)2, BMP7, glial cell line-derived neurotrophic factor (GDNF), retinoic acid, phosphoinositide 3-kinase (PI3K) inhibitor, and ras homologue gene family member A (RhoA) inhibitor. Cisplatin-induced kidney injury was used as an AKI mouse



Stage 1	Stage 2	Stage 3	Stage 4
Suspension culture	Adhesion culture		
DMEM/F12 Glutamax			
2% FBS, 0.5% P/SM	10% KSR, 50 μM 2ME, 1% NEAA, 0.5% P/SM		
activin A (100 ng/ml)	BMP7 (100 ng/ml)	TGFβ1 (5 ng/ml)	TGFβ1 (5 ng/ml)
CHIR99021 (1 μM)	CHIR99021 (1 μM)	TTNPB (1 μM)	DMH1 (0.5 μM)

Fig. 1 Differentiation protocol for inducing human iPSCs into $OSR1^+SIX2^+$ renal progenitors. The differentiation method used to generate $OSR1^+SIX2^+$ renal progenitors with embryoid body (EB)-based 3D cultures. *CHIR99021* an inhibitor of glycogen synthase kinase 3 (GSK3), *TTNPB* an analog of retinoic acid, *DMH1* a selective BMP antagonist, *FBS* fetal bovine serum, *P/SM* penicillin streptomycin, *KSR* knockout serum replacement, *2ME* 2-mercaptoethanol, *NEAA* non-essential amino acid, *BMP* bone morphogenetic protein, *TGF* transforming growth factor. Adapted from Toyohara et al. [41]

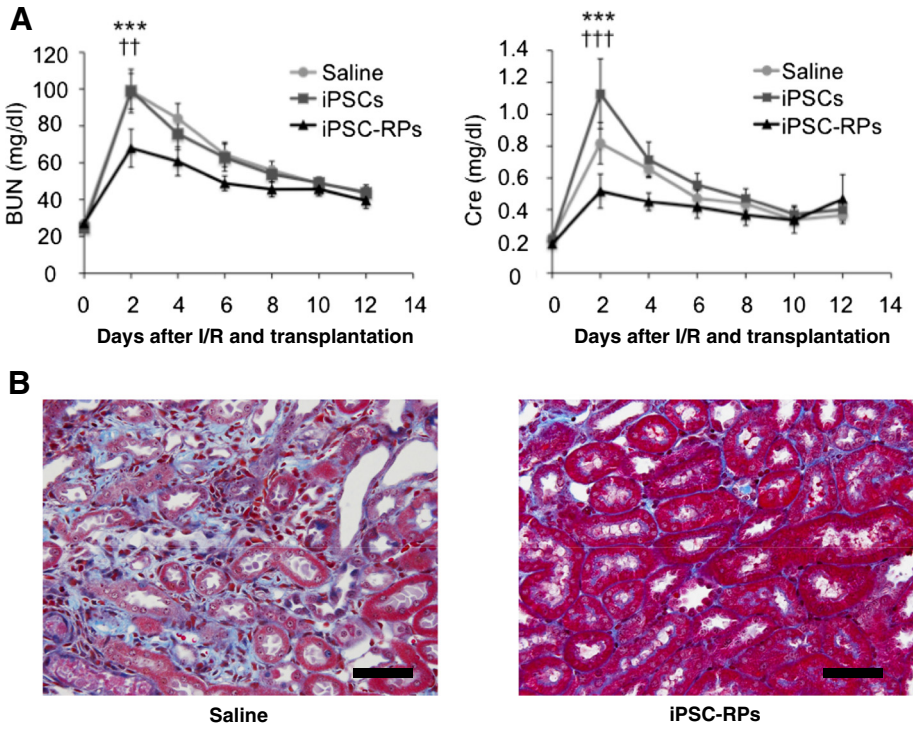


Fig. 2 Cell therapy using human iPSC-derived renal progenitors ameliorates acute kidney injury in mice. **a** Time course of blood urea nitrogen (BUN) and plasma creatinine (Cre) levels in acute kidney injury (AKI) mouse models induced by ischemia/reperfusion (I/R) injury that received the renal subcapsular transplantation of human iPSC-derived renal progenitors (iPSC-RPs, *triangle*), undifferentiated iPSCs (iPSCs, *square*), or saline (*circle*). Statistically significant: $^{***}p < 0.001$ versus saline, $^{\dagger\dagger}p < 0.01$ versus iPSCs, $^{\dagger\dagger\dagger}p < 0.001$ versus iPSCs. **b** Section images of representative kidney tissue samples from host mice that received an injection of saline (*left panel*) or the transplantation of iPSC-RPs (*right panel*). Masson's trichrome staining images on day 12 after I/R injury and transplantation. *Scale bars*, 20 μm. Adapted from Toyohara et al. [41]

model. While the differentiation protocol and the type of AKI mouse model were different from our report, the transplantation of renal progenitors in their study also improved AKI. Other large differences between the two studies include the transplantation method and the integration of the renal progenitors into the host mouse kidney. The group injected the renal progenitors through the mouse tail vein and confirmed that most cells remained in the host kidney and integrated into the host renal tubules. Although there is still the possibility that paracrine factors secreted from the progenitors may be mainly responsible for the observed therapeutic effects, it was suggested that the amelioration of AKI was the result of the integration of the renal progenitors into host renal tubules.

These two studies show the potential of regenerative therapy for kidney diseases using human iPSC-derived renal progenitors. However, optimization of the directed differentiation and transplantation of progenitor cells is needed. Further characterization of human iPSC-derived renal progenitors is also required.

Kidney reconstruction

Although the reconstruction of the whole kidney might solve the problem of donor organ shortage in kidney transplantation and become a radical treatment for kidney disease, there remain a number of technical obstacles to overcome. Some may be solved using innovative strategies, such as self-organization, blastocyst complementation, decellularized kidney, and bioartificial kidney.

Self-organization

Self-organization is the process where stem/progenitor cells spontaneously reconstitute mini-organs in a 3D culture. This ability makes self-organization a potentially powerful tool for generating 3D models of human organ development. Recently, several groups have differentiated human iPSCs or human embryonic stem cells (ESCs) into renal progenitor cells and reconstituted 3D kidney-like structures using self-organization.

Our group has reported methods for inducing renal progenitor cells from human iPSCs/ESCs using stepwise differentiation and chemical biology strategies [41, 45, 46]. By screening growth factors and chemical compounds that can induce renal progenitor cells from human iPSCs, we could induce $OSR1^+$ IM cells and $OSR1^+SIX2^+$ renal progenitors. We also reconstituted 3D nephron-like structures in vitro that mainly contain proximal renal tubules [41, 45, 46].

Xia et al. reported a protocol by which human iPSCs/ESCs were differentiated into the progenitors like ureteric bud (UB), an embryonic cell population that gives rise to the collecting system and the lower urinary tract from the renal pelvis to a part of the urinary bladder [52]. Consistently, UB lineage markers, such as HOXB7, RET, and GFRA1, were upregulated in the induced cells. These UB-like progenitors could also assemble the chimeric 3D kidney-like structure with mouse metanephric cells. Two other studies performed by Lam et al. and Kang et al. have also developed original differentiation protocols to generate nephron progenitor cells using 2D adherent cultures and generated 3D nephron-like structures containing renal tubules [53, 54]. However, the recapitulation of 3D kidney structures containing glomeruli, renal tubules, and collecting ducts in vitro has remained challenging.

Significant advances in the generation of 3D kidney-like structures using renal progenitor cells derived from human iPSCs/ESCs have also been made [55–58]. These studies established stepwise protocols that mimic kidney development. Taguchi et al. demonstrated by the lineage analysis of mouse embryonic kidney that nephron progenitors in MM are derived from the cell population in posterior nascent mesoderm at embryonic day (E) 8.5 where Brachyury, a marker of primitive streak, is expressed while OSR1 is not [55]. They also found that UB is derived from the cell population in anterior IM, which expresses OSR1 but not Brachyury at E8.5. Accordingly, they established stepwise protocols to differentiate both mouse ESCs and human iPSCs into nephron progenitors. These progenitors could reconstitute 3D nephron-like structures containing glomeruli and renal tubules by coculture with the mouse embryonic spinal cord [55]. Takasato et al. induced primitive streak from human ESCs. They then used FGF9 as a factor to induce IM from the primitive streak and generated a self-organizing structure that included both MM and UB lineage cells [56]. The same group modified their differentiation protocol to increase the proportion of MM and succeeded in generating 3D kidney-like structures from human iPSCs that contained glomeruli, renal tubules, and collecting ducts surrounded by renal interstitium and vascular endothelial cells [57]. Furthermore, endocytosis of dextran and apoptosis in response to cisplatin were observed in the proximal renal tubules within the kidney-like structures, indicating that the 3D kidney-like structures could be used for drug nephrotoxicity tests. This simple differentiation protocol can generate the most complete kidney-like structures to date. Morizane et al. induced $SIX2^+SALL1^+WT1^+PAX2^+$ nephron progenitors from both human ESCs and iPSCs at 90 % induction efficiency and used the self-organization of these progenitors to reconstruct 3D nephron-like structures that expressed markers for podocytes, proximal renal tubules, loops of Henle, and distal renal tubules [58]. The kidney-like structures also showed the upregulated expression of kidney injury molecule (KIM)-1, a marker of AKI, in response to treatment with gentamicin or cisplatin. Although these 3D kidney-like structures are still immature and resemble human fetal kidney [57], they have tremendous potential for the study and treatment of AKI.

Blastocyst complementation

In blastocyst complementation, ESCs/iPSCs are injected into the blastocysts, which are embryonic structures formed after fertilization. Injected ESCs/iPSCs synchronize with the development of the host embryo to generate a chimeric animal. When ESCs/iPSCs are injected into the blastocysts of animals in which the essential gene for the development of certain organs is knocked out, they can complement the organ formation. In the first report of blastocyst complementation, Chen et al. injected wild-type mouse ESCs into the blastocysts of knockout mice for recombination-activation gene 2 (Rag2), an indispensable enzyme for the rearrangement of immunoglobulin and T cell receptor genes [59]. In these chimeric mice, T and B cells were derived from the injected ESCs. Kobayashi et al. reported that whole pancreas was formed in $Pdx1^{-/-}$ pancreatogenesis-disabled mice by blastocyst complementation injecting wild-type mouse ESCs and iPSCs [60]. In the same study, they also showed that rat iPSCs could generate rat pancreas in $Pdx1^{-/-}$ mouse by blastocyst complementation. These results indicate human iPSC-derived pancreas tissues could be generated in other animals, such as pig, by blastocyst complementation, although the stromal components, which include the vessels and nerves in the generated pancreas, are derived from the host animal. Blastocyst complementation has also been applied to kidney reconstruction as well [61]. Renal tissues derived from MM were generated by blastocyst complementation using knockout mice of the Sall1 gene, which is essential for the development of MM. However, renal tissues derived from UB and the vascular and nervous systems originated from the hosts, not from the injected mouse iPSCs. Additionally, unlike pancreas, rat iPSCs failed to form rat kidneys by blastocyst complementation using mouse blastocysts. Still, although there remain a number of technical hurdles to overcome, blastocyst complementation is expected as a viable method to reconstruct whole kidney organs.

Decellularized scaffold

The extracellular matrix (ECM) provides a scaffold specific for certain cell types within an organ and enables normal organ function [62]. Several studies have reported that the perfusion of detergents decellularizes the organs and generates 3D scaffolds. The first report using this strategy was on the rat heart, which was decellularized by coronary perfusion and repopulated with neonatal

cardiac cells, fibrocytes, endothelial cells, and smooth muscle cells. After 8 days of the repopulation, contraction and pump function of the heart were observed [63]. Other organs, such as the liver and lung, have also been reconstructed using decellularized scaffolds [64, 65], as too has kidney. Ross et al. decellularized rat kidneys and reseeded them with undifferentiated mouse ESCs. The ESCs subsequently lost their embryonic appearance and expressed renal markers, including Pax2 and Ksp-cadherin [66]. Song et al. decellularized rat, porcine, and human kidneys by detergent perfusion and then reseeded the decellularized rat kidneys with endothelial and epithelial cells. The construct was perfused with media in a sterilized organ bioreactor that acted as a closed system. The reconstructed kidneys expressed markers for podocytes and renal tubules. Furthermore, urine was generated from the reconstructed kidney after orthotopic transplantation into rat [67]. In general, the key to successful decellularized scaffold methods is identifying which cells should be used for the reseeding.

Bioartificial kidney

Hemodialysis and hemofiltration therapies have substantially contributed to curing patients who suffer from AKI or CKD. Although these treatments can excrete small molecules and substitute for some renal functions, they cannot completely replace the transport and endocrinologic functions of renal tubule cells. Therefore, the replacement of renal tubular functions has been investigated using bioartificial kidneys. Humes et al. developed a therapeutic device in which porcine renal tubule cells were attached inside polysulfone hollow fibers that were installed in an extracorporeal perfusion circuit [68]. Importantly, clinical trials using this approach reported better survival and more rapid recovery from AKI [69, 70]. Saito et al. also reported a bioartificial renal tubule device that reduces the expression levels of inflammatory cytokines and increased the life span of AKI goats [71]. These results suggest bioartificial devices may diminish the morbidity and mortality of AKI patients [72, 73], but the type(s) of renal tubule cells used needs further consideration.

Hurdles to overcome

Although regenerative therapy for AKI has been vigorously examined, there remain many hurdles for its clinical application. As for cell transplantation, the route of cell delivery and the volume of cells to be transplanted need to be optimized. Currently, deliver methods include directly injecting cells into the renal parenchyma and renal subcapsular space and also using transvascular approaches, but all have flaws. Injection into the renal parenchyma allows cells to directly approach the injured kidney tissues, which might be advantageous for the cells to integrate into the host kidney tissues. However, this approach injures the host kidneys themselves, which may cause severe bleeding and deterioration of renal functions. Transplantation into the renal subcapsular space, on the other hand, is relatively safe and suitable for exerting paracrine effects, but integration of the transplanted cells into the host kidney tissues might occur at low efficiency. Finally, although transvascular approaches may be technically the easiest, the transplanted cells are distributed into other organs and risk embolization. The integration of transplanted cells into the host kidney tissues is also difficult with this approach.

While studies using mice should help optimize cell therapies, mouse AKI models may have different etiologies from the human disease. Additionally, species differences, such as body size and anatomy, make it difficult to extrapolate disease model results to clinical practice. Preclinical studies using animals that are closer to human, like monkeys and pigs, would be needed before clinical trials.

Kidney reconstruction could be a radical treatment and applied to almost all kinds of AKI, including those resulting from pre- and post-renal failure as well as ATN. However, there are still several obstacles before we could replace failed kidneys with reconstructed ones. Function and safety for clinical application of reconstructed kidneys need to be confirmed. The reconstruction of the urinary drainage tracts must also be addressed. Recently, Yokote et al. developed an excellent urine excretion strategy [74]. They transplanted pig and rat MM with the cloaca into host animals and then allowed the grafts to develop into functional kidney that filtrated urine from the host blood stream. They connected the host animal's ureter to the bladder developed from the transplanted cloaca. This strategy avoided hydronephrosis, and the developed kidney consequently discharged urine via the recipient's ureter.

Conclusions

In this review, we summarize the two main regenerative medicine strategies for the treatment of AKI: cell transplantation and kidney reconstruction. Of the two, cell transplantation is far more established and can be used in AKI cases that are not severe. Cell therapy operates by replacing the injured renal tubule cells with renal stem/progenitor cells or by exerting paracrine effects with trophic factors secreted from renal stem/progenitor cells that help the injured renal tubules regenerate. Candidate cells include HSCs, MSCs, or renal progenitors isolated from adult kidneys or induced from iPSCs. Of these, iPSCs show the promise, as they are relatively easy to establish and infinitely proliferate. However, efficient differentiation protocols for renal progenitors remain to be established before iPSCs can be used to treat AKI clinically. In the most severe cases of AKI, a more

ambitious strategy, kidney reconstruction, is being considered. Here, whole kidneys are being reconstructed in the lab for replacing the diseased kidney. This strategy aims to comprehensively recapitulate renal function. In cell transplantation and kidney reconstruction, considering recent progress made in these fields, the next decades will see tremendous advances.

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Authors' contributions
TT and KO drafted the manuscript. Both authors read and approved the final manuscript.

Competing interests
KO is a founder and member without salary of the scientific advisory boards of iPS Portal, Japan.

Author details
[1]Department of Stem Cell and Regenerative Biology and Harvard Stem Cell Institute, Harvard University, 7 Divinity Ave, Cambridge, MA 02138, USA. [2]Center for iPS Cell Research and Application (CiRA), Kyoto University, 53 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan.

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