Regenerative medicine in kidney disease

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The treatment of renal failure has changed little in decades. Organ transplantation and dialysis continue to represent the only therapeutic options available. However, decades of fundamental research into the response of the kidney to acute injury and the processes driving progression to chronic kidney disease are beginning to open doors to new options. Similarly, continued investigations into the cellular and molecular basis of normal kidney development, together with major advances in stem cell biology, are now delivering options in regenerative medicine not possible as recently as a decade ago. In this review, we will discuss advances in regenerative medicine as it may be applied to the kidney. This will cover cellular therapies focused on ameliorating injury and improving repair as well as advancements in the generation of new renal tissue from stem/progenitor cells.

Kidney International (2016) **90,** 289–299; http://dx.doi.org/10.1016/j.kint.2016.03.030

KEYWORDS: directed differentiation; kidney development; kidney organoid; kidney repair; progenitor; regeneration; stem cell

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Received 19 February 2016; revised 10 March 2016; accepted 17 March 2016; published online 24 May 2016

The term "regenerative medicine" is used to describe any biomedical approach to the replacement or regeneration of human tissues or organs for therapeutic purposes. Advances in our understanding of normal tissue development, turnover, regeneration, and repair have all contributed to the concept of regenerative medicine as a translational clinical approach. The generation of pluripotent stem cells (PSCs) from human somatic cells, the isolation of tissue-specific stem cells, and the reprogramming of nonstem cells to a stem cell state all bring the prospect of regrowing an entire organ via bioengineering. The presence of stem cell niches within adult organs has allowed us to understand normal turnover events. Hence, in its broadest sense, regenerative medicine also encompasses interventions to improve the ability of the body to repair itself, either via immunomodulation or the administration of biologicals. All of these options can be envisaged for any organ; however, the barriers to success increase as histological and functional complexity increases. Hence, the replacement of skin or the bioengineering of simple epithelial structures, such as bladder wall, have seen substantial progress. The kidney has been a far greater challenge. However, the last decade has seen significant changes in what we can achieve with this organ. Whereas all options were previously prophetic,[1] many are now reaching proof of concept. Much of this advance rests on fundamental biological insights into normal kidney development and postnatal repair.

Postnatal kidney repair

The kidney is not a static organ, rather it is repairing and remodeling itself throughout life. Relatively quiescent when unchallenged, acute injury can rapidly trigger extensive cellular proliferation.[2] This endogenous repair potential gives the kidney a capacity to repopulate and repair damaged structures, even though nephron formation has ceased shortly before birth.[3] This repair process has been clearly observed in several animal models subjected to acute injury. The most commonly used experimental model of acute kidney injury is the induction of transient ischemia via clamping of the renal artery. Performed unilaterally (provides a capacity to investigate the contralateral organ as a control) or bilaterally, this mimics clinical ischemia reperfusion injury.[4] This injury model in mice is known to result in a rapid inflammatory response with substantial epithelial cell death followed by a proliferation repair response that, over a 7-day period, results in a return to normal histology. A more chronic injury, unilateral ureteral ligation, results in tubular atrophy, interstitial expansion, and loss of renal parenchyma. However,

once the obstruction is removed, the tissue can remodel to repair damaged tubules without forming new nephrons.[5] This repair is accompanied by substantial cell proliferation within the epithelium.

The last decade has seen many efforts aimed at elucidating the precise cellular origin of the cells involved in this kidney repair, with this knowledge likely to form the foundation for new cell- or factor-based treatments. Potential cell types of value will be discussed herein. However, the innate ability of the kidney to repair itself is limited and reaches a barrier when faced with repetitive episodes of injury or chronic damage. This process, termed "maladaptive kidney repair," results in interstitial fibrosis, parenchymal loss, and therefore an irreversible loss of nephrons. To investigate the capacity of the renal epithelium to respond to repeated injury, Grgic *et al.*[6] tagged all renal epithelium derived from the $Six2^+$ cap mesenchyme to produce a receptor to diphtheria toxin. This allowed for the induction of selective and repetitive injury of the nephron epithelium of the postnatal kidney in these mice. A single damage event triggered the anticipated macrophage infiltration and tubular epithelial cell proliferation, with a complete resolution of pathology within 7 days. However, repeated injury resulted in a maladaptive response including myofibroblast proliferation, loss of vasculature (rarefaction), glomerulosclerosis, and fibrosis. Hence, whereas the primary response of the tubular epithelial is proliferation to elicit repair, repeated epithelial injury can also trigger a fibrotic response.[6,7] Overall, events such as DNA damage, increasing age, previous episodes of acute kidney injury, and sustained cells stress may induce tubular cells to enter into cycle arrest at the G2/M phase mediating the secretion of cytokines and growth factors that promote an inflammatory response. The molecular changes associated with this loss of repair suggest that the reexpression of a number of molecular pathways that are initially critical in nephron formation and patterning can contribute to the tubulointerstitial response to chronic injury. Pathways implicated include the notch and Wnt-signaling pathways and, more recently, the transcription factor Sox9.[8–10] Improvements in the regulation of this endogenous renal repair process or optimization of any cell therapy going forward will rely on understanding repair as well as unveiling the molecular mechanism of maladaptive kidney repair.

Cellular approaches to improving repair

A number of cell types are considered to be able to either contribute directly to renal repair after injury or substantially ameliorate renal injury without directly contributing to the renal epithelium. The successful delivery or regulation of these cell types is likely to be crucial for the development of new regenerative treatments for kidney disease. Experimental nephrology has focused on 4 possible origins for cells contributing to postnatal renal repair: (i) interstitial cell transdifferentiation to epithelium,[11] (ii) recruitment of cells from the bone marrow,[12–16] (iii) tubular cell dedifferentiation and proliferation in response to injury,[2,11,17] and (iv) repopulation of the renal tubules by an adult resident kidney stem/progenitor cell population.[18–20] Options (i) and (ii) involve nonepithelial cells presumably transdifferentiating into renal epithelium whereas options (iii) and (iv) propose a repair process involving epithelial cells within the renal epithelium itself. After many years of careful studies, there is little evidence for the first 2 options occurring.[21] The most definitive proof that renal repair involves cells within the renal epithelium of the nephron was provided by lineage studies showing no evidence of dilution of the cap mesenchyme-derived ($Six2^+$) tubular epithelium with a nonepithelial cell source.[22] This did not resolve whether any cell within the epithelium can contribute to repair or whether repair relied on a resident stem cell population within the tubules. This debate has become a major focus over recent years.

Endogenous tubular kidney progenitors. The presence of a resident tubular stem cell population ($CD133^+$ and $CD24^+$) within the human adult kidney has been proposed, with this viewed as a defined subpopulation resident within the tubular compartments.[18–20,23] Over the last decade, many studies have investigated the existence, location, and contribution of these renal progenitor cells (RPCs) to epithelial repair.[19,20,23,24] The delivery of such cells has also been reported to act as a successful therapy for both acute and chronic animal models.[18,20,23,24] Based on specific markers, it has also been possible to isolate and culture RPCs from human urine, providing a pathway for personalized disease modeling and drug screening.[25] As well as progenitors involved in tubular turnover, there is also evidence for progenitors with overlapping protein signatures that can contribute to the turnover of podocytes within the glomerulus.[26,27]

Some view the RPC population of the postnatal renal tubules as potentially representing a retained tubular progenitor, in part based on expression of markers such as Pax2 seen in the developing organ.[24] Although it is clear that RPCs do not have a capacity to regenerate an entire nephron, the concept is that each segment might contain a distinct tubular progenitor able to selectively contribute to repair via differential proliferation in response to injury. Evidence for this was seen in a mouse model by Rinkevich *et al.*[28] who reported clonal proliferation of epithelial cells in all nephron segments with each cell type only contributing to the turnover of that segment. This study did not prove that this proliferation involved the activity of a stem/progenitor cell versus all epithelial cells. Indeed, there is an ongoing debate around whether RPCs correspond to a stable subpopulation within the tubular epithelium or a transient cell state that appears in response to a homeostatic imbalance within the kidney (Figure 1). The confusion has come in part from an inability to directly compare between human and mouse. The CD24 epitope used in association with CD133 to identify a specific double positive population in humans is not present in the mouse. Conversely, the capacity to lineage trace, though available in the mouse, is not in the human. It is also possible that mouse and human kidneys are not using the same

Figure 1 | Models for turnover of the tubular epithelium in response to postnatal injury. Two models have been proposed to explain the capacity for the tubular epithelium to repair in response to acute injury. (**a**) Tubular stem cell/progenitors exist within the mature tubular epithelium. These preferentially respond to damage via proliferation to replace cells from the epithelium. As a result, an increasing proportion of the resulting repaired epithelium will have been derived from a progenitor. It is not clear whether these also retain progenitor status. (**b**) Acute injury results in epithelial apoptosis but also promotes the proliferation of any mature epithelial cell within the tubule. These proliferating cells contribute new epithelial cells to elicit repair after injury. No cell has any preferential contribution to this process. Figure adapted from Rabelink and Little.[21]

approach to repair. However, recent lineage studies in mice have demonstrated podocyte turnover from a progenitor pool within the Bowman capsule,[29] as had been reported in humans,[26,27] but at a low level making it difficult to detect.

Repair via proliferation of mature renal epithelial cells. The differences between human and mouse with respect to the evidence for a renal progenitor population has vexed the field. Lineage tracing studies in mice suggest that the proliferating populations after renal injury were fully mature renal epithelial cells rather than a specific progenitor subpopulation,[30] whereas analysis of cell division events using unbiased DNA labeling also suggested randomness in the cells reentering cell division within the tubule.[31] Careful anatomical reanalysis of human tubular epithelium has described a subpopulation of cells referred to as scattered tubular cells.[32] Such cells show a distinct morphology suggestive of a distinct subpopulation within the tubules. However, Berger *et al.*[33] reported the first tracking study of scattered tubular cells providing evidence that this population is not a defined intratubular progenitor population of fixed morphology but may rather arise from any tubular cell as a result of spontaneously dedifferentiation triggered by local injury. Indeed, it has been known for decades that cells within the proximal tubule can express mesenchymal markers such as vimentin after an injury episode,[17] suggesting a dedifferentiation process can occur in response to injury.

Irrespective of the eventual outcome of this debate, evidence is required for a capacity to positively influence the repair process of the endogenous kidney for therapeutic purposes. As noted, repeated kidney injury eventually leads to a loss of the capacity for the kidney to repair with an apparently inappropriate cytokine environment resulting in

progressive renal fibrosis. The cellular origin of these fibrotic regions has also been a controversial area of research; however, there is now strong evidence in mice, again from lineage tracing studies, that the pericytic compartment is a strong contributor to renal fibrosis[34] with a specific population of interstitial cells positive for Gli1 representing the fibrogenic population in many tissues, including kidney and lung.[35] Indeed, ablation of this population in animals significantly reduces the fibrotic response to chronic injury.[35]

Mesenchymal stem cells

In the early 1990s, a number of studies proposed that cells from the bone marrow were able to home to sites of injury around the body, including the kidney.[16] Initially, it was thought that such bone marrow–derived cells might even have a capacity to generate new tissue in remote sites; however, this has not been shown to be the case.[36,37] Whereas many distinct cell types exist within the bone marrow, we will focus here on the mesenchymal stem cells. "Mesenchymal stem cells" (MSCs), or mesenchymal stromal cells, is a term used to describe a population of cells present within the bone marrow that are clonogenic (able to regenerate clonal colonies from a single cell), able to undergo long-term proliferation and to differentiate into a variety of mesenchymal lineages—including bone, cartilage, and fat—*in vitro*.[38] Though initially investigated as a support population within the bone marrow, they have also been investigated for the therapeutic potential with respect to mesenchymal tissue replacement such a bone and cartilage. As a result of their apparent immune protection and proreparative immune modulatory activities, it is possible to deliver nonautologous MSCs into a patient without immune rejection, providing the possibility of an off-the-shelf stem cell product for cell therapy.[21]

In the context of the kidney, many studies suggest that bone marrow–derived MSCs are able to ameliorate tissue damage in response to kidney injury and to reduce allograft rejection. The effectiveness of MSCs in ameliorating renal acute damage has been shown in many animal models of acute kidney injury, including glycerol, mercury, and cisplatin or ischemia-induced injury.[39–42] However, this is not regarded as occurring as a result of integration into the damaged epithelium or transdifferentiation into an epithelial cell type. As in other settings, such beneficial effects are attributable to their proven immunomodulatory capacity[43–46] and the release of paracrine factors.[38,47–49] Indeed, several reports show that these cells release growth factors such as hepatocyte growth factor, insulin-like growth factor 1, vascular endothelial growth factor, and epidermal growth factor, which are widely considered to improve renal function.[39–42] Indeed, the delivery of conditioned media from MSCs in culture has been reported to have an equivalent effect in animal models,[39] supporting the paracrine mode of action of these cells. The utility of these cells is less clear in animal models of chronic kidney disease. In a progressive model of renal failure, it was shown that MSCs underwent maldifferentiation into cells with adipocyte features within the glomeruli.[50] This raises concerns about the safety and efficacy of MSC-based cell therapy for chronic disease.

The preclinical data using MSCs in models of acute renal injury have prompted worldwide interest in MSCs for a variety of clinical indications, including in relation to nephrology. Currently, human MSCs have been administrated to more than 1000 patients either by autologous or allogenic transplantation.[21,49,51–54] Tan *et al.*[55] showed that patients who were given autologous infusion of MSCs showed reduced transplant rejection and improvement in renal function at 1 year. However, it is still unclear whether those cells have long-term efficacy or adverse side effects, and the guidelines for isolation, expansion, and characterization protocols need be uniform in order to extend their use in different trials.[21] Although originally identified in the bone marrow (bmMSCs), MSC-like cells have been also identified to reside in many adult tissues including the kidney, possibly representing a perivascular cell population involved in normal tissue repair.[43,44,47] In 2012, an endogenous kidney MSCs population (kMSCs) was characterized from the adult murine kidney, showing the characteristic gene and protein expression profile, clonogenicity, and trilineage (fat, bone, cartilage) potential of an MSC.[56] Compared with bmMSCs, this population showed an enrichment for kidney-specific genes suggesting potential tissue-specific roles. It is interesting to note that the fibrogenic Gli1$^+$ population described recently as the source of interstitial fibrotic lesions in mice also behaves as an MSC population *in vitro*, raising a far less promising role for a kMSC.[35]

Macrophages. Other important players in mediating kidney repair are macrophages. Derived from the monocytic lineage, it is widely known that macrophages promote the fibrotic and inflammation reaction during renal disease. However, over the last 15 years, they have been also described to possess a protective role in acute and chronic kidney diseases.[57] In addition, macrophages have shown a capacity to ameliorate allograft rejection.[58] These findings identify macrophages as interesting cell-based mediators favoring tissue repair.

Macrophages have been classically described as the guardians of the body due to their role in innate immunity. Classically, macrophages are thought to clear foreign pathogens that can disrupt tissue homeostasis.[59] However, macrophages represent a heterogeneous population with distinct functions depending on resident tissue and phase of tissue injury or disease. In some kidney diseases, macrophages display classical activation through the release of proinflammatory cytokines, reactive oxygen species, and nitric oxide. This is termed an M1 response or M1 phenotype.[60,61] In different contexts or stages of the disease, macrophages can also display antifibrotic and anti-inflammatory features, termed an "M2 phenotype."[62,63] M2 macrophages exert a reparative role in ischemia reperfusion injury[64,65] and unilateral ureteric obstruction.[66,67] Numerous reports have shown that changes in the local microenvironment are critical

to trigger an M2 phenotype. For example, the tubular epithelial cells can stimulate this process by releasing colony-stimulating factor-1 (macrophage colony-stimulating factor).[68,69] Based on these findings, researchers have specifically directed macrophages toward an M2 phenotype via genetic manipulation[70–73] or cytokine alteration,[74–77] resulting in improved tissue repair or renal function. The former has included the overexpression of interleukin (IL)-4, IL-10, inducible nitric oxide synthase or heme-oxygenase,[73–75,78] all of which improved renal function. A further subdivision of the M2 responses into M2a and M2c has provided additional molecular understanding of the efficacy with respect to ameliorating renal damage.[76] Taken together, the therapeutic potential of macrophages is currently opening new exciting options for cellular or cytokine/growth-factor based-therapies.

Regeneration

Whereas understanding and regulating the capacity for the epithelium within the adult kidney to repair is a major objective for the treatment of kidney injury, patients presenting with chronic kidney injury are often well advanced in their disease at diagnosis with existing evidence of interstitial fibrosis and a significant loss of nephrons. In the developing organ, a nephron progenitor population gives rise to all of the cellular components of the forming nephrons;[1,79] however, no such cell remains in the postnatal kidney. Hence, the formation of new nephrons after birth does not occur.[3] For this reason, considerable focus has been placed on the development of approaches in which kidney tissue is regenerated from other sources of stem cells. Extensive research into the systems biology of murine kidney development[80–82] has been performed that, coupled with our understanding of early embryonic patterning of the mesoderm,[83] provides a "protocol" upon which attempts to recreate the process of kidney organogenesis have been based. The objectives here include the production of specific renal cell types, such as proximal tubular epithelial cells, for use in cellular therapy, or the recreation of complex multicellular kidney tissue with the long-term potential for use in renal replacement. A variety of stem cell sources have been investigated for their capacity to form kidney cell types. These include human fetal kidney stem cells, cells from the amniotic fluid, or the directed differentiation of human PSCs (both embryonic stem cells [ESCs] and induced pluripotent stem cells [iPSCs]) to kidney endpoints. The generation of kidney cell types from available stem cell populations will also facilitate *in vitro* applications such as drug screening. As PSCs can now readily be derived from adult somatic cells, such as those available from any patient, the directed differentiation of these stem cells would allow for personalized medicine approaches including disease modeling and personalized drug efficacy screening. Patient-derived PSCs also provide the option of autologous regenerative treatments. Here we will discuss the different approaches that have been taken to date for the generation of kidney cell types from stem cells and describe how these have been applied.

Generating kidney tissue from human fetal stem cells. As noted, the postnatal human kidney no longer contains the progenitor population capable of forming a new nephron. It had also been demonstrated that adult nonrenal cell types, including bone marrow and blood stem cells, cannot generate nephron cells or incorporate into existing nephrons to elicit repair.[36,37] One regenerative approach that has been investigated, therefore, has been the use of nephron progenitor populations sourced from human fetal kidney tissue. Although this presents ethical challenges in some countries, it has also provided some technical challenges in that we do not yet have a good understanding of how to maintain a human nephron progenitor population outside the stem cell niche in which it normally resides. Indeed, this is only just becoming possible in mouse cells.[84] Additionally, it has been difficult to identify specific markers for the purification of this cell type. In mice, the capacity to label a population in transgenic animals provides an avenue for specific cellular enrichment that it not possible from human fetal tissue. In order to investigate whether nephron progenitors could generate new structures after transplantation, initial studies transplanted fragments of human fetal kidney (14–22 weeks gestation) into immunodeficient mice.[85,86] These embryonic transplants continued to differentiate,[85,86] as had previously been shown when embryonic mouse kidneys were transplanted from one animal to another.[87] However, whereas the transplanted tissue could draw in a vasculature, they did not integrate into the host kidney.[85,86] Studies were then initiated with the objective of isolating specifically the nephrogenic cells from the human fetal kidney to evaluate both to their capacity to integrate into existing kidney tissue but also to generate new structures. In order to achieve this, a subfractionation strategy was required. Initial studies examining whether a $CD24^{+}CD133^{+}$ population, similar to that described in the adult kidney as having renal tubule progenitor capacity, showed that 35% to 50% of cells in the early kidney were positive for these markers.[34] However, these were shown not to represent a renewable nephron progenitor population.[88,89] By comparing the expression of human fetal kidney, the pediatric kidney cancer Wilms tumor, which is enriched for mesenchymal progenitors,[85–90] and adult kidney, a population of neural cell adhesion molecule (NCAM) $(CD56)^{+}$ cells were identified as potentially representing nephron progenitors in human fetal kidney.[36,88,89] This fraction is enriched for presumed nephron progenitor genes (e.g., *SIX2*, *OSR1,* and *PAX2*); however, NCAM is also present within the cortical stroma of the fetal kidney.[89] Further fractionation was made based on coexpression of epithelial CAM, as the latter is not present in the stroma but is present in developing nephrons from the pretubular aggregate stage.[89] The transplantation of this enriched fraction of human fetal kidney onto the chorioallantoic membrane of a developing chick showed that these cells were able to differentiate into epithelial structures expressing identifiable markers of distinct nephron segments, including

the proximal tubule, distal tubule, and loop of Henle.[91] Notably, it was subsequently shown that the repeated intraparenchymal administration of NCAM1$^+$ cells resulted in improved renal function (increased creatinine clearance is a model of chronic kidney injury [5/6 nephrectomy]).[91]

Amniotic fluid-derived nephron progenitors. Another approach to sourcing cells capable of acting as nephron progenitors has been the use of amniotic fluid stem cells. Cultured cells accessed from amniotic fluid have been described to be able to self-renew in a chromosomally stable fashion for >350 doublings.[92] Such stem cells have been isolated from human amniotic fluid collected between 12 and 18 weeks of gestation and display a capacity for long-term expansion in culture. Initial studies showed a capacity for such cells, virally tagged with green fluorescent protein, to integrate into embryonic mouse kidney cultures *in vitro*.[93] As this material was collected as a part of routine amniocentesis, this is a more readily available source of fetal human stem cells, but remains unlikely to provide an autologous source of cells for treatment. Initial studies into the properties of amniotic stem cells in renal injury concluded that the injection of such cells into animal models of kidney disease delayed progression of fibrosis.[94] More recent studies have investigated whether these stem cells can generate renal cell types *in vitro*, with evidence for a capacity to differentiate into podocyte-like cells in comparison to immortalized human podocyte lines.[95] Moreover, it is proposed that the cells can be propagated in a nephron progenitor state for many passages in order to provide an expandable source of cells able to give rise to this specific kidney cell type.[95] Such cells may be valuable resources for the study of podocyte biology as well as for the screening of drugs to establish their effects on the podocyte.

Kidney cells derived from PSCs. The use of human fetal stem cells, whether derived from human fetal kidney or more readily accessible sources such as the amniotic fluid, does require access to this material. In addition, these stem cells have not proven to be able to generate the diversity of cell types required for the bioengineering of a replacement organ. In 2006, the door was opened on an option that solved both of these barriers. First in mouse and then in human cells, Takahashi *et al.*[96,97] demonstrated that any somatic cell can be returned to pluripotency; a state equivalent to that of the epiblast of the early embryo and hence equivalent to an embryonic stem cell line. The overexpression of 4 critical transcription factors, *Oct3/4, Sox2, Klf14,* and *c-myc*, was sufficient to induce the formation of PSC colonies at low frequency. Such cell lines are referred to as iPSCs. The capacity to culture iPSC colonies and to direct their differentiation into cell types representative of derivatives of all 3 germ layers was also provided.[97] This finding represents a watershed in cell biology and most particularly in regenerative medicine. For the first time, the option to recreate any desired cell type from any available starting cell is possible. With advances in the use of episomal and retroviral cassettes for the introduction of the reprogramming genes, iPSCs can now rapidly be generated without any genetic footprint from the reprogramming process, from any patient. Recent advances in gene editing technologies, including the transcription activator-like effector nuclease (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR) gene editing techniques, also provide a capacity to repair mutations responsible for single gene disorders within the patient-derived iPSCs.[98] With time, regenerative medicine approaches will enable the loop to be closed such that a patient can be treated with tissues generated from their own cells, but without the underlying genetic disease they initially presented with. While these are revolutionary advances, there remains a long gap between making a cell that is pluripotent and using that cell to make the cells that are required to treat kidney disease. However, the last 3 years have seen major advances in protocols designed to direct the differentiation of iPSC to kidney.

Directed differentiation of human PSCs to kidney endpoints has been demonstrated by a number of laboratories, with most protocols drawing from our understanding of normal kidney development (Figure 2).[79] Mae *et al.*,[99] using an iPSC reporter line into tagged with green fluorescent protein within the OSR1 locus, reported the formation of intermediate mesoderm (IM) in response to culture with activin A, bone morphogenetic protein (BMP)7, and the Wnt agonist, CHIR99021. This approach drew on prior evidence of a role for activin A and Wnt signaling in mesoderm patterning and BMP7 in metanephric mesenchyme survival. They reported early evidence that these cells could further differentiate into glomerular podocytes and renal tubule epithelia when cocultured with embryonic mouse tissue. Similarly, Lam *et al.*[100] treated human ESCs cells with CHIR99021 to induce BRACHYURY$^+$MIXL1$^+$ mesendoderm with the subsequent addition of fibroblast growth factor

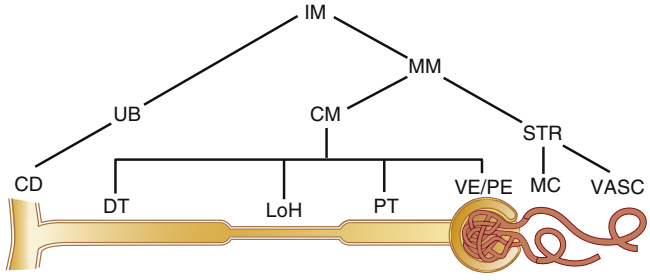

Figure 2 | Cellular origins of the kidney cell types required for repair or regeneration. The kidney arises from the intermediate mesoderm (IM), which forms both the ureteric bud (UB) and the metanephric mesenchyme (MM). The UB gives rise to all of the epithelium of the collecting ducts (CDs). The MM gives rise to the stroma (STR) and the cap mesenchyme (CM). All epithelial cells along the nephron other than the collecting duct arise from the CM. This includes the parietal epithelium (PE) and visceral epithelium ([VE], podocytes) of the glomeruli, the proximal tubules (PTs), loops of Henle (LoHs), and distal tubules (DTs). The STR gives rise to the mesangial cells (MCs), pericytes, and interstitium. It is also thought that some of the vascular endothelium (VASC) arises from this compartment.

(FGF)2 and retinoic acid to generate $PAX2^+$ $LHX1^+$ IM cells. Again, the resulting IM cells showed evidence of a capacity to form tubular epithelium (LTL^+CDH16^+) and integrate into embryonic kidney explants. At a similar time, using monolayer culture with BMP4 and FGF2, followed by retinoic acid, activin A, and BMP2, Xia *et al.*[101] reported the formation of $HOXB7^+RET^+GFRA1^+$ ureteric bud-like progenitors from human ESCs/iPSCs. When cocultured with dissociated embryonic mouse kidney, these were seen to contribute to the ureteric tree, which gives rise to the collecting ducts of the kidney.

A major challenge had been to robustly generate the nephron progenitor population. The nephron progenitor population is derived from the metanephric mesenchyme (Figure 2) and is located in the periphery of the growing organ surrounding or capping each of the tips of the branching ureteric epithelial tree. Taguchi *et al.*[102] dissecting the lineage of the ureteric epithelial progenitors from the nephron progenitors, developed a complicated stepwise induction protocol for nephron progenitors that they applied to both mouse ESCs and human iPSCs. This demonstrated appropriate *in vitro* transitions through nascent mesoderm, posterior nascent mesoderm, and posterior IM to nephrogenic mesenchyme (*WT1, PAX2*, *SALL1*, *SIX2*), which, when cocultured with a source of Wnt signaling, formed complex 3-dimensional models of developing nephrons comprising both glomeruli and renal tubules. This group has gone on to show substantial functional maturation of these *in vitro*–generated nephrons after transplantation under the renal capsule of recipient animals.[103] Although this was the first, a number of more recent studies now also report the generation of segmenting nephrons from iPSC, including evidence of proximal tubular differentiation sufficient to report specific nephrotoxic injury.[104,105] One of these studies[105] used a quite distinct approach to directed differentiation with initial culture of human iPSC in Matrigel (Corning, Corning, NY) to induce the formation of an epiblast-like structure. These epiblast-like spheres gave rise to nephron-like structures, including evidence of nephron segmentation and differentiation, if canonical Wnt signaling was activated.

The formation of nephrons alone does not represent the entire kidney. Recently, we reported the spontaneous formation from human iPSC of very complex multicellular structures highly reminiscent of entire developing kidneys referred to as "organoids."[106] Again, this involved the stepwise addition of growth factors/agonists selected to mimic appropriate steps of normal kidney development.[106] $Mixl1^+Brachyury^+$ posterior primitive streak was induced via culture in either high BMP4/low activin A or a canonical Wnt agonist. Subsequent culture in FGF9 induced the coexpression of the IM markers PAX2, LHX1, and OSR1 with continued $FGF9^{+/-}$ BMP7 and retinoic acid generating a ureteric epithelium ($GATA3^+PAX2^+RET^+DBA^+CDH1^+$) within a field of nephrogenic mesenchyme ($SIX2^+WT1^+PAX2^+HOXD11^+$). Experimental studies in mice had previously shown that the developing kidney can reform even after enzymatic dissociation, suggesting a strong self-organization capacity during embryogenesis.[107,108] Hence, human iPSC-derived cultures were dissociated and reaggregated in 3 dimensions for subsequent culture.[109] This resulted in the formation of kidney organoids containing collecting duct and >100 individual patterned and segmented nephrons. The forming nephrons show connections with collecting ducts epithelium and the formation of loops of Henle ($UMOD^+$). Strikingly, surrounding the nephrons there was an appropriate renal stromal population ($MEIS1^+$) and a forming vascular network ($SOX17^+PECAM^+$), providing evidence for the simultaneous additional induction of progenitors for these tissue components.[109] On rare occasions, this vasculature was found within the immature glomeruli. The accuracy of this as a model for the human fetal kidney remains to be established, although initial transcriptional profiling established significant congruence with human fetal kidney.[109]

Another approach to the challenge of building a complex multicellular organ from human PSCs involved mixing together distinct cell types for coculture. First described for liver,[110] this approach involved creating 3-dimensional organ buds via coculture of endothelium, MSCs, and iPSC-derived kidney progenitors.[111] The resulting organ buds were transplanted into the cranial window of immunodeficient mice where they formed nephrons and vascularized.[111] Using i.v. injection of a dye known to be filtered by the glomeruli, a labeled urinary filtrate was demonstrated. Indeed, the podocytes formed slit diaphragms, suggesting substantial maturation after transplantation.

In summary, the capacity to direct the differentiation of human PSCs to kidney is now significant. Given the first report of the generation of any cell type resembling a human kidney component dates back to only 2012, with evidence that podocyte-like cells can be formed *in vitro* from human ESC,[112] this has been a remarkably rapid application of the technology to the field of nephrology. The advantage of using human PSCs lies in the ability to maintain and expand such cells long term, providing a renewable supply of material, and the capacity to derive the lines from any individual, providing the future with the potential of autologous treatments. In the interim, the generation of nephrons, or indeed kidney organoids, represents a major opportunity for their application in screening of pharmaceuticals for nephrotoxicity, personalized disease modeling of genetic kidney disease, and even personalized drug screening, as is being applied to lung organoids from cystic fibrosis patients. The use of proximal tubule cells generated from iPSC in nephrotoxicity screening has already been reported and several studies in which nephrons have been generated from hPSC have demonstrated an appropriate response of the proximal tubules within these nephrons to nephrotoxicants such as cisplatin.[113,114] iPSC-derived nephrons have also been generated from lines in which CRISPR editing has mutated both copies of PKD1, with preliminary evidence of cyst formation *in vitro*.[105] Several studies have also begun to evaluate the capacity of

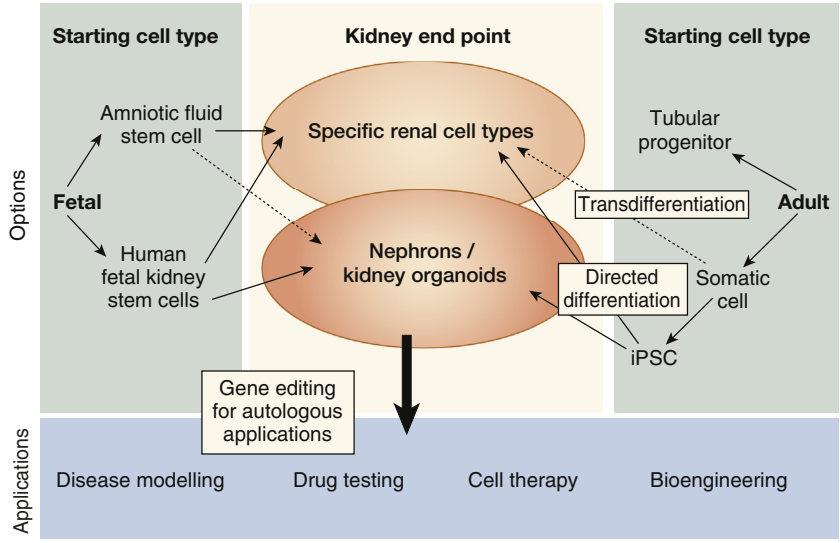

Figure 3 | Options for the regeneration of kidney tissue. The available cell options from which specific renal cell types or kidney organoids can be generated include those isolated from human fetal material, including amniotic fluid stem cells and fetal kidney stem cells, or adult tissue, including kidney tubular progenitors or any adult somatic cell type. The latter can be reprogrammed to a pluripotent state for directed differentiation to kidney tissue or transdifferentiated directly to a renal endpoint. The generation of renal tissue in this way can be applied in disease modeling, drug testing, cell therapy, and bioengineering applications. The capacity to edit the genome of a patient cell type, when an underlying genetic mutation is known, provides the possibility of autologous cell therapies. iPSC, induced pluripotent stem cells.

iPSC-derived kidney cell types to reduce renal damage in models of acute renal injury, with conflicting results. Toyohara *et al.*[115] reported reduced injury without evidence of cellular integration whereas Imberti *et al.*[116] reported integration. The eventual utility of such stem cell–derived embryonic structures in dissecting kidney diseases with postnatal onset remains to be investigated. Similarly, the capacity for these cells integrate back into a patient organ or be used for bioengineering tissue for renal replacement therapy is still far off. Nevertheless, these are remarkable advances in kidney regeneration.

Reprogrammed adult cells. The discovery that any somatic cell can be returned to a pluripotent state via the enforced expression of as few as 4 genes has completely revolutionized regenerative medicine. However, the concept of master transcription factors able to impose a specific cell fate onto a cell dates back by a further 20 years to the studies showing the capacity of the *MyoD* gene to transform a fibroblast into a myocyte.[117] Although the term "reprogramming" is often now reserved for the generation of PSCs—able to differentiate into any cell type—the use of overexpression of key genes within tissue-specific transcriptional regulatory networks is now being exploited to redirect the identity of one cell type into another. This is sometimes referred to as transdifferentiation or direct reprogramming. The latter term highlights the fact that this approach bypasses the requirement to take the cell right back to a state of pluripotency for subsequent directed differentiation, instead converting the cells' identity from one to another directly. Indeed, the last 5 years have seen an explosion in studies in which the genes required to change a fibroblast into another cell type have been identified. These include direct reprogramming of fibroblast to neuron, cardiac muscle, and neural progenitor.[118] In many of these studies, the key discovery has rested with the identification of the minimal genes required for a successful outcome. As for directed differentiation, this relies upon an understanding of the molecular networks involved in determining the target cell type of interest and hence frequently draws on an understanding of embryology. In the kidney, the identification of a transcriptional regulatory network of 6 genes capable of transdifferentiating proximal tubules to a cap mesenchymal state has been reported.[119] The absence of culture conditions suitable to support this tissue have restricted the utility of this approach. Although no direct reprogramming to any other kidney cell type has been achieved to date, this may prove to be a simpler approach for the generation of specific renal cell types, such as podocyte or proximal tubule, for personalized screening of drugs or disease modeling. Figure 3 presents options for regenerating a kidney.

Conclusions

In this review, we have described the state of play with respect to the development of regenerative medicine options for kidney disease. From a very low base, this has become a major area of focus over the past decade. New insights into the processes controlling endogenous renal repair and the capacity to isolate progenitor from the postnatal renal epithelium are providing possible options for the treatment of acute kidney injury. Further understanding of why such endogenous repair processes fail and approaches for the modulation of the fibrotic response are likely to provide the key to the challenge of chronic kidney disease. Major advances in the directed differentiation of stem cells to a large range of kidney

cell types have also brought significant hope. Though this has yet to deliver a cellular therapy, the kidney is not alone in this challenge, with the successful delivery of PSC-derived tissues into patients currently restricted to retinal pigmented epithelia and certain neural subtypes. Simply with respect to having cells reach the appropriate site within an adult organ, let alone functionally integrate, the obstacles are perhaps higher for the kidney than for most organs. This represents a challenge whether the source of the regenerated kidney cells are primary fetal progenitors, PSCs, or reprogrammed adult cells. In the meantime, the regeneration of kidney cell types provides new approaches for drug screening, whether to identify nephrotoxicity or to personalize therapeutic treatments.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

MHL is a Senior Principal Research Fellow of the National Health and Medical Research Council (APP1042093). PK is a CONICYT Becas-Chile postgraduate scholar. The laboratory is supported by funding from the NHMRC, National Institute of Health and the Australian Research Council. The Murdoch Childrens Research Institute is supported by the Victorian Government's Operational Infrastructure Support Program.

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