Reprogramming the kidney: a novel approach for regeneration

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Nuclear reprogramming has reshaped stem cell science and created new avenues for cell-based therapies. The ability to bestow any given phenotype upon adult cells regardless of their origin is an exciting possibility. How can this powerful tool be harnessed for the treatment of kidney disease? Many approaches, including induced pluripotent stem cell (iPSC) production, direct lineage conversion, and reprogramming to a kidney progenitor, are now possible. Indeed, the generation of iPSC lines from adult kidney-derived cells has been successfully achieved. This, however, is just the beginning of the challenge. This review will discuss the fundamental concepts of transcription factor–based reprogramming in its various forms, highlighting recent advances in the field and how these are applicable to the kidney. The relative merits of each approach will be discussed in the context of what is a realistic and feasible strategy for kidney regeneration via reprogramming.

Kidney International (2012) **82,** 138–146; doi:10.1038/ki.2012.68; published online 21 March 2012

KEYWORDS: dedifferentiation; directed differentiation; induced pluripotency; nephron progenitor; reprogramming; transdifferentiation

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Received 5 October 2011; revised 3 January 2012; accepted 11 January 2012; published online 21 March 2012

Nuclear reprogramming can be defined as a directed change in cell phenotype that would not otherwise occur naturally.[1,2] Nuclear reprogramming is famously exemplified by the creation of induced pluripotent stem cells (iPSCs): embryonic stem cell (ESC)–like cells generated from a variety of cell types via the delivery of specific transcription factors.[3–5] Originally, Takahashi and Yamanaka[3] showed that overexpression of just four transcription factors could reprogram mouse embryonic fibroblasts to iPSCs. These iPSCs expressed ESC markers, gave rise to teratomas when transplanted subcutaneously, and contributed to embryo development when injected into a blastocyst. iPSC reprogramming experiments have been repeated and extended to include a wide variety of donor tissues, including skin, blood, fat, and skeletal myoblasts.[6–9] A recent addition has been the generation of iPSCs from kidney, either using adult kidney mesangial cells[10] or renal epithelial cells shed into the urine.[11] This and many other examples of transcription factor–based reprogramming have raised the possibility that lineage conversion between any two cell types, without the need to return to pluripotency, may be possible. Indeed, it is possible to reprogram directly to a variety of functional cell types, including neurons, cardiomyocytes, and pancreatic β-islet cells.[1,12,13] Despite this impressive display of phenotypic conversion, the reprogramming to cells within the kidney lineages remains elusive. This is an important issue to address, as the kidney has a limited capacity to regenerate after injury. Chronic kidney disease is a major global health problem, and there is an acute need to develop treatment options that can restore endogenous kidney function, as opposed to providing dialysis or transplantation. Although researchers have extensively investigated the presence of stem cells in the adult kidney,[14–16] including the identification of progenitors in the Bowman's capsule able to replace podocytes *in vivo*,[17–19] to date there have been no reports of a postnatal kidney progenitor cell capable of giving rise to all the cell types of the mammalian nephron. As such, no new nephrons can form in the adult mammalian kidney. Reprogramming therefore represents an attractive strategy for the generation of nephron progenitors in the postnatal mammalian kidney.

CROSSING THE BOUNDARIES: HOW DOES REPROGRAMMING WORK?

Reprogramming need not be restricted to dedifferentiation (return to a prior differentiative state) and certainly not

restricted to the reimposition of a pluripotent state. There are many reports of forced conversion between two unrelated differentiated cell types that does not involve a pluripotent intermediate state (termed lineage reprogramming or transdifferentiation[20]). What appears critical for both dedifferentiation and transdifferentiation is the forced expression of genes critical to the maintenance of the desired end-point phenotype. In the case of iPSCs, these genes include the key ESC pluripotency regulators, *Oct4* and *Sox2*, and so it follows for the generation of specific differentiated cell types that the choice of reprogramming factors will be specific to that cell type. This was indeed the case in an impressive example of lineage reprogramming *in vivo* where Zhou *et al.*[1] converted mature exocrine cells of an adult mouse to glucose-responsive, insulin-secreting pancreatic β-islet cells capable of ameliorating hyperglycemia in streptozotocin-induced diabetic mice. They used the transcription factors *Ngn3*, *Pdx1*, and *Mafa*, all of which are important for β-islet cell specification with pancreatic developmental defects arising in their corresponding mutant mice. More recently, additional studies have reported the reprogramming of fibroblasts into cardiomyocytes[13] and neurons.[12] Again, the reprogramming factors used in these studies were lineage instructive for the end-point phenotype, which supports the rationale for transcription factor–based reprogramming.

The hematopoetic field is an ideal system to study lineage conversion, owing to both the cellular diversity and the relative ease of isolation and culture of these different cell types. Lineage reprogramming is relatively advanced in the hemotopoetic field and predates much of the excitement generated by the recent iPSC technology. Early experiments in this field focused on the lineage-instructive role of *GATA1* and *PU.1* in the erythroid–megakaryocyte and monocyte cell lineages, respectively. Overexpression of *GATA1* in monocytes caused conversion to an erythroid–megakaryocyte lineage as assessed by lineage-specific markers.[21,22] Conversely, overexpression of *PU.1* in an erythroid–megakaryocyte cell line induced a phenotypic conversion to a monocytic cell population, facilitated via repression of GATA1.[23] This phenotypic switching between monocytes and erythroid–megakaryocytes in response to GATA1 or PU.1 overexpression highlights the importance of the binary cell fate choices that exist during normal development, as cross-antagonism between GATA1 and PU.1 proteins defines one of the most primitive steps in hematopoietic development.[24–26]

CELL FATE DETERMINATION, WADDINGTON'S LANDSCAPE, AND ATTRACTOR STATES

The concept that a differentiated cell can be reprogrammed into another state has captured the imagination of the biomedical community. How can this occur? During embryogenesis, progressive development of epigenetic barriers allows organized differentiation to occur, giving rise to a highly complex organism. Unique chromatin marks such as DNA methylation and histone acetylation control the activity of specific genes, thereby acting as modulators of cell fate. This has been conceptualized as the 'epigenetic landscape' first described by Conrad Waddington[27] (Figure 1). It is using this framework that the principles of not only differentiation but also reprogramming can be understood.

Waddington depicted the differentiation of a pluripotent cell as akin to a ball rolling from a hilltop through a landscape of valleys to its final destination (Figure 1a). In doing so, the ball will encounter alternative pathways, with its final 'differentiated' destination dependent on which pathway is chosen. Similarly, a progenitor cell may have multiple fates available to it. The differentiated phenotype it adopts will depend upon the epigenetic barriers that either permit or prevent certain outcomes. If a powerful fate-determining or lineage-instructive transcription factor is activated in a progenitor cell, or an alternative fate repressed by another regulatory network, this will provide a potent attraction toward a particular lineage.[28] Once a ball enters such a stable basin, it may not move further because of the barriers around it (Figure 1a). This represents entry into an 'attractor state' or a discrete cell phenotype resulting from the sum of the transcriptional activity present in the cell at that time.[29] Such attractor states may be intermediate resting points during differentiation or a final destination. Either way, an attractor state is regarded as stable such that a less stable cell type will converge upon a nearby attractor state over time.[29,30] Once a cell is in a particular attractor state, it is energetically unfavorable for that cell to change its transcriptional activity and move toward a different attractor state. However, if a cell is forcibly disrupted via the imposition of a different gene regulatory network, as occurs during reprogramming, it may be pulled toward a different attractor state to reach another energetically favorable resolution. Biological evidence for the attractor state hypothesis was reported in a number of studies.[31–33] For example, treatment of HL60 cells with either DMSO or all-trans retinoic acid results in transdifferentiation into neutrophils, but this attractor state is reached via different pathways in each case (Figure 1b). If the stimulus was removed before the neutrophil phenotype was reached, the cells reverted back to the initial phenotype, demonstrating the strength of the initial attractor state.[31]

Given that an attractor state is a stable solution of gene regulatory activity that results in a defined cell phenotype, reprogramming must destabilize this gene regulatory network to the point where the original attractor state is no longer energetically favorable and the cell is forced to move into a new attractor state. In the context of Waddington's landscape, reprogramming factors must force the ball over one or more hills (epigenetic barriers) into an alternative valley (attractor state) or back up a valley to an alternative attractor state (Figure 1d). The key to efficient reprogramming is to know which valleys lead to specific attractor states for specific cell phenotypes and which combinations of lineage-instructive genes are required for convergence to a given attractor state. Gene expression studies or single-gene-perturbation experiments can provide clues to appropriate lineage-instructive transcription factors. Importantly, studies have shown that cells can converge to

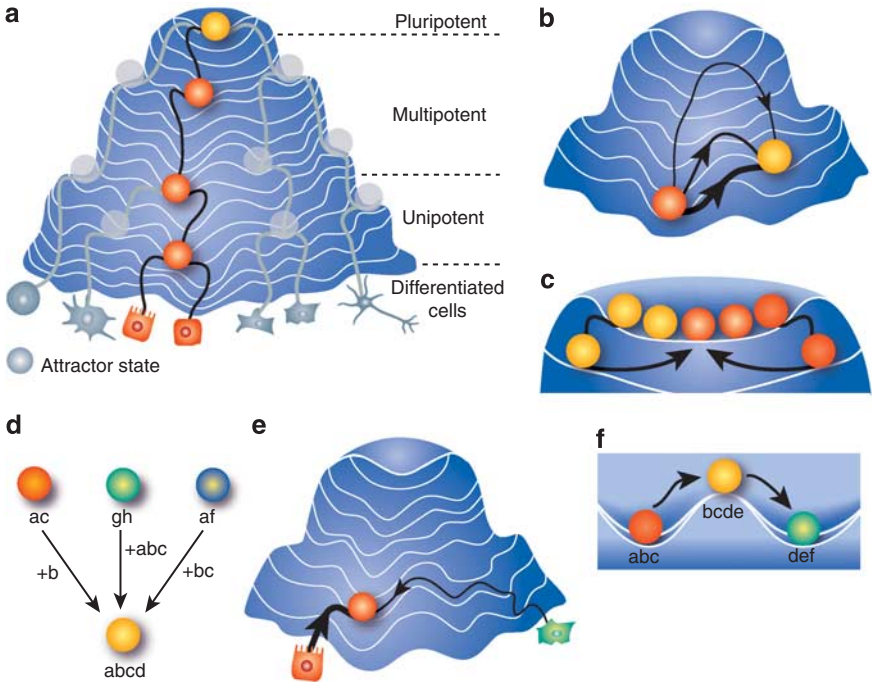

Figure 1 | The key principles of reprogramming as viewed within the analogy of Waddington's landscape. Waddington's landscape effectively represents cell lineage specification and fate determination. It can also be used to illustrate the principles of the attractor state and explain what might be required for reprogramming from one state to another. Waddington's landscape has some key features. (**a**) The landscape has direction: once the ball has committed to its descent, it cannot roll back up of its own accord. The more plastic the cell, the higher up the hill it is. Hence, pluripotent is at the top, multipotent further down, unipotent below that, and differentiated at the bottom. (**b**) The many hills and valleys represent the myriad of potential differentiative pathways available. Indeed, a particular basin may be approached from more than one pathway. When transdifferentiating (moving from one cell type to another), you can take different approaches to reach the same outcome. (**c**) Cells converge back into an attractor state, and hence reprogramming requires a considerable shift away from that attractor basin. (**d**) Forced reprogramming requires the overexpression of genes instructive for an alternative attractor state (e.g., abcd) to the starting attractor state. In this example, three different starting cells in different attractor states (expressing different sets of genes, indicated as letters) require the overexpression of differing genes (indicated as +) to reach the same end-point attractor state. Only some of these genes may be required, and hence different combinations of genes can reach the same destination. (**e**) It is easier to reprogram from a related cell type (as shown by thick arrow) as opposed to a more distant attractor state (as shown by thin arrow). (**f**) During reprogramming via the overexpression of lineage-instructive genes, there is no requirement to retrace a prior developmental pathway. There can be direct conversion from one attractor state to another with the cell undergoing reprogramming displaying an intermediate gene expression pattern between the two states during reprogramming.

a single attractor state. For example, when hematopoetic progenitor cells were FACS separated into Sca1$^{very\ lo}$ or Sca1$^{very\ hi}$ and cultured in identical conditions, the Sca1 profile of the two populations converged over time to the same hematopoetic attractor state[32] (Figure 1c). This means that reprogramming can be imprecise: as long as the imposed transcriptional activity brings the cell near the desired attractor state, the cell will converge to that attractor state (Figure 1d). It also means that there may be more than one possible combination of reprogramming factors that will work for different cell types: a finding that has been demonstrated multiple times (Figure 1d).[34–36]

Because an attractor state is characterized by its gene regulatory network, cells that are more closely related in lineage are predicted to have more similar attractor states than cells further apart in lineage (Figure 1e). This appears to be the case when comparing the time and efficiency of reprogramming the relatively distant fibroblasts with iPSCs (10–20 days; 0.5%) vs. hematopoietic reprogramming of pre-B cells to macrophages (2–3 h; approaching 100% efficiency).[37,38] Adult progenitor cells such as myeloid progenitor cells or pro-B cells can be reprogrammed to iPSCs with 300 times greater efficiency than their more differentiated progeny.[39] Similarly, reprogramming efficiency is increased 50-fold when converting neural stem cells to iPSCs.[40] Another prediction is that a cell undergoing reprogramming need not dedifferentiate and then redifferentiate; rather, it may directly transdifferentiate with the intermediate cell phenotype expressing markers of both the original and final cell types (Figure 1f). Hence, the ball can be pushed over a hill from one valley to the next without having to go back up to the valley and down another. Indeed, reprogramming from pre-B cell to macrophage does not involve the reexpression of pre-B cell precursor markers, but instead a gradual shift in gene expression from the start to end state.[38] This is accompanied by the downregulation of chromatin-associated factors, illustrating that reprogramming involves chromatin reorganization.[41–43]

REPROGRAMMING THE KIDNEY: WHAT ARE THE OPTIONS?

Given the myriad of cell types affected during kidney disease, what cell type should we aim to create? In diseases such as

type 1 diabetes or Parkinson's disease, replacement or restoration of the function of a single cell type (β-islet cell or dopaminergic neuron, respectively) may be sufficient to rescue the phenotype. In contrast, the treatment of renal disease would require the replacement or restoration of the many functionally distinct cell types of the nephron. In addition to the cellular complexity of the nephron, the pathology of the various forms of kidney disease can vary widely. In some cases, this may lend itself to distinct approaches for distinct disease presentations, but where complete nephron replacement is required a large number of different cell types would be needed. It would not seem feasible to generate each of these via reprogramming and then redeliver all *in vivo*, nor would it seem feasible to generate so many different cell types with the one *in vivo* reprogramming strategy as each phenotype would require different genes to elicit the appropriate attractor state. Ideally, the creation of a nephron stem/progenitor cell population would circumvent this problem by giving rise to all the cell types of the nephron. Subsequent differentiation to multiple end points would then be possible. Even here, there is more than one strategy for induction. These include directing the differentiation of a pluripotent stem cell toward a renal lineage or dedifferentiating/transdifferentiating an adult cell directly to a renal cell. For each of these options, there are a variety of starting and target cell options, as illustrated in Figure 2.

In the case of *directed differentiation* to a renal lineage, the best starting cell population would be patient-derived iPS cells, which represent a genetic match (see Table 1). The recent studies by Song *et al.*[10] and Zhou *et al.*[11] have for the first time demonstrated that it is feasible to take an adult kidney-derived cell back to a pluripotent state. This observation, in and of itself, does not provide a way of treating kidney disease. An iPSC derived from a mesangial cell is likely to be functionally equivalent to an iPSC derived from blood or skin, and it is easier to obtain patient samples for the latter. There is some evidence of a retention of epigenetic memory, suggesting that a kidney cell–derived iPSC is more easily returned to the kidney than a neural-derived iPSC and vice versa;[44] however, this may represent incomplete reprogramming. Even if this is the case, the delivery of undifferentiated iPSCs or hESCs back into a patient would result in teratoma formation, and thus onward differentiation from the iPSC state is required. The literature is reporting an increasing number of protocols for the directed differentiation of human pluripotent cells (ESCs and iPSCs) toward a variety of lineages and cell types. Perhaps of importance for the kidney is the reported success of directed differentiation of human ESCs and iPSCs to specific hematopoietic lineages.[45,46] The hematopoietic system is mesodermal in origin and arises from the intermediate mesoderm, as does the kidney. Piggybacking on some of the early steps of blood differentiation may open the way for directed differentiation to kidney cell types. There have also been a small number of reports indicating that kidney may be a feasible destination based upon the induction of appropriate gene expression in mouse ESCs treated with RA/Activin-A and BMP7[47] and evidence for spontaneous formation of Podxl1+ glomerular structures in teratomas formed from human ES cells.[48] As encouraging as these studies are, it is important to note that the differentiated cells are not analogous to their *in vivo* counterparts. As such, further optimization of these procedures is necessary before stepwise differentiation of the mesoderm into functional kidney cells can be claimed.

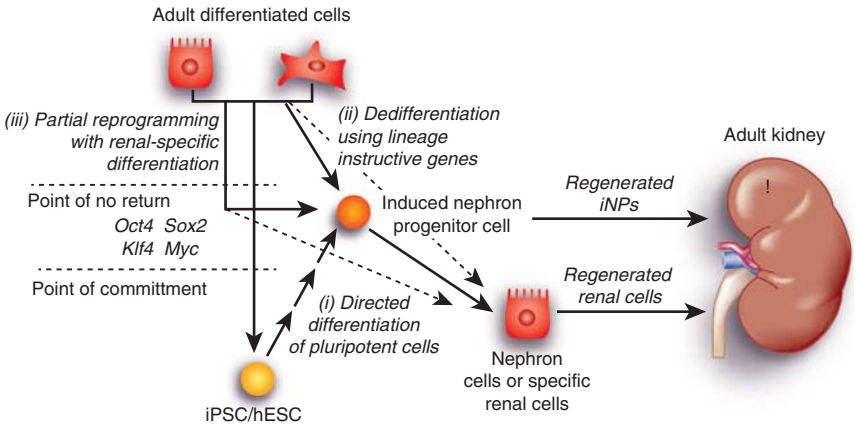

Figure 2 | The application of reprogramming to the kidney, indicating the feasible starting cells and target phenotypes. (i) Reprogramming may involve the directed differentiation of human embryonic stem cells (hESCs)/induced pluripotent stem cells (iPSCs) to a renal lineage. The iPSCs may be recipient-derived and may be derived from adult kidney cells or any other available adult cell type using the same factors. Directed differentiation is likely to recapitulate development; hence, it is likely to require differentiation through a nephron progenitor intermediate (induced nephron progenitor cell; iNP) but may continue on to more specific mature renal cell types. (ii) A specific renal lineage may also be achieved via lineage-instructive reprogramming directly to that state from an adult cell type. Again, this may be the renal epithelium, renal stroma, or any other available differentiated adult cell type; however, this is likely to be more successful if the attractor states of the starting and target cell type are as close as possible. Reprogramming may be to the iNP state or directly to a more mature renal cell fate. (iii) Finally, reprogramming may use the classical Yamanaka factors until the cells pass the point of no return, after which a renal lineage may be reached via the application of the appropriate environmental cues. Such cues may once again target the iNP state or aim to directly induce a more mature renal cell type.

Table 1 | Three different options for cellular reprogramming

	Directed differentiation	Lineage reprogramming	Culture-induced transdifferentiation of partially reprogrammed cells
Source of starting cells	ESC or iPS cells	Any cell; most commonly fibroblast or hematopoietic cell	Any cell; most commonly fibroblast or hematopoietic cell
Genes involved	Nil. Usually involves small compounds or growth factors thought to recapitulate normal differentiation toward the desired end point	Specific transcription factors able to direct a cell to a specific target attractor state	Yamanaka factors (Oct4, Sox2 ± Klf4, c-myc)
Advantages	Entirely based on culture conditions, therefore no genetic manipulation Stepwise induction allows for optimization of efficiency at each step If starting with iPS, possible to generate from the target patient thereby ensuring a genetic match Ultimately, may include genetic repair of defective genes before differentiation and delivery	Knowledge of intermediate cell culture conditions is not required Cells can transit directly from one phenotype into another Starting cell population could be directly and readily sourced from target patient	Does not rely on knowledge of target cell gene regulatory network Starting cell population could be directly and readily sourced from target patient
Disadvantages	No established differentiation protocols for moving from human pluripotent state to functional kidney cell Optimal final culture conditions for target cell undefined May include multiple steps each requiring different factors to induce a stepwise differentiation	Optimal final culture conditions for target cell must be defined Technique relies on knowledge of the target cell gene regulatory network Requires introduction or reactivation of genes. This would ultimately need to be a removable system to be approved for use in man	Specific culture conditions able to select for target cell type must be established Requires knowledge of the kinetics of partial reprogramming in order to define the time window for transdifferentiation Has the potential to also generate unwanted pluripotent cells Requires introduction or reactivation of genes. This would ultimately need to be a removable system to be approved for use in man

Abbreviations: ESC, embryonic stem cell; iPS cell, induced pluripotent stem cell.

GOING DIRECTLY FROM NON-KIDNEY TO KIDNEY

The alternative approach, *lineage reprogramming* to the preferred renal cell type directly from an adult cell, is growing in credibility (see Table 1). Here the starting cell is an adult cell, frequently a skin fibroblast, but the generation of a pluripotent intermediate is not required. Instead, lineage-instructive genes are overexpressed to drive the starting cell toward the target attractor state. There are some caveats to the current approach. Although most studies reporting iPSC induction have started with primary cell isolates, much of the classic lineage reprogramming studies have been conducted in immortalized cell lines. Indeed, immortalized fibroblasts are easier to reprogram than their primary counterparts.[49] If the ultimate intention is a cellular therapy, reprogramming must be performed using primary cells. Another key feature of the field has been the focus on generating target cell types that are mature differentiated cells. This has the obvious advantage that the target cells have readily identifiable phenotypic characteristics (e.g., beating cardiomyocyte; action potential–positive neurons) and frequently a well-characterized attractor state, thereby identifying the specific genes required. The generation of a progenitor cell type, such as a nephron progenitor in the case of the kidney, has been less common; however, several studies have now reprogrammed fibroblasts directly to tissue-specific progenitor cells using a combination of forced gene expression of carefully controlled culture media conditions.[50,51]

CHOOSING A TARGET CELL TYPE: THE NEPHRON PROGENITOR

Having discussed the reprogramming options now available, we return to the issue of deciding what target cell to make (Figure 2). In diseases such as type 1 diabetes or Parkinson's disease, replacement or restoration of the function of a single cell type (β-islet cell or dopaminergic neuron, respectively) may be sufficient to rescue the phenotype. For the kidney, the choice of a target cell type is a major challenge. The entire epithelium of the nephron arises from a single cell type, the nephron progenitors of the developing kidney. The nephron progenitor population is a developmentally specific pool of condensed mesenchymal cells called cap mesenchyme (CM) located around the tips of the branching collecting duct epithelium.[52,53] These cells behave as stem cells, undergoing self-renewal in order to maintain the CM. The nephrons form from these cells via a mesenchyme-to-epithelial transition (MET) to form an epithelial renal vesicle. This structure elongates to form comma-shaped and S-shaped bodies, which then pattern and segment to form the functional nephron comprising glomerulus, proximal tubule, loop of Henle, and distal tubule. In the mammalian kidney, these nephron progenitors are exhausted via differentiation before birth.[54,55] Although this population does not persist in the postnatal kidney,[56] lineage reprogramming of human cells to the nephron progenitor phenotype represents an attractive possibility for the creation of a cell source either able to elicit *de novo* nephrogenesis or be

differentiated further into multiple renal cell types. The first barrier is to define the attractor state of this target cell type.

WHAT GENES WILL BE REQUIRED TO INDUCE NEPHRON PROGENITORS?

The molecular networks thought to regulate CM specification, self-renewal, and differentiation are dynamic and complex and have been reviewed extensively elsewhere.[57] Some of these genes will be involved in specification of the CM, whereas others may promote self-renewal, prevent death, or prevent epithelialization. For reprogramming purposes, it will be important to distinguish between transcriptional regulators that are necessary for nephron progenitors and those that are both necessary and lineage instructive. For example, *Sall1* is expressed in nephron progenitors[58] and is necessary for kidney development but may not be lineage instructive, as overexpression of *Sall1* in the committed Wnt4$^+$ portion of the CM had no effect on the differentiation of this subcompartment.[59] Fortunately, it is unlikely that every lineage-instructive factor will be required for reprogramming. As long as sufficient stimuli are provided to activate the endogenous gene regulatory network, the cells may be gradually pulled into the correct attractor state. Indeed, this has been the case in iPSC reprogramming, where certain factors can be replaced or even eliminated altogether.[4,60,61]

Many successful reprogramming studies have commenced with a large number of potential lineage-instructive genes. Indeed, the initial identification of factors able to induce pluripotency started with a candidate list of 24 genes that were ultimately narrowed to 4.[3] As this will necessarily involve screening many combinations, the development of a high-content, robust screen of the target phenotype is a major priority. This represents a barrier to the induction of any target renal cell type, including nephron progenitors. The CM 'niche' is largely undefined, and there have been no successful reports of the long-term survival of isolated CM *in vitro*.[56,62,63] Both fibroblast growth factor and bone morphogenetic protein signaling appear to be important in survival of the CM.[64–66] However, successful reprogramming does depend on the provision of an appropriate environment.[1,67] If the culture conditions do not replicate the niche present within the nephrogenic zone of the developing kidney, then even an appropriate set of reprogramming genes may not induce nephron progenitor formation.

IT IS NOT THE JOURNEY BUT KNOWING WHEN YOU GET THERE

According to reprogramming theory, there is no limit to the distance that cells may traverse or the lineage boundaries they may cross in order to change phenotype. However, there is variability in the efficiency of iPSC reprogramming depending upon the starting cell type, and the force with which you need to impose a specific attractor state may vary. What is critical is to prove that your reprogramming genes deliver the desired phenotype and not some other end point. The key to success appears to depend on two things: (i) the specificity of the reprogramming genes to the desired attractor state and (ii) the specificity of the assay for end point. Many transcription factors expressed within the CM have other developmental roles both in kidney, non-renal, and, indeed, non-mesodermal tissues. Pax2, Eya1, and Six1, for example, have all been implicated in the development of the inner ear,[68–70] as well as the brain and cranial placodes.[71,72] Six1 and Eya1 are both implicated in the development of the thymus[73,74] and in somitogenesis.[75] This again emphasizes the need for a stringent readout of the phenotype desired at the end of the reprogramming event. Complete reprogramming to an induced nephron progenitor phenotype must be assessed functionally, as well as morphologically. However, there is a lack of informative functional assays for nephron potential in the field. Most assays have been developed to assess the ability of an adult stem cell type to integrate into an existing nephron in response to renal injury or to form renal tubular structures under the renal capsule.[76–78] This approach is not ideal, as it does not necessarily provide the correct developmental context for nephron progenitor survival and differentiation. We and others have developed recombination assays in which dissociated embryonic kidney is used to provide the necessary developmental context to test integration and contribution of nephron progenitors to an endogenous CM.[56,79] By using this assay, we have been able to analyze the functional contribution of test cell populations to the nephron progenitor compartment of *ex vivo* organoid cultures.[56]

MATCHING CELL TYPE TO DISEASE STATE

A major challenge would be whether induced nephron progenitors were able to survive in a postnatal kidney, let alone contribute to existing adult nephrons and/or generate new nephrons. The observation that neonephrogenesis from a persistent nephron progenitor population does occur in the adult zebrafish, including normal patterning, segmentation, and fusion with the collecting duct, brings hope.[80] In mammals, the reactivation of key developmental genes, such as Pax2 (ref. 81) and others,[82] does imply that recapitulation of development within the tubular epithelium itself might be feasible, but neonephrogenesis may not. If an induced nephron progenitor population cannot survive and differentiate in the adult kidney, it may be necessary to choose a different target cell. As noted previously, different cells may be required for different renal disease states. Table 2 identifies the distinct cell types affected in a variety of hereditary renal diseases. Coupling gene correction with reprogramming to the specifically required differentiated cell type may finally provide a strategy for the treatment of inherited renal disorders, including ciliopathies and nephrotic syndromes. However, the introduction of such cell types into the mature kidney remains a formidable barrier. Although we know that the Six2$^+$ CM gives rise to all other cells within the mature nephron, whether there is a subsequent hierarchy of segment-specific or pan-nephron epithelial progenitors that exist between the CM state and the mature cell types is not known. However, such intermediate cell types (immature podocyte, immature proximal tubule

Table 2 | Defining the appropriate target cell for different renal diseases

Renal disease	Podocyte[a]	Pan-nephron epithelial progenitor[a]	Proximal tubular epithelium[a]	Distal tubular/ LOH tubular epithelium[a]	Collecting duct epithelium	Mesangial cells
Polycystic kidney disease (ADPKD, ARPKD)		■	■	■	■	
Alport syndrome, nephrotic syndrome (NPHS1/2), other podocyte defects	■					
Cystic diseases; ciliopathies		■	■	■	■	
Dent's disease, cystinosis, Lowe syndrome			■			
Gitelman syndrome, distal tubular acidosis				■		
Diabetes insipidus					■	
Denys Drash syndrome; Frasier syndrome, other inherited forms of FSGS or MS	■					■

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; FSGS, focal segmental glomerulosclerosis; LOH, loop of Henle; MS, mesangial sclerosis.
Different hereditary defects affect different renal cell types.[86] Shading represents the preferred target cell type to be generated via reprogramming for the treatment of each of these forms of renal disease.
[a]Derived from cap mesenchyme/nephron progenitors.

cells, pan-nephron epithelia) may also represent promising target cells for reprogramming strategies. Such intermediate cell types that are already epithelia may be more easily encouraged to adopt appropriate differentiated states if reintroduced into an adult tissue. We have begun to define the expression profile of intermediate states of early nephron epithelia[83] potentially defining appropriate lineage-instructive genes for such reprogramming targets. The knowledge in the field with respect to the growth factors able to support distinct tubular segments will also be valuable in attempts to reprogram to these more specific target cell types.

CUTTING TO THE CHASE: ANOTHER OPTION

To date, we have proposed directed differentiation or lineage reprogramming as options for generating nephron progenitors. There is, however, a third option now proving feasible: *culture-induced transdifferentiation of partially reprogrammed cells* using the four iPSC factors (see Table 1). The induction of pluripotency using *Oct2*, *Sox2*, *c-myc*, and *Klf4* does not appear to involve an ordered retracing of the specific differentiative events experienced by that starting cell population during embryogenesis. Indeed, the expression of the reprogramming factors is no longer required once the epigenetic state of the donor cell moves that cell out of its initial attractor state. The withdrawal of the reprogramming factors after this 'point of no return' places the cells in a state of indecision facetiously referred to as 'Area 51'.[84] Guaranteed progression to the successful generation of iPSCs requires a longer period of expression of the four key reprogramming factors past a point referred to as the 'point of commitment'. Nagy and Nagy[84] predicted that rather than these genes specifically imposing pluripotency, they created an epigenetic state in which other factors played a role in dictating the outcome for individual cells. This is supported by observations of occasional colonies of beating cardiomyocytes arising spontaneously during the generation of iPSCs.[85] This also predicts that cells in this state may be diverted to alternative fates, potentially allowing the generation of a wide variety of cell phenotypes using the same four genes. This prediction has proven accurate with the conversion of fibroblasts to cardiomyocytes, neuronal progenitors, and hematopoietic progenitors using some or all of the same genes as those used to generate iPSCs.[50,51,85] In such studies, the key determinant of ultimate cellular phenotype was the culture conditions provided to these cells during reprogramming, not the transcription factors themselves. For example, hematopoetic progenitors were generated via Oct4-mediated reprogramming with subsequent culture in stem cell factor and FMS-like tyrosine kinase 3 ligand.[50] The implications for the kidney are exciting. This approach removes the need to define the specific transcriptional network required for reprogramming to a specific attractor state, requiring instead an appropriate media able to select for the renal cell type required. An understanding of the growth factors involved in defining and maintaining the required cell type is needed for this approach. A strong identifying phenotype, such as beating in a culture dish in the case of cardiomyocytes, would also make this approach more likely to succeed. In some instances, such information is already available for more mature renal target cell populations, such as the podocyte. In contrast, the challenge of this approach for the generation of nephron progenitors is again our lack of understanding of the growth factors able to support and sustain this phenotype.

CONCLUSION

We have discussed here a variety of nuclear reprogramming options for the generation of nephron progenitors or more mature renal cell types. Although these would have been deemed fiction 5 years ago, the pace with which the field advances makes it likely that one or more such approaches will prove fruitful for the renal field in the short term. There remains a chasm between such scientific breakthroughs and

clinical delivery. Nevertheless, for the renal failure patient, the hope that comes with potential changes in treatment options is substantial.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

CEH was supported by a Rosamond Siemon Postgraduate Scholarship. MHL is a Principal Research Fellow of the National Health and Medical Research Council supported by the National Institutes of Digestion Diabetes and Kidney, NIH (DK070136, DK070136), the NHMRC (ID631362), and Australian Stem Cell Centre funding to MHL.

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