BRIEF REVIEW www.jasn.org

Human Kidney Cell Reprogramming: Applications for Disease Modeling and Personalized Medicine

Adam C. O'Neill* and Sharon D. Ricardo†

*Department of Pediatrics, Dunedin School of Medicine, Otago University, Dunedin, New Zealand, and †Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia

ABSTRACT

The ability to reprogram fully differentiated cells into a pluripotent embryonic state, termed induced pluripotent stem cells (iPSCs), has been met with great excitement. iPSC technology has advanced the fundamental study of disease modeling with the potential for cell-replacement therapy, especially in the neuronal and cardiac fields. However, renal medicine as of yet has not benefited from similar advancements. This review summarizes the unique characteristics of iPSCs and their potential applications for modeling kidney disease. Pioneering such endeavors could yield constructs that recapitulate disease phenotypes, open avenues for more targeted drug development, and potentially serve as replenishable sources for replacement of kidney cells in the setting of human disease.

J Am Soc Nephrol 24: 1347–1356, 2013. doi: 10.1681/ASN.2012121199

Through experimentation involving nuclear fusion came the realization that differentiated somatic cells have potential to show a plasticity that is not unidirectional.[1–3] Subsequent studies suggested the transfer of a single somatic cell nucleus into an enucleated unfertilized egg possessed the ability to not only form all three germ layers but also produce viable offspring.[4–6] Decades later, the direct reprogramming of fibroblasts into a pluripotent state, so-called induced pluripotent stem cells (iPSCs),[7,8] has renewed interest in what constitutes the reprogramming process. An explosion of subsequent studies confirms that a large variety of somatic cells can be efficiently reprogrammed into iPSCs[9–13] and subsequently redifferentiated into other cell types that recapitulate disease phenotypes.[14–16] Such information offers proof-of-principle for the use of iPSCs as useful *in vitro* modeling systems that could ultimately lead to novel drug development and testing. Additionally, as iPSCs are produced from individual patients, the derivation of patient-specific stem cell lines could provide a limitless source of clinically useful immune and genetically matched cells.

Since the pioneering discovery by Takahashi and Yamanaka,[7,8] iPSCs have now been successfully generated from a wide array of human tissues.[14,16–20] Despite such advances, cell reprogramming with respect to the kidney remains in its infancy. Only recently has it been possible to derive iPSCs from kidney mesangial cells[21] or epithelial cells sourced from urine.[22] Furthermore, the directed differentiation of mesangial cell-derived iPSCs to podocyte-like cells (iPSC-podocyte)[23] and the generation of iPSCs from kidney disease patients has only recently been reported.[24]

Here we review our current knowledge regarding the use of pluripotent stem cells targeted at kidney disorders. Specifically, it will address certain shortcomings of traditional model systems, current knowledge regarding the differentiation of pluripotent stem cells into the kidney mesodermal lineage, and the advantages of reprogramming for *in vitro* disease modeling and therapeutic interventions. Finally, the efficiency and safety of iPSC technology that governs the prospective applications and clinical promise for kidney regeneration will also be discussed.

KIDNEY REGENERATION AND CELL REPLACEMENT

The kidney is a highly complex organ with many different cell types, including tubular epithelial, glomerular, and interstitial cells. Additional complexity exists within distinct compartments of the nephron, which possess divergent regenerative capabilities after kidney insult. For example, the tubular epithelium has the highest potential for self-renewal,[25,26] whereas replacing glomerular cells, in particular podocytes, remains challenging.[27,28] Glomerular podocytes display a complex cytoarchitecture and appear to enter cell quiescence after birth, a characteristic that makes podocyte replacement after injury difficult.[29–31] Podocyte damage results from many

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Prof. Sharon D. Ricardo, Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria 3800, Australia. Email: Sharon.ricardo@monash.edu

factors, including genetic, immunologic, toxicologic, and mechanical insults, and as such podocyte depletion remains a hallmark of a broad spectrum of clinical syndromes termed podocytopathies.[32–34] In addition to the primary insult to podocytes, secondary damage of neighboring podocytes may result in a vicious cycle of progressive damage.[35]

Despite the minimal regenerative potential of glomerular podocytes, the kidney has an inherent ability for endogenous remodeling of tissue architecture and cellular replacement after injury.[36] The kidney's repair response, consisting of cellular replacement of the injured tubular epithelium, does not involve specialized kidney progenitors.[37] Epithelial cell replacement most likely arises from surviving cells capable of intrinsic proliferation and expansion. Moreover, endogenous tubular epithelial cellular replacement and tissue remodeling may be accelerated by administering bone marrow–derived mesenchymal stem cells that home to the sites of damage and modulate the inflammatory responses to facilitate tissue repair.[38–43] Furthermore, $CD133^+$ and $CD24^+$ renal progenitors committed toward a podocyte or tubular lineage reside at the urinary pole of the Bowman capsule and provide a source of replacement cells during normal homeostasis and after injury.[44–47]

Understanding the process of endogenous kidney regeneration is important for the development of new therapeutic strategies aimed at cellular replacement and reversal and/or attenuation of fibrosis. As such, the reprogramming of adult cells to generate iPSCs[7,8,48] with high proliferative ability and broad differentiation capacity represents a major advance for both preclinical and clinical applications.

PLURIPOTENT STEM CELLS TARGETED AT THE KIDNEY

Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent and thus could offer important alternative avenues for regenerative therapies through differentiation into distinct kidney cell types. Pioneering studies in *Xenopus laevis* show presumptive ectoderm (animal cap) can be induced to produce all three components—tubules, glomus, and ducts—of the pronephros when cultured with known mesoderm-inducing factors, such as activin A and retinoic acid.[49–51] In light of such success, subsequent studies using the same mesoderm-inducing factors have been applied to the mammalian metanephric kidney.[52–55] It was apparent not only that the same mesoderm-inducing factors could direct the differentiation of embryonic bodies into various renal progenitors *in vitro*[52–55] but also that such cells when injected *ex vivo* into developing mouse kidneys could integrate into tubules with at least 50% efficiency (reviewed in Table 1).[53,56] Nonetheless, whether the ESC-derived progeny contributes to function of the nephron is not yet evident.

Because of the vast pluripotent potential of undifferentiated human ESCs (hESCs), teratoma formation results after their injection *in vivo*.[54] Thus, the therapeutic applications resulting from this technology have been slow to develop. In an attempt to circumvent deleterious consequences of pluripotency, a study by Vigneau and colleagues differentiated ESCs into renal progenitors and subsequently enriched for such populations. Injection of these enriched kidney progenitors into newborn kidneys reduced the risk of teratoma formation.[55] Since these discoveries, more recent work has identified the role of other lineage-specifying factors, such as bone morphogenic protein (BMP)-2, BMP-4, and BMP-7[57] and other small molecules[58] in the establishment of intermediate mesoderm, from which most nephron-specific cell types are derived. Furthermore, the exploration of specific culture conditions and cell markers demonstrates that it is possible to enrich for cells resembling a kidney progenitor state (Table 1).[59]

Extending previous *in vitro* work,[60] a recent report by Xinaris and colleagues provides novel advancement toward the therapeutic application of embryonic tissue in the potential treatment of renal diseases.[61] In this report, renal aggregates (organoids) constructed from single cell suspensions of fully dissociated embryonic day (E) 11.5 mouse kidneys grown in culture were subsequently transplanted into immune-deficient rats. Remarkably, the implanted organoids were capable of generating vascularized nephrons consisting of functional glomerular and tubular tissue (Table 1).[61] Although the transplanted tissue survived for only 3–4 weeks, the ability to grow and mature developing fetal kidney tissue *in situ* offers advantages, including the ability to better understand the gene regulatory networks required for both early and late kidney lineage specification and ascertainment of the local microenvironment necessary for renal stem cell differentiation and nephrogenic maintenance. With the organoids already committed down a lineage of kidney development, such an application relieves the need for an in-depth knowledge of the gene regulatory networks necessary for direct reprogramming, a feature that is a prerequisite for any directed differentiation of pluripotent cells into kidney lineages. Despite these advances, ethical constraints surrounding hESCs have limited their widespread use in disease modeling and other applications, forcing the investigation into alternative solutions.

iPSCs

The landmark discovery by Takahashi and Yamanaka in mice[7] and humans,[8] involving the expression of four transcription factors (*Sox2*, *Oct4*, *c-Myc,* and *Klf4*) that direct the reprogramming of fibroblasts into a pluripotent state, has revolutionized the field of stem cell biology (Figure 1). Since the discovery of iPSCs, extensive investigation has focused on the derivation of new cocktails of factors that give rise to such pluripotent cells more efficiently. Such cocktails involve both integrating and nonintegrating methods, combined with growth factors and chromatin-modifying elements.[62]

Although iPSCs and hESCs show similarities in terms of transcription, epigenetics, self-renewal, and pluripotent

Table 1. Summary of renal progenitor cell production from ESCs and iPSCs

Source	Factors Involved*	Findings	Reference
Xenopus	Activin A and RA	*In vitro* culture of presumptive ectoderm with factors involved facilitated pronephric tubules production.	49
Xenopus	Activin A, RA, FGF	*In vitro* treatment of presumptive ectoderm with factors involved induced pronephric glomus production.	50
Xenopus	Activin A, RA	*In vitro* culture of presumptive ectoderm treated with factors involved caused pronephric ducts production.	51
Human	Activin A, bNGF, HGF, RA, bFGF, EGF, BMP4, TGF-β1	*In vitro* culture of hESCs treated with the factors involved form cells of the mesodermal lineage and express renal markers WT-1 and renin.	52
Mouse	Activin A, RA, BMP7	*In vitro* EBs cultured with the factors involved form renal epithelial progenitor cells. *Ex vivo* injection of these EBs into developing kidney contributed to the generation of tubular epithelia with near 100% efficiency.	53
Mouse	Activin A, Wnt4, HGF	Wnt4 expressing EBs cultured *in vitro* with the factors involved can promote differentiation into renal tubular cells. *In vivo* injection of these cells into mouse kidney resulted in teratoma formation.	54
Mouse	Fetal mouse kidney microenvironment	*Ex vivo* injection of ESCs into mouse embryonic kidneys can integrate into tubules with 50% efficiency.	56
Mouse	Activin A	*In vitro* culture of EB with factor involved coupled with FACS selection enabled enrichment for progenitor cells in proximal tubule progenitors and collecting tubule production. *In vivo* injection of enriched renal progenitor cells in newborn mouse kidneys allowed integration into renal tubular structures without teratoma formation.	55
Mouse	BMP2, BMP4, BMP7	*In vitro* differentiation of ESCs into intermediate mesoderm.	57
Mouse	Combination of small molecules	*In vitro* culture of the factors involved induced differentiation of ESCs to intermediate mesoderm, which forms a vast number of renal cell types.	58
Human	Low serum concentration, reduced density of MEF feeders	*In vitro* culture of hESCs coupled with FACS selection on certain renal markers. Transcriptional profiling further showed enrichment for kidney development genes to be apparent.	59
Human	Activin A, RA, BMP7	*In vitro* culture of the factors involved induced differentiation of human iPSCs into cells with podocyte features. Such cells shared morphologic and functional characteristics similar to that of primary human podocytes and were also able to integrate into developing glomeruli.	23
Mouse	VEGF, mouse kidney microenvironment	Single cell suspensions produced from fully dissociated embryonic day 11.5 mouse kidneys were cultured *in vitro* to form aggregates (organoids). Organoids were treated with VEGF and implanted beneath the renal capsule of immune-deficient rats. Markers of mature nephron segments were appropriately expressed, and nephron-specific functions were observed in the implanted tissue. Organoids were only viable *in vivo* for 3–4 wk.	61

RA, retinoic acid; FGF, fibroblast growth factor; BMP7, bone morphogenic protein 7; bNGF, β nerve growth factor; HGF, hepatocyte growth factor; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; BMP2, bone morphogenic protein 2; BMP4, bone morphogenic protein 4; WT-1, Wilms tumor 1; EB, embryonic body; VEGF, vascular endothelial growth factor.

potential, they are not identical.[63] Growing evidence demonstrates that genetic, epigenetic, and transcriptional differences exist among these pluripotent cells.[64–66] One obvious difference is the presence of epigenetic memory, whereby the reprogrammed cell retains a profile reminiscent of its somatic cell of origin.[67,68] Large-scale comparisons between iPSCs and hESCs further show that the former fails to efficiently silence the expression pattern of the somatic cells from which they were derived, potentially affecting the models reliability for disease modeling and drug discovery.[63] The extent of this memory has been reported to diminish, however, with extended passaging.[67]

Functional consequences for this memory also persist, whereby an inability to silence the original expression pattern equates to a tendency for iPSCs to preferentially differentiate back to the parental cell type.[69] Thus, the potential exists for the residual epigenetic memory to be exploited in cases where limited knowledge regarding the gene regulatory networks involved in the production of renal progenitor cells is present—like that for the nephron. However, the difficulty in collecting viable kidney tissue hinders the ability to exploit such features. Innovative approaches, such as the isolation of various kidney cells from urine for cell reprogramming,[22] have overcome such obstacles, making the ability to derive numerous patient specific iPSCs more feasible.

A recent report representing the largest of its kind to investigate factors that

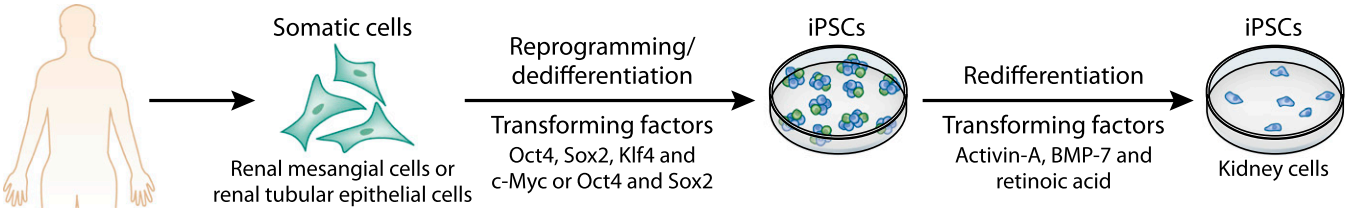

Figure 1. Disease-in-a-dish: recent advances in somatic cell reprogramming of kidney cells. iPSCs can be derived from both mesangial cells and urinary epithelial cells using either the traditional four factor (*Oct4*, *Sox2*, *Klf4*, and *c-Myc*) or two factor (*Oct4* and *Sox2*) methods. The reprogramming of kidney cells to iPSCs that have a high proliferative ability and broad differentiation potential holds promise for both preclinical and clinical applications. For example, the directed differentiation of iPSCs to podocytes using conditioned media and morphologic selection has been successfully reported.

may affect the ability of pluripotent cells in modeling potential diseases shows that genomic sites previously thought to be stable, in effect, imprinted loci and X-chromosome inactivation, actually differ from the reprogrammed somatic cells.[70] Because of the scope of the study, the authors were able to attribute many of the abnormal DNA methylation sites to the reprogramming process itself or specific culture conditions. Such genomic instabilities thus warrant a degree of caution, particularly in cases where the disease is sensitive to imprinting or X chromosome inactivation status.[70]

IPSCS FOR DISEASE MODELING AND MORE TARGETED DRUG THERAPY

Disease Modeling

Given the numerous proof-of-principle studies showing the ability of iPSCs to recapitulate the molecular basis and pathogenesis of multiple diseases,[14–20,71–75] it is increasingly apparent that direct reprogramming might allow for the effective generation of pluripotent cells that carry the disease-associated genotype.

The surrogate application of iPSCs as representative of kidney diseases is increasingly becoming reality given (1) recent advances involving the production of iPSC from both mesangial[21] and epithelial cells derived from urine[22] using the four traditional reprogramming factors (*Sox2*, *Oct4*, *Klf4*, and *c-Myc*), (2) the identification that certain kidney diseases do not hinder the reprogramming process,[24] (3) the generation of iPSCs from renal proximal tubular cells with only two factors (*Sox2* and *Oct4*), and the recent reports of reprogramming of iPSCs into iPSC-podocytes (Figure 1).[23] Primary human podocytes show rapid dedifferentiation *in vitro*, and because of their limited capacity to proliferate, represented by irreversible growth arrest, long-term propagation remains difficult.[76] In contrast, iPSC-podocytes maintain differentiated features for at least 3 months.[23]

The production of iPSC-podocytes, and potentially epithelial cells, allows an alternative culture method that facilitates both quantitative and qualitative interrogation of kidney cell function in disease. Despite these advances, numerous challenges hinder the development of *in vitro* iPSC-kidney disease models. These include the immense complexity owing to the involvement of myriad interwoven kidney cell types, the pathologic heterogeneity among various forms of kidney diseases, and a lack of knowledge regarding the developmental gene networks involved in directing the efficient production of specific kidney cells. Despite such complexity, it is essential to focus efforts on whether iPSC-kidney cell lines recapitulate disease processes across a range of renal disorders (Figure 2A).

Aside from avoiding the ethical dilemmas associated with use of hESCs, iPSCs provide particular advantages for modeling disorders where the cause of disease is unknown. Patient-specific iPSCs allow for the study of disease-specific pathogenesis *in vitro* and in the long term could conceivably provide alternatives for cellular replacement therapies (Figure 2D). Using lentiviral transduction, iPSCs have been generated from kidney transplant recipients with a history of ESRD due to autosomal dominant polycystic kidney disease, systemic lupus erythematosus, and Wilms tumor.[24] Importantly, the autosomal dominant polycystic kidney disease–derived iPSCs obtained from skin keratinocytes were confirmed to maintain the *PKD1* gene mutations, although any additional somatic mutations in the tubular epithelium that are important for cyst development may not exist.[24]

It would be necessary for disease-specific iPSCs to retain the disease-related mutation to recapitulate their pathogenic phenotypes. Furthermore, to allow for reliable measurements and effective extrapolation of data to be useful in disease modeling, the choice of controls to compare the diseased line is essential. Such approaches could involve using identical genetic backgrounds to avoid confounding the interpretation of results. Alternatively, correction for the genetic trait in the diseased iPSC line by overexpression of the affected protein or pharmacologic intervention could act as a sufficient control, as could knockdown of the product under investigation by RNA interference in control cells.[77]

Finally, for the purposes of disease modeling, an assay of gene expression should be used at various time points after differentiation. A study conducted by Nguyen and colleagues[74] reported that iPSC-derived neurons obtained from a patient with Parkinson disease showed varying gene expression profiles dependent on the stage of differentiation. Thus, iPSC-derived patterns of gene expression may be similar to that of the developing human counterpart.

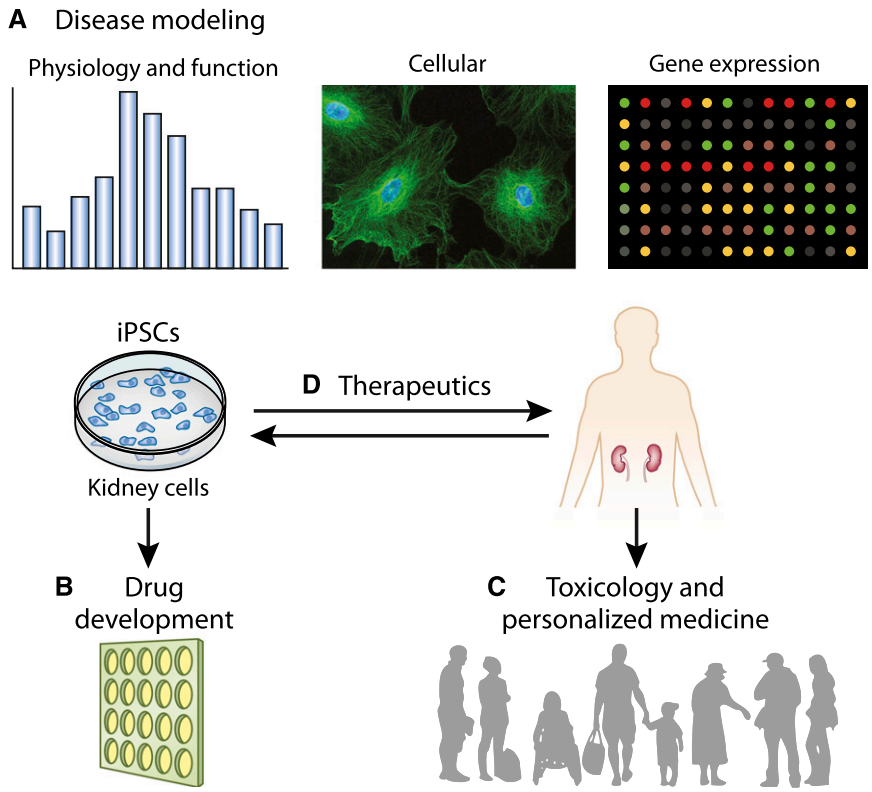

Figure 2. Multiple potentials exist for iPSC-derived kidney cells for studying disease development *in vitro* in order to develop and screen new therapeutics and deliver modified treatment regimes. iPSC-derived kidney cells from controls and patients with kidney disease could be used to measure the qualitative and quantitative aspects of the disease, effectively recapitulating the phenotype *in vitro* (A). Further, iPSC-kidney cells have the potential to become multipurpose research and clinical tools for high-throughput toxicology screening and drug development (B), might be used in population-based screening for toxicologic aspects and personalized medicine (C), and hold potential for disease iPSC-podocyte lines to be corrected and used in cell replacement therapy (D).

The ability for cells derived from iPSCs to recapitulate the disease phenotype could be influenced by such processes.

Therapeutics and Targeted Drug Therapy

The incidence of AKI and CKD is rising and reaching epidemic proportions. In patients with CKD, the progressive decline in renal function is multifactorial and attributable to a variety of mechanisms, including failure to resolve the inciting proinflammatory injury leading to the onset of self-perpetuating damage and ultimately the development of fibrosis and tissue attenuation. With such complexity, the need for disease models that recapitulate human kidney disorders is paramount. Mouse models that are genetically manipulated, bred, and maintained have been vital to advancing medical research and the study of disease pathogenesis. However, given the species-specific differences in physiology, genomics, and metabolic demands that exist between mice and humans,[78–81] application of discoveries in rodents cannot be easily translated, especially with respect to therapeutics, where direct translation is vital.

Equally critical for understanding fundamental mechanisms of disease is the role of cultured cells providing controlled environments for the elucidation of disease mechanisms. Improvements in microimmunodissection techniques have made purification and isolation of specific kidney cells possible.[82,83] Disadvantages exist, however, in primary cell culture, whereby certain cells, such as podocytes, exhibit rapid growth arrest and dedifferentiation, losing their ability to replicate and maintain certain characteristics as effective models.[84] Advances in immortalization of cells have overcome the detrimental growth arrest characteristics, facilitating their use in *in vitro* dissection of physiologic and disease-specific mechanisms.[76,85,86] Despite this, immortalization of cell lines requires genetic manipulation that invariably leads to deleterious downstream consequences and a failure to conserve the native characteristics of the original cell type. For example, proximal convoluted tubules lose their brush-border microvilli upon repeated passaging.[87]

There are many advantages of developing pluripotent cells as a therapeutic strategy for patients with a variety of kidney disorders. Compared with other human kidney cell lines, iPSCs derived from mesangial cells have long-term proliferative ability and potentially a broad range of differentiation capacities, including podocytes.[21,23] Furthermore, noninvasive procedures can be used, such as the collection of urine to obtain the necessary cells for reprogramming.[22,88] However, it remains uncertain whether iPSC-derived kidney cells can be differentiated from target cells of other germ lineages, for example, skin fibroblasts that originate in the ectoderm. Nonetheless, iPSC technology provides a valuable tool to interrogate human disease and offers an unprecedented opportunity to develop self-renewing models that will facilitate mechanistic studies of disease.

The screening of genetic mutations in disease-derived iPSCs and the development of functional assays in cells can also serve as a fundamental step for future studies to correct the genetic defects in selected tubular epithelium or in podocytes that maintain a proliferative capacity. For example, several genes encoding for podocyte nuclear and cytoplasmic proteins, slit diaphragm, and cell membranes have been identified as driving podocyte phenotype and functional abnormalities in subsets of patients with FSGS and nephrotic syndrome. The generation of iPSC-derived kidney cell lines from these patients that maintain

their genotype and phenotype will be a valuable tool for better understanding how mutations cause disease, for screening new drug compounds, for developing disease-modifying assays, and potentially for developing autologous cell replacement therapies. These cell lines may provide information to optimize an affected individual's personalized medical care and open up new sources for stem cell banking. Moreover, in the long term, the autologous transplantation of patient-derived iPSC-kidney cells, with correction of the underlying genetic defect, may provide an innovative approach for kidney cellular replacement.

Finally, the discovery of unexpected adverse reactions and toxicities to new medicines before and during phase I–III clinical trials remains a challenge. With pharmaceutical companies facing increased pressure to speed drug discovery and reduce costs, the development of patient-derived iPSC kidney cells may enhance both productivity of drug development and patient safety. Therefore, the generation of iPSC cells from patients with genetic and nongenetic kidney disease will open new avenues for toxicology testing using high-throughput platforms earlier in the drug discovery process, with high translational power back to the individual (Figure 2B). The ability to easily obtain large numbers of patient-specific tissue could allow for the assessment of why factors cause certain individuals to benefit from a given drug, while others have toxic adverse effects. Personalized drug treatment could then be facilitated by the stratification of populations based on certain factors, such as genetic determinants that result in patients being nonresponders versus responders (Figure 2C).[89,90]

FUTURE CHALLENGES AND CONSIDERATIONS

Although promising in the field of disease modeling and therapeutic discovery, current iPSC technology may not be of any direct therapeutic benefit due to the oncogenic potential of some of the reprogramming factors (*c-Myc* and *Klf4*). Ideally, the use of small molecules that induce reprogramming is favored.[91–93] However, it should be noted that chemicals used could also induce toxicity, including carcinogenicity.

Given that the injection of undifferentiated iPSCs *in vivo* inadvertently results in teratoma formation, additional refinements need to occur for their effective use therapeutically. Investigations into the production of somatic stem cells with a more limited potential for differentiation could circumvent this. The generation of induced neural stem cells by lineage reprogramming[94] (explained below) is a useful example because such somatic stem cells have the ability to differentiate into their daughter cell types.[95] Importantly, induced neural stem cells, when injected into immunosuppressed mice, failed to induce teratoma formation but instead were able to self-renew and also differentiate into numerous neuronal cell types;[94] such a feature is important in their application therapeutically. Additionally, such cells also provide an unlimited source of neurons and other neural cell types for studying.

With respect to the kidney, however, the possibility of a mammalian nephron progenitor stem cell, such as the cap mesenchyme, which gives rise to all epithelium of the kidney,[96,97] being able to survive, differentiate, and contribute to repair after nephrogenesis is distant.[98] Nevertheless, the identification of nephron stem/progenitors in the adult zebrafish provides optimism for a multipotent self-renewing progenitor in the adult kidney.[99]

In addition to iPSC reprogramming, other avenues for kidney cell reprogramming are being investigated,[98] such as those involving lineage reprogramming.[100] Although providing benefits over direct reprogramming of iPSCs, one disadvantage of lineage reprogramming is the requirement for information about target gene regulatory networks in order to transform one cell type into another. The use of six genes in the direct reprogramming of adult proximal tubule cells to nephron progenitors, reminiscent of the embryonic kidney, has recently been reported.[100] This extends the future regenerative potential of stem cells in renal medicine. Direct reprogramming is arguably best suited to the kidney because it relies on information of the optimal culture conditions,[98] which, in the case for certain cell types (such as podocytes), is already available.[23] Another potential advantage of direct reprogramming is differentiation into desired cell types that can potentially be facilitated by the exploitation of the residual epigenetic memory favoring spontaneous differentiation to the parental cell type (discussed above).[69] Future endeavors should aim to determine whether kidney-derived iPSCs show a tendency to redifferentiate back into the parental cell type. Understanding what genes are necessary for this spontaneous redifferentiation may provide further information on the regulatory networks sufficient for nephron and progenitor cell production. Although limited, methods aimed at efficiently inducing mouse ESCs into renal progenitors and fully differentiated cell types have already been developed (Table 1).[53–55,57–59] Hijacking the knowledge of such technology to generate mesodermal and renal lineage cells from iPSCs could be possible.

Finally, it is yet to be explored whether dedifferentiation and redifferentiation processes induce changes in the resulting somatic cell that are different from the parental cell type, such as changes involved in proliferation and senescence that give iPSC a more immature phenotype. Differentiation of iPSCs into cardiomyocytes provides evidence of this,[101] where these differences could potentially confound any results obtained in the disease modeling and drug discovery potentials.

CONCLUDING REMARKS

Although knowledge that differentiated cells can be reprogrammed into a pluripotent state, or indeed another lineage, has been known for decades, the recent interest in this phenomenon can be attributed to the latest developments in

somatic cell reprogramming over the past several years. Effective disease models and drug screens are the current focus, but more ambitious applications with respect to regenerative medicine will follow. In contrast to many other fields, the kidney is yet to substantially gain from such technology. The accumulation of recent reports describing the manipulation of iPSCs for applications in kidney disease modeling act to address certain shortcomings that hinder their widespread use, such as the derivation of iPSCs from epithelial cells isolated from the urine. With so much left to learn, future challenges will involve mapping the redifferentiation of iPSCs into the many cell types of the kidney and investigate potentials into gaining new insights into disease processes and therapeutic interventions.

DISCLOSURES

None.

REFERENCES

1. Blau HM: How fixed is the differentiated state? Lessons from heterokaryons. *Trends Genet* 5: 268–272, 1989
2. Gurdon JB, Laskey RA, Reeves OR: The developmental capacity of nuclei transplanted from keratinized skin cells of adult frogs. *J Embryol Exp Morphol* 34: 93–112, 1975
3. DiBerardino MA, Hoffner NJ: Gene reactivation in erythrocytes: Nuclear transplantation in oocytes and eggs of Rana. *Science* 219: 862–864, 1983
4. Gurdon JB, Byrne JA: The first half-century of nuclear transplantation. *Proc Natl Acad Sci U S A* 100: 8048–8052, 2003
5. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH: Viable offspring derived from fetal and adult mammalian cells. *Nature* 385: 810–813, 1997
6. Hochedlinger K, Jaenisch R: Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* 415: 1035–1038, 2002
7. Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676, 2006
8. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S: Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131: 861–872, 2007
9. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, Bilić J, Pekarik V, Tiscornia G, Edel M, Boué S, Izpisúa Belmonte JC: Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 26: 1276–1284, 2008
10. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC: Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. *Proc Natl Acad Sci U S A* 106: 15720–15725, 2009
11. Ahmed RP, Haider HK, Buccini S, Li L, Jiang S, Ashraf M: Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infarcted heart. *Circ Res* 109: 60–70, 2011
12. Cai J, Li W, Su H, Qin D, Yang J, Zhu F, Xu J, He W, Guo X, Labuda K, Peterbauer A, Wolbank S, Zhong M, Li Z, Wu W, So KF, Redl H, Zeng L, Esteban MA, Pei D: Generation of human induced pluripotent stem cells from umbilical cord matrix and amniotic membrane mesenchymal cells. *J Biol Chem* 285: 11227–11234, 2010
13. Haase A, Olmer R, Schwanke K, Wunderlich S, Merkert S, Hess C, Zweigerdt R, Gruh I, Meyer J, Wagner S, Maier LS, Han DW, Glage S, Miller K, Fischer P, Schöler HR, Martin U: Generation of induced pluripotent stem cells from human cord blood. *Cell Stem Cell* 5: 434–441, 2009
14. Dimos JT, Rodolfa KT, Niakan KK, Weisenthal LM, Mitsumoto H, Chung W, Croft GF, Saphier G, Leibel R, Goland R, Wichterle H, Henderson CE, Eggan K: Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321: 1218–1221, 2008
15. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, Seyfarth M, Sinnecker D, Schömig A, Laugwitz KL: Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N Engl J Med* 363: 1397–1409, 2010
16. Ebert AD, Yu J, Rose FF Jr, Mattis VB, Lorson CL, Thomson JA, Svendsen CN: Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature* 457: 277–280, 2009
17. Lee G, Papapetrou EP, Kim H, Chambers SM, Tomishima MJ, Fasano CA, Ganat YM, Menon J, Shimizu F, Viale A, Tabar V, Sadelain M, Studer L: Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature* 461: 402–406, 2009
18. Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR: A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* 143: 527–539, 2010
19. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L: Modelling the long QT syndrome with induced pluripotent stem cells. *Nature* 471: 225–229, 2011
20. Brennand KJ, Simone A, Jou J, Gelboin-Burkhart C, Tran N, Sangar S, Li Y, Mu Y, Chen G, Yu D, McCarthy S, Sebat J, Gage FH: Modelling schizophrenia using human induced pluripotent stem cells. *Nature* 473: 221–225, 2011
21. Song B, Niclis JC, Alikhan MA, Sakkal S, Sylvain A, Kerr PG, Laslett AL, Bernard CA, Ricardo SD: Generation of induced pluripotent stem cells from human kidney mesangial cells. *J Am Soc Nephrol* 22: 1213–1220, 2011
22. Zhou T, Benda C, Duzinger S, Huang Y, Li X, Li Y, Guo X, Cao G, Chen S, Hao L, Chan YC, Ng KM, Ho JC, Wieser M, Wu J, Redl H, Tse HF, Grillari J, Grillari-Voglauer R, Pei D, Esteban MA: Generation of induced pluripotent stem cells from urine. *J Am Soc Nephrol* 22: 1221–1228, 2011
23. Song B, Smink AM, Jones CV, Callaghan JM, Firth SD, Bernard CA, Laslett AL, Kerr PG, Ricardo SD: The directed differentiation of human iPS cells into kidney podocytes. *PLoS ONE* 7: e46453, 2012
24. Thatava T, Armstrong AS, De Lamo JG, Edukulla R, Khan YK, Sakuma T, Ohmine S, Sundsbak JL, Harris PC, Kudva YC, Ikeda Y: Successful disease-specific induced pluripotent stem cell generation from patients with kidney transplantation. *Stem Cell Res Ther* 2: 48, 2011
25. Humphreys BD, Valerius MT, Kobayashi A, Mugford JW, Soeung S, Duffield JS, McMahon AP, Bonventre JV: Intrinsic epithelial cells repair the kidney after injury. *Cell Stem Cell* 2: 284–291, 2008
26. Bonventre JV: Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. *J Am Soc Nephrol* 14[Suppl 1]: S55–S61, 2003
27. Pavenstädt H, Kriz W, Kretzler M: Cell biology of the glomerular podocyte. *Physiol Rev* 83: 253–307, 2003
28. Shankland SJ, Eitner F, Hudkins KL, Goodpaster T, D'Agati V, Alpers CE: Differential expression of cyclin-dependent kinase inhibitors in human glomerular disease: Role in podocyte proliferation and maturation. *Kidney Int* 58: 674–683, 2000
29. Griffin SV, Hiromura K, Pippin J, Petermann AT, Blonski MJ, Krofft R, Takahashi S, Kulkarni AB, Shankland SJ: Cyclin-dependent kinase 5 is a regulator of podocyte differentiation, proliferation, and morphology. *Am J Pathol* 165: 1175–1185, 2004
30. Kriz W: Progressive renal failure—inability of podocytes to replicate and the consequences for development of glomerulosclerosis. *Nephrol Dial Transplant* 11: 1738–1742, 1996

31. Barisoni L, Mokrzycki M, Sablay L, Nagata M, Yamase H, Mundel P: Podocyte cell cycle regulation and proliferation in collapsing glomerulopathies. *Kidney Int* 58: 137–143, 2000
32. Wiggins RC: The spectrum of podocytopathies: A unifying view of glomerular diseases. *Kidney Int* 71: 1205–1214, 2007
33. D'Agati VD: Podocyte injury in focal segmental glomerulosclerosis: Lessons from animal models (a play in five acts). *Kidney Int* 73: 399–406, 2008
34. Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohno K, Holzman LB, Wiggins RC: Podocyte depletion causes glomerulosclerosis: Diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 16: 2941–2952, 2005
35. Matsusaka T, Sandgren E, Shintani A, Kon V, Pastan I, Fogo AB, Ichikawa I: Podocyte injury damages other podocytes. *J Am Soc Nephrol* 22: 1275–1285, 2011
36. Cochrane AL, Kett MM, Samuel CS, Campanale NV, Anderson WP, Hume DA, Little MH, Bertram JF, Ricardo SD: Renal structural and functional repair in a mouse model of reversal of ureteral obstruction. *J Am Soc Nephrol* 16: 3623–3630, 2005
37. Humphreys BD, Czerniak S, DiRocco DP, Hasnain W, Cheema R, Bonventre JV: Repair of injured proximal tubule does not involve specialized progenitors. *Proc Natl Acad Sci U S A* 108: 9226–9231, 2011
38. Wise AF, Ricardo SD: Mesenchymal stem cells in kidney inflammation and repair. *Nephrology (Carlton)* 17: 1–10, 2012
39. Reinders ME, Fibbe WE, Rabelink TJ: Multipotent mesenchymal stromal cell therapy in renal disease and kidney transplantation. *Nephrol Dial Transplant* 25: 17–24, 2010
40. Bi B, Schmitt R, Israilova M, Nishio H, Cantley LG: Stromal cells protect against acute tubular injury via an endocrine effect. *J Am Soc Nephrol* 18: 2486–2496, 2007
41. Semedo P, Palasio CG, Oliveira CD, Feitoza CQ, Gonçalves GM, Cenedeze MA, Wang PM, Teixeira VP, Reis MA, Pacheco-Silva A, Câmara NO: Early modulation of inflammation by mesenchymal stem cell after acute kidney injury. *Int Immunopharmacol* 9: 677–682, 2009
42. Tögel F, Hu Z, Weiss K, Isaac J, Lange C, Westenfelder C: Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms. *Am J Physiol Renal Physiol* 289: F31–F42, 2005
43. Yuan L, Wu MJ, Sun HY, Xiong J, Zhang Y, Liu CY, Fu LL, Liu DM, Liu HQ, Mei CL: VEGF-modified human embryonic mesenchymal stem cell implantation enhances protection against cisplatin-induced acute kidney injury. *Am J Physiol Renal Physiol* 300: F207–F218, 2011
44. Angelotti ML, Ronconi E, Ballerini L, Peired A, Mazzinghi B, Sagrinati C, Parente E, Gacci M, Carini M, Rotondi M, Fogo AB, Lazzeri E, Lasagni L, Romagnani P: Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. *Stem Cells* 30: 1714–1725, 2012
45. Ronconi E, Sagrinati C, Angelotti ML, Lazzeri E, Mazzinghi B, Ballerini L, Parente E, Becherucci F, Gacci M, Carini M, Maggi E, Serio M, Vannelli GB, Lasagni L, Romagnani S, Romagnani P: Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol* 20: 322–332, 2009
46. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, Elger M, Kriz W, Floege J, Moeller MJ: Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol* 20: 333–343, 2009
47. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, Ronconi E, Meini C, Gacci M, Squecco R, Carini M, Gesualdo L, Francini F, Maggi E, Annunziato F, Lasagni L, Serio M, Romagnani S, Romagnani P: Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol* 17: 2443–2456, 2006
48. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ: Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451: 141–146, 2008
49. Moriya N, Uchiyama H, Asashima M: Induction of pronephric tubules by activin and retinoic acid in presumptive ectoderm of Xenopus laevis. *Dev Growth Differ* 35: 123–128, 1993
50. Brennan HC, Nijjar S, Jones EA: The specification and growth factor inducibility of the pronephric glomus in Xenopus laevis. *Development* 126: 5847–5856, 1999
51. Osafune K, Nishinakamura R, Komazaki S, Asashima M: In vitro induction of the pronephric duct in Xenopus explants. *Dev Growth Differ* 44: 161–167, 2002
52. Schuldiner M, Yanuka O, Itskovitz-Eldor J, Melton DA, Benvenisty N: Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. *Proc Natl Acad Sci U S A* 97: 11307–11312, 2000
53. Kim D, Dressler GR: Nephrogenic factors promote differentiation of mouse embryonic stem cells into renal epithelia. *J Am Soc Nephrol* 16: 3527–3534, 2005
54. Kobayashi T, Tanaka H, Kuwana H, Inoshita S, Teraoka H, Sasaki S, Terada Y: Wnt4-transformed mouse embryonic stem cells differentiate into renal tubular cells. *Biochem Biophys Res Commun* 336: 585–595, 2005
55. Vigneau C, Polgar K, Striker G, Elliott J, Hyink D, Weber O, Fehling HJ, Keller G, Burrow C, Wilson P: Mouse embryonic stem cell-derived embryoid bodies generate progenitors that integrate long term into renal proximal tubules in vivo. *J Am Soc Nephrol* 18: 1709–1720, 2007
56. Steenhard BM, Isom KS, Cazcarro P, Dunmore JH, Godwin AR, St John PL, Abrahamson DR: Integration of embryonic stem cells in metanephric kidney organ culture. *J Am Soc Nephrol* 16: 1623–1631, 2005
57. Bruce SJ, Rea RW, Steptoe AL, Busslinger M, Bertram JF, Perkins AC: In vitro differentiation of murine embryonic stem cells toward a renal lineage. *Differentiation* 75: 337–349, 2007
58. Mae S, Shirasawa S, Yoshie S, Sato F, Kanoh Y, Ichikawa H, Yokoyama T, Yue F, Tomotsune D, Sasaki K: Combination of small molecules enhances differentiation of mouse embryonic stem cells into intermediate mesoderm through BMP7-positive cells. *Biochem Biophys Res Commun* 393: 877–882, 2010
59. Lin SA, Kolle G, Grimmond SM, Zhou Q, Doust E, Little MH, Aronow B, Ricardo SD, Pera MF, Bertram JF, Laslett AL: Subfractionation of differentiating human embryonic stem cell populations allows the isolation of a mesodermal population enriched for intermediate mesoderm and putative renal progenitors. *Stem Cells Dev* 19: 1637–1648, 2010
60. Unbekandt M, Davies JA: Dissociation of embryonic kidneys followed by reaggregation allows the formation of renal tissues. *Kidney Int* 77: 407–416, 2010
61. Xinaris C, Benedetti V, Rizzo P, Abbate M, Corna D, Azzollini N, Conti S, Unbekandt M, Davies JA, Morigi M, Benigni A, Remuzzi G: *In vivo maturation of functional renal organoids formed from embryonic cell suspensions. J Am Soc Nephrol* 23: 1857–2012
62. González F, Boué S, Izpisúa Belmonte JC: Methods for making induced pluripotent stem cells: reprogramming à la carte. *Nat Rev Genet* 12: 231–242, 2011
63. Chin MH, Mason MJ, Xie W, Volinia S, Singer M, Peterson C, Ambartsumyan G, Aimiuwu O, Richter L, Zhang J, Khvorostov I, Ott V, Grunstein M, Lavon N, Benvenisty N, Croce CM, Clark AT, Baxter T, Pyle AD, Teitell MA, Pelegrini M, Plath K, Lowry WE: Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 5: 111–123, 2009
64. Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, Lynch C, Harness JV, Lee S, Barrero MJ, Ku S, Martynova M, Semechkin R, Galat V, Gottesfeld J, Izpisua Belmonte JC, Murry C, Keirstead HS, Park HS, Schmidt U, Laslett AL, Muller FJ, Nievergelt CM, Shamir R, Loring JF: Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs

during reprogramming and time in culture. *Cell Stem Cell* 8: 106–118, 2011
65. Bock C, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, Ziller M, Croft GF, Amoroso MW, Oakley DH, Gnirke A, Eggan K, Meissner A: Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144: 439–452, 2011
66. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, Guo T, Qi Z, Downey SL, Manos PD, Rossi DJ, Yu J, Hebrok M, Hochedlinger K, Costello JF, Song JS, Ramalho-Santos M: Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat Cell Biol* 13: 541–549, 2011
67. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K: Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol* 28: 848–855, 2010
68. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J, Hanna J, Murakami P, Jaenisch R, Weissleder R, Orkin SH, Weissman IL, Feinberg AP, Daley GQ: Epigenetic memory in induced pluripotent stem cells. *Nature* 467: 285–290, 2010
69. Hu Q, Friedrich AM, Johnson LV, Clegg DO: Memory in induced pluripotent stem cells: Reprogrammed human retinal-pigmented epithelial cells show tendency for spontaneous redifferentiation. *Stem Cells* 28: 1981–1991, 2010
70. Nazor KL, Altun G, Lynch C, Tran H, Harness JV, Slavin I, Garitaonandia I, Müller FJ, Wang YC, Boscolo FS, Fakunle E, Dumevska B, Lee S, Park HS, Olee T, D'Lima DD, Semechkin R, Parast MM, Galat V, Laslett AL, Schmidt U, Keirstead HS, Loring JF, Laurent LC: Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. *Cell Stem Cell* 10: 620–634, 2012
71. Liu GH, Barkho BZ, Ruiz S, Diep D, Qu J, Yang SL, Panopoulos AD, Suzuki K, Kurian L, Walsh C, Thompson J, Boue S, Fung HL, Sancho-Martinez I, Zhang K, Yates J 3rd, Izpisua Belmonte JC: Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature* 472: 221–225, 2011
72. Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE: Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature* 471: 230–234, 2011
73. Zhang J, Lian Q, Zhu G, Zhou F, Sui L, Tan C, Mutalif RA, Navasankari R, Zhang Y, Tse HF, Stewart CL, Colman A: A human iPSC model of Hutchinson Gilford progeria reveals vascular smooth muscle and mesenchymal stem cell defects. *Cell Stem Cell* 8: 31–45, 2011
74. Nguyen HN, Byers B, Cord B, Shcheglovitov A, Byrne J, Gujar P, Kee K, Schüle B, Dolmetsch RE, Langston W, Palmer TD, Pera RR: LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell* 8: 267–280, 2011
75. Agarwal S, Loh YH, McLoughlin EM, Huang J, Park IH, Miller JD, Huo H, Okuka M, Dos Reis RM, Loewer S, Ng HH, Keefe DL, Goldman FD, Klingelhutz AJ, Liu L, Daley GQ: Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. *Nature* 464: 292–296, 2010
76. Shankland SJ, Pippin JW, Reiser J, Mundel P: Podocytes in culture: Past, present, and future. *Kidney Int* 72: 26–36, 2007
77. Tiscornia G, Vivas EL, Izpisúa Belmonte JC: Diseases in a dish: Modeling human genetic disorders using induced pluripotent cells. *Nat Med* 17: 1570–1576, 2011
78. Bankir L, de Rouffignac C: Urinary concentrating ability: Insights from comparative anatomy. *Am J Physiol* 249: R643–R666, 1985
79. Kriz W, Koepsell H: The structural organization of the mouse kidney. *Z Anat Entwicklungsgesch* 144: 137–163, 1974
80. Cotte N, Balestre MN, Phalipou S, Hibert M, Manning M, Barberis C, Mouillac B: Identification of residues responsible for the selective binding of peptide antagonists and agonists in the V2 vasopressin receptor. *J Biol Chem* 273: 29462–29468, 1998
81. Susztak K, Bitzer M, Meyer TW, Hostetter TH: Animal models of renal disease. *Kidney Int* 73: 526–528, 2008
82. Jans F, Vandenabeele F, Helbert M, Lambrichts I, Ameloot M, Steels P: A simple method for obtaining functionally and morphologically intact primary cultures of the medullary thick ascending limb of Henle's loop (MTAL) from rabbit kidneys. *Pflugers Arch* 440: 643–651, 2000
83. Wagner CA, Lükewille U, Valles P, Breton S, Brown D, Giebisch GH, Geibel JP: A rapid enzymatic method for the isolation of defined kidney tubule fragments from mouse. *Pflugers Arch* 446: 623–632, 2003
84. Mundel P, Reiser J, Kriz W: Induction of differentiation in cultured rat and human podocytes. *J Am Soc Nephrol* 8: 697–705, 1997
85. Reiser J, von Gersdorff G, Loos M, Oh J, Asanuma K, Giardino L, Rastaldi MP, Calvaresi N, Watanabe H, Schwarz K, Faul C, Kretzler M, Davidson A, Sugimoto H, Kalluri R, Sharpe AH, Kreidberg JA, Mundel P: Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 113: 1390–1397, 2004
86. Saleem MA, O'Hare MJ, Reiser J, Coward RJ, Inward CD, Farren T, Xing CY, Ni L, Mathieson PW, Mundel P: A conditionally immortalized human podocyte cell line demonstrating nephrin and podocin expression. *J Am Soc Nephrol* 13: 630–638, 2002
87. Racusen LC, Monteil C, Sgrignoli A, Lucskay M, Marouillat S, Rhim JG, Morin JP: Cell lines with extended in vitro growth potential from human renal proximal tubule: Characterization, response to inducers, and comparison with established cell lines. *J Lab Clin Med* 129: 318–329, 1997
88. Zhou T, Benda C, Dunzinger S, Huang Y, Ho JC, Yang J, Wang Y, Zhang Y, Zhuang Q, Li Y, Bao X, Tse HF, Grillari J, Grillari-Voglauer R, Pei D, Esteban MA: Generation of human induced pluripotent stem cells from urine samples. *Nat Protoc* 7: 2080–2089, 2012
89. Woodcock J: The prospects for "personalized medicine" in drug development and drug therapy. *Clin Pharmacol Ther* 81: 164–169, 2007
90. Chun YS, Byun K, Lee B: Induced pluripotent stem cells and personalized medicine: current progress and future perspectives. *Anat Cell Biol* 44: 245–255, 2011
91. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA: Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol* 26: 1269–1275, 2008
92. Shi Y, Desponts C, Do JT, Hahm HS, Schöler HR, Ding S: Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell* 3: 568–574, 2008
93. Ichida JK, Blanchard J, Lam K, Son EY, Chung JE, Egli D, Loh KM, Carter AC, Di Giorgio FP, Koszka K, Huangfu D, Akutsu H, Liu DR, Rubin LL, Eggan K: A small-molecule inhibitor of tgf-Beta signaling replaces sox2 in reprogramming by inducing nanog. *Cell Stem Cell* 5: 491–503, 2009
94. Han DW, Tapia N, Hermann A, Hemmer K, Höing S, Araúzo-Bravo MJ, Zaehres H, Wu G, Frank S, Moritz S, Greber B, Yang JH, Lee HT, Schwamborn JC, Storch A, Schöler HR: Direct reprogramming of fibroblasts into neural stem cells by defined factors. *Cell Stem Cell* 10: 465–472, 2012
95. Reynolds BA, Weiss S: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 255: 1707–1710, 1992
96. Rumballe BA, Georgas KM, Combes AN, Ju AL, Gilbert T, Little MH: Nephron formation adopts a novel spatial topology at cessation of nephrogenesis. *Dev Biol* 360: 110–122, 2011

97. Hartman HA, Lai HL, Patterson LT: Cessation of renal morphogenesis in mice. *Dev Biol* 310: 379–387, 2007
98. Hendry CE, Little MH: Reprogramming the kidney: A novel approach for regeneration. *Kidney Int* 82: 138–146, 2012
99. Diep CQ, Ma D, Deo RC, Holm TM, Naylor RW, Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman B, Zhu H, Ikenaga T, Ono F, Englert C, Cowan CA, Hukriede NA, Handin RI, Davidson AJ: Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. *Nature* 470: 95–100, 2011
100. Hendry CE, Vanslambrouck JM, Ineson J, Suhaimi N, Takasato M, Rae F, Little MH: Direct transcriptional reprogramming of adult cells to embryonic nephron progenitors [published online ahead of print June 13, 2013]. *J Am Soc Nephrol* doi: 10.1681/ASN.2012121143
101. Beqqali A, Kloots J, Ward-van Oostwaard D, Mummery C, Passier R: Genome-wide transcriptional profiling of human embryonic stem cells differentiating to cardiomyocytes. *Stem Cells* 24: 1956–1967, 2006