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Generating Kidney from Stem Cells

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Abstract

Human kidney tissue can now be generated via the directed differentiation of human pluripotent stem cells. This advance is anticipated to facilitate the modeling of human kidney diseases, provide platforms for nephrotoxicity screening, enable cellular therapy, and potentially generate tissue for renal replacement. All such applications will rely upon the accuracy and reliability of the model and the capacity for stem cell–derived kidney tissue to recapitulate both normal and diseased states. In this review, we discuss the models available, how well they recapitulate the human kidney, and how far we are from application of these cells for use in cellular therapies.

INTRODUCTION

A pluripotent cell is defined as a cell with a capacity to form any cell type in the body. In vivo, pluripotent stem cells (PSCs) exist only transiently in the developing blastocyst before subsequently differentiating into the many specialized cell types that make up the entire human body. Nearly four decades ago, culture conditions were identified that facilitated both the derivation and in vitro expansion of PSCs from mouse embryos (1, 2). This was a major breakthrough in mouse genetics, as the transfer of genetically modified mouse PSCs into recipient blastocysts enabled the generation of knockout mice, providing a powerful and systematic approach for investigating gene function. Although this was the major use of mouse PSCs, it was clear that these cells had a capacity to contribute to all germ layers and, hence, if available from humans, they might serve as a source of human tissue. In fact, there was a considerable delay before the derivation of the first human embryonic stem cells (hESCs) in 1998 (3). Less than a decade later in 2007, the Yamanaka and Thomson labs independently identified a robust method to derive hPSCs without the use of human embryos. This involved the forced expression of a defined set of transcription factors to reprogram terminally differentiated cells back to an "induced" pluripotent state (4, 5). Remarkably, these induced pluripotent stem cells (iPSCs) are equivalent to hESCs in that they can be expanded indefinitely in the appropriate culture conditions to maintain pluripotency or can be induced to differentiate into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. The in vitro–directed differentiation of hESCs and iPSCs has been a major area of interest in the stem cell field. In this review, we focus on the current state of play with respect to the generation of human kidney tissue using hPSCs. Almost all such protocols have in some way drawn on our understanding of mammalian kidney development.

DEVELOPMENT OF THE MAMMALIAN KIDNEY

The kidney in the human, as in all mammals, represents only one of three excretory organs that form during embryogenesis. This final permanent kidney, the metanephros, contains on average 1 million epithelial structures (6) referred to as nephrons, which filter the blood via a capillary network within the glomeruli of each nephron. The resulting urinary filtrate passes down a functionally segmented epithelial tubule comprising >20 cell types, with each playing distinct roles in fluid and nutrient (e.g., amino acids, glucose, protein) reabsorption. The nephrons are connected to a branching collecting duct network that empties via multiple calyces into the renal pelvis before exiting the kidney via the ureter.

The regulation of kidney morphogenesis in mammals has long been studied in animal models, notably the rat and mouse. The most comprehensive cellular and molecular atlases of gene expression, as well as temporospatial studies of lineage relationships and quantitative cellular morphogenesis (7–10), have been completed in the mouse. While there are many similarities between the postnatal mouse and human kidney with respect to key segment-specific genes and proteins, there are also significant differences in scale, overall structure, nephron number, and timing of differentiation. The human kidney is composed of a number of conical lobes, each with a papilla, whereas the mouse kidney is effectively a single lobe (11, 12) (**Figure 1*a***). Hence, the mouse has a unipapillate kidney, whereas the human kidney has multiple papillae. Kidney development in the mouse is completed within approximately 10 days, while in the human it extends across >30 weeks (from week 5 to 36) (11). Despite these differences, it is considered that all nephron formation occurs before birth in humans (12, 13) and by the first few days after birth in mice (14, 15). Although all nephrons are formed during this period, there are substantial tubular maturation and elongation in the postnatal organ, such that recent single-cell profiling of the adult mouse kidney at the transcriptomic level suggested that 60.5% of all cells in the adult kidney are

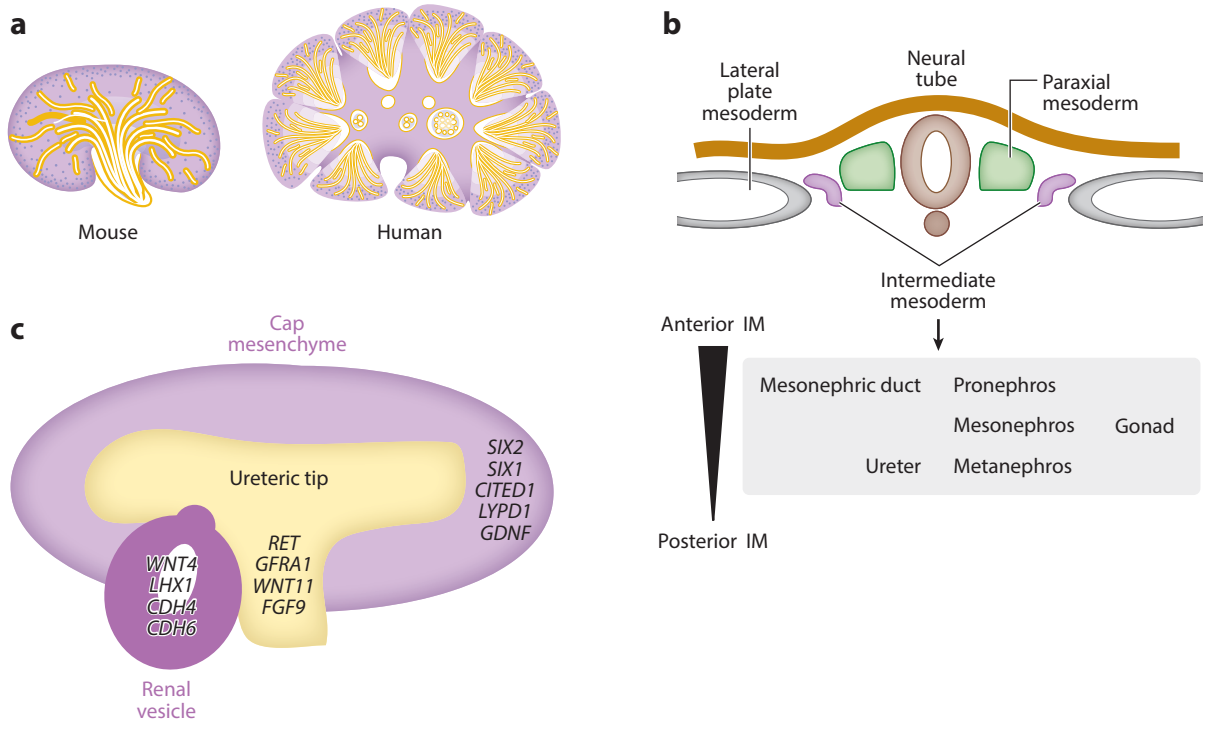

Figure 1

Development of the mammalian kidney. (*a*) Diagram of a unipapillate mouse kidney versus a multipapillate human kidney. (*b*) Mediolateral view of the early developing embryo showing the relative position of the paraxial, intermediate and lateral plate mesoderm and the derivatives of the intermediate mesoderm (IM). (*c*) Diagram of an individual nephrogenic niche illustrating the relationship between the ureteric tip and surrounding cap mesenchyme as well as the location of a nascent nephron at the renal vesicle (stage 1 nephron) stage of differentiation. Key marker genes for developing human are indicated in these three compartments. Adapted with permission from Reference 11.

proximal tubules, whereas the renal interstitium represented 1.26% (16). In contrast, histology of both developing mouse and human kidney shows nephrons forming within an abundant stromal field (17).

Although many studies have examined the early stages of mouse metanephric development, this stage of human development is rarely accessible even for anatomical examination. Moreover, lineage analyses are not possible in the setting of human development. There is sufficient histological similarity, however, to assume that our understanding of kidney morphogenesis in the mouse is likely to inform similar processes in the human. As the purpose of this review is to consider how we are able to generate kidney tissue via the directed differentiation of hPSCs, we first describe what is understood about the process of kidney morphogenesis in the mouse. In the mouse, all three pairs of excretory organs—the pronephros, mesonephros, and metanephros—are regarded as derivatives of the intermediate mesoderm (IM). This subcompartment of the definitive mesoderm forms as cells stream from the posterior primitive streak to form the definitive mesoderm of the elongating body (18). As the name suggests, the IM is positioned mediolaterally between the paraxial mesoderm that will give rise to the axial skeleton, limb muscles, and dermis, as well as the lateral plate mesoderm that will give rise to the heart, body wall, endothelia, and viscera of both mesoderms (**Figure 1*b***). The IM will give rise to the kidney, gonads, and ureter. The

excretory organs arising from the IM form in a rostral-caudal order initiated by the formation of the mesonephric or Wolffian duct (also referred to as the nephric duct) (19). The flanking IM gives rise to the pro-, meso- and metanephric mesenchyme from which the tubular epithelium forms.

Little is known about the pronephros, but in the mouse, mesonephric tubules appear to arise as the result of a mesenchyme-to-epithelial (MET) inductive event, with the resulting structures patterning and segmenting as they elongate. The most rostral of these tubules connect to the nephric duct as it extends caudally, whereas the more caudal tubules do not (20). The mesonephros arises immediately next to the somatic component of the gonads, but it completely degenerates except for persistent rostral tubules that contribute in males to the efferent seminiferous tubules of the testis (21). Formation of the metanephros is anatomically quite distinct from that of the pro- or mesonephros in that metanephros formation is initiated by the outgrowth of a side branch of the nephric duct, the ureteric bud (UB). The UB grows toward the metanephric mesenchyme (MM) in response to secretion of glial-derived neurotrophic factor (GDNF) produced by the MM. As the UB reaches the MM, it begins to dichotomously branch to give rise to the collecting ducts of the kidney (22). The unbranched portion of the UB will form the ureter. The mesenchymal cells immediately surrounding the branching ureteric tips represent a $SIX2^+$ self-renewing progenitor population referred to as the cap mesenchyme (9) (**Figure 1*c***).

Lineage tracing has definitively shown that these mesenchymal cells are the nephron progenitors (NPs) that give rise to all epithelial cells within the forming nephrons (7, 9). A nephrogenic niche is formed between each ureteric tip and the surrounding NPs (**Figure 1*c***). Signals from the mesenchyme support ureteric tip proliferation and ureteric tree branching, while signals from both the ureteric tips and the surrounding stroma can stimulate self-renewal of NPs or initiate commitment to nephron formation (22). The initiation of each nephron occurs in a stereotypic region below the ureteric tip and, similar to the mesonephros, arises through MET. Here, a pretubular aggregate gives rise to a renal vesicle, also referred to as a stage 1 nephron (17, 23) (**Figure 1*c***). This renal vesicle is immediately polarized such that the distal renal vesicle will invade the adjacent ureteric tip to form a patent lumen between the two structures, and the proximal end will give rise to the visceral and parietal epithelium of the glomeruli (23). What follows is a process of elongation and segmentation through comma- and S-shaped body stages (stage 2 nephrons) until the glomerular end of the nascent nephrons draws in endothelial progenitors from the surrounding stroma to form a capillary loop stage nephron. Although there has been significant focus on the reciprocal inductive events occurring between the UB and the $Six2^+$ NPs, the MM also contributes to both the cortical and some portion of the medullary stroma. There is also evidence that endothelial progenitors (24) and even tissue macrophages (25) are also present within the stroma of the early metanephros. *Foxd1* lineage tracing has shown that this cortical stromal population gives rise to the perivascular cells of the renal endothelial network, including the cells of renin lineage (26). Finally, additional stromal cell populations, including the $Tbx18^+$ stroma surrounding the invading UB, contribute to the medullary stroma and form the muscular and adventitial cells along the ureter (27).

The molecular pathways known to control the process of nephron formation have also been well studied in mice. Canonical Wnt signaling is responsible for both NP self-renewal (28) and induction of nephron formation (29). Expansion of the NPs can be supported by fibroblast growth factor (FGF)9 and FGF20 (30), and there are now several media described, including FGF9, Smad inhibition, notch inhibition, and low canonical Wnt signaling that are able to support isolated NPs in culture (31–33). It is also known that nephron formation can be induced from a nephron-competent mesoderm in vitro via a strong canonical Wnt source, such as spinal cord, recombinant Wnt3A, or small molecular activators of canonical signaling, such as CHIR99201 or

BIO. The formation, extension, and patterning of the nephric duct are known to require retinoic acid and FGF signaling (34), and ultimately this population expresses the Ret tyrosine kinase, making it responsive to GDNF. Lineage tracing and gene expression analyses have also identified characteristic genes marking specific stages of differentiation and progenitor cell types in the developing mouse kidney. By way of example, expression of *Osr1* marks IM, but also lateral plate mesoderm, while coexpression of *Pax2*, *Lhx1*, and *Osr1* more definitively marks IM. The nephric duct expresses *Gata3*, *Lhx1*, *Pax2*, and *Pax8*, whereas the tips of the ureteric epithelium express *Ret*, *Gfrα1*, and *Wnt11*. In mice, the NPs express *Cited1* and *Six2*; then during early stages of nephron commitment, they downregulate *Six2* and upregulate *Wnt4*, *Cdh6*, *Cdh4*, *Dll1*, and *Lhx1*. In combination, our understanding of gene expression markers, critical signaling pathways, cell–cell signaling events, and lineage relationships has provided a set of mileposts with which to recreate kidney embryogenesis in vitro. Very recently, the first detailed anatomical, immunohistochemical, and profiling data on the developing human kidney became available (35–37). Although this does identify some subtle differences in gene expression in the NPs of the human developing kidney, such as the expression of both *SIX1* and *SIX2* and the novel expression of *LYPD1*, many of the accepted markers of differentiation appear conserved at least within trimester 1 human kidney (37) (**Figure 1*c***). This is likely why protocols based on mouse development have succeeded in humans. Continued analysis of gene expression in bona fide human tissue will, however, be absolutely critical to stringently assess the fidelity of hPSC-derived models of human kidney.

Approaches for Generating Human Kidney Cell Types from Pluripotent Stem Cells

One of the most stringent tests for the pluripotency of hPSCs is the teratoma assay where undifferentiated hPSCs are injected into immunocompromised mice and allowed to spontaneously differentiate in vivo (3, 5). Indeed, the first evidence that hPSCs were capable of giving rise to renal tissue came from such assays, where renal tubules and glomeruli can be observed in teratomas harvested 6–8 weeks postinjection (3, 38, 39). Although this is an important assay for evaluating pluripotency, teratomas typically comprise many different cell types that form simultaneously in a manner that is not well defined or controlled. In contrast, the directed differentiation of hPSCs in vitro can selectively generate specific cell types through careful manipulation of the cell's environment, typically through the addition of soluble morphogens to the culture medium. Since the derivation of the first hESCs over two decades ago, these types of approaches have been used to effectively direct the differentiation of hPSCs toward many different cell lineages, including hematopoietic, cardiac, neural, and pancreatic fates (40). Yet the reliable and efficient in vitro differentiation of renal cell types has been a much more recent outcome, with the most substantial advances occurring in the last 3–4 years (**Figure 2**).

One of the earliest reports of the generation of kidney tissue from PSCs was that of Taguchi et al. (41), who reasoned that the dearth of effective kidney differentiation protocols was, at least in part, due to an insufficient understanding of how the kidney is specified in vivo. Using lineage tracing techniques in mice, they showed that the MM that gave rise to the nephrons of the metanephros originated from the posterior IM, whereas the nephric duct from which the ureter arises initiates within anterior IM (41). This information was then used to induce MM from both mouse and human PSCs. In their protocol, the initial specification of posterior primitive streak was achieved by exposing aggregates of PSCs, referred to as embryoid bodies, to bone morphogenetic protein (BMP)4 and high levels of WNT pathway activity using the GSK3β inhibitor, CHIR99021. A switch to a medium with BMP4, activin A, retinoic acid, and moderate CHIR99021 was used to promote subsequent derivation of the posterior IM, followed by the addition of FGF9 and low

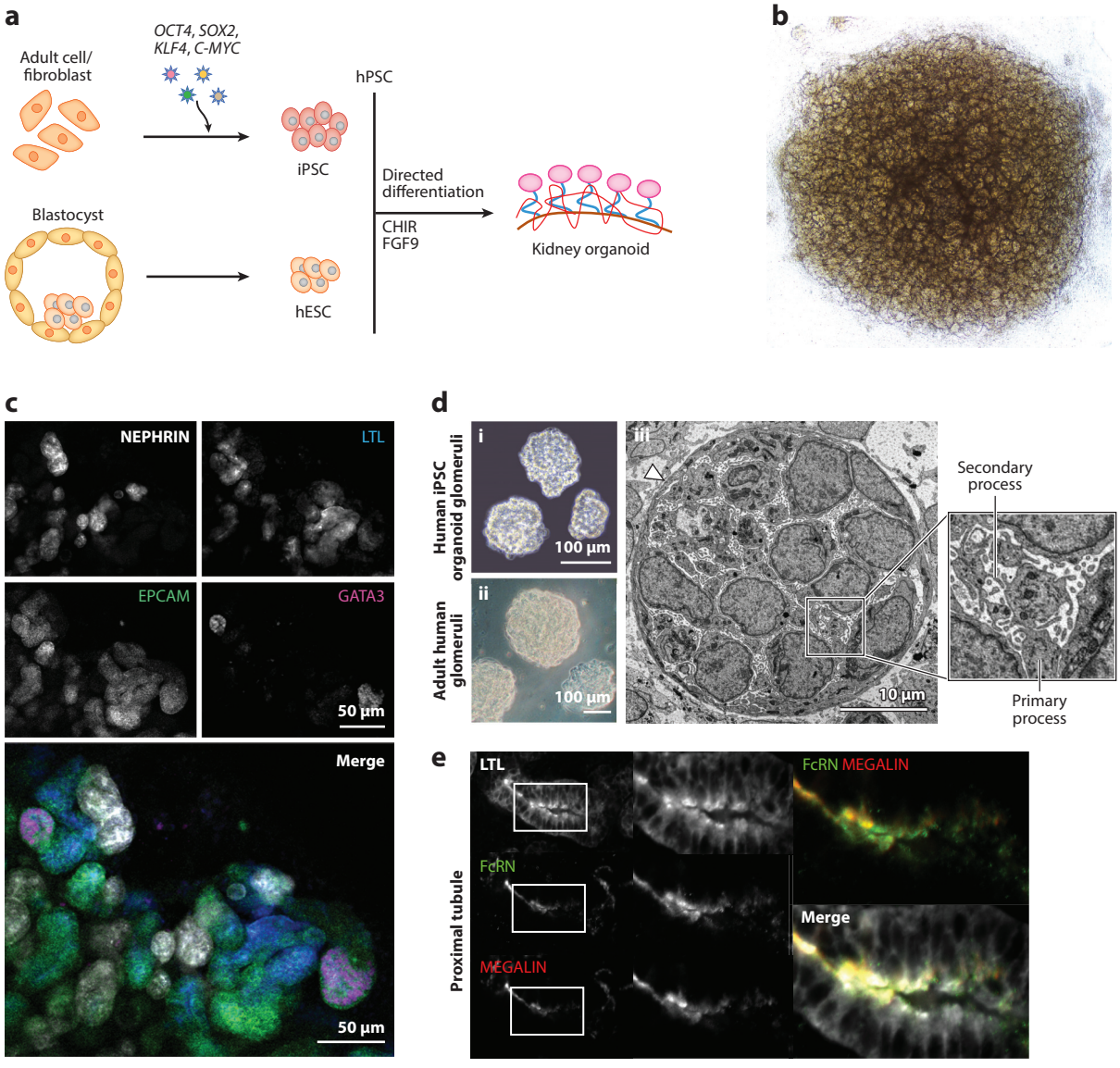

Figure 2

Kidney organoids derived from human pluripotent stem cells (hPSCs) contain patterning and segmenting nephrons. (*a*) Human kidney organoids can be generated via the directed differentiation of human embryonic stem cells (hESCs) or human-induced pluripotent stem cells (iPSCs). hESCs are derived from the inner cell mass of the blastocyst. Human iPSCs are generated via transcriptional reprogramming from adult somatic cells, such as fibroblasts. Reprogramming factors include the genes *OCT4*, *KLF4*, *SOX2*, and *C-MYC*. (*b*) Brightfield image of a kidney organoid derived using the protocol of Takasato et al. (44). (*c*) Immunofluorescent images of nephrons within a kidney organoid showing the presence of podocytes (NEPHRIN; *white*), tubular epithelium (EPCAM; *green*), proximal tubule (LTL; *blue*), and collecting duct/distal tubule (GATA3; *purple*) together with an overlay of all channels (*bottom*). (*d*) Brightfield images of glomeruli isolated via sieving from human iPSC-derived organoids (*i*) versus adult human glomeruli (*ii*). Middle panel (*iii*) shows transmission electron microscopy through a glomerulus within a kidney organoid illustrating the presence of primary and secondary foot processes. (*e*) Immunofluorescent staining of a proximal tubule within an hPSC-derived kidney organoid stained with lotus tetranoglobus lectin (LTL; *white*), neonatal Fc receptor (FcRN; *green*) and MEGALIN (*red*).

CHIR99021 to promote NP formation. These NPs underwent epithelialization following co-culture with mouse embryonic spinal cords, with the resulting structures resembling early-stage S-shaped nephrons and expressing markers for podocytes and tubules with proximal and distal regions (41).

In contrast to embryoid bodies, which are often associated with uncontrolled and heterogeneous differentiation, monolayer hPSC culture was used by Takasato et al. (42). Here, high BMP4/low activin A or high CHIR99021 alone was sufficient for efficient derivation of posterior primitive streak, as assessed by expression of *MIXL1* and *BRACHYURY*. High levels of FGF (either by FGF2 or FGF9) were then used for subsequent specification of IM. These monolayer cultures supported simultaneous differentiation of both ureteric epithelium and nephrogenic mesenchyme; the latter was shown to give rise to renal vesicles or early nephrons (42). In a later modification to this protocol, monolayer cultures were dissociated to single cells at day 7 (corresponding to IM stage of differentiation), aggregated by centrifugation, and transferred to Transwell^{TM} filters to facilitate three-dimensional (3D) culture at an air–liquid interface (43, 44). This was performed in an attempt to permit the formation of more complex and anatomically accurate multicellular structures, termed organoids (**Figure 2*a***). At the time, 3D organoid cultures had been developed following the directed differentiation of hPSCs toward various different lineages, including eye, brain, pituitary, intestine, stomach, liver, and heart (reviewed in 45). In a further modification to their earlier protocol, Takasato et al. (44) also used a short CHIR99021 pulse following the aggregation step to actively trigger nephron formation. The cell pellet was subsequently cultured in the presence of high FGF9 for five days, then without additional growth factors for a further 13 days (**Figure 2*b***). Remarkably, kidney organoids generated using this protocol contained not only derivatives of the nephrogenic mesenchyme but also putative ECAD$^+$/GATA3$^+$ collecting duct epithelium, renal interstitium, and an extensive endothelial network. The study also showed that it was possible to move between anterior and posterior IM, and hence between collecting duct and nephron, by extending the duration of the initial CHIR99021 induction. When well balanced, the resulting kidney organoids contained up to 100 segmented and patterning nephrons per organoid, each containing distal and proximal nephron segments and glomeruli containing tightly interdigitated podocytes with both primary and secondary foot processes (**Figure 2*c–e***). Morphologically, these nephrons underwent an elongation process similar to that seen in the mouse embryo, progressing from an S-shaped body toward a capillary loop stage. Expression profiling of these organoids in comparison with human fetal tissues showed the highest transcriptional congruence with trimester 1 human kidney.

Contemporary with Takasato et al. (44) were two other reports of protocols for the generation of kidney tissue from hPSCs. Morizane et al. (46) reported the generation of primitive streak again using a high dose of CHIR99021, which was then followed by subsequent treatment with activin A to promote posterior IM (46). A subsequent switch to medium containing relatively low levels of FGF9 was then used to obtain a highly enriched population of SIX2$^+$ NPs. With continuous FGF9 treatment in monolayer culture, these NPs spontaneously differentiated into round, polarized cell clusters reminiscent of renal vesicles. Upon FGF9 withdrawal, these structures were reported to elongate into tubular structures that expressed markers of proximal tubules and podocytes. The formation of epithelial structures was also facilitated by 3D culture and transient CHIR99021 treatment following the dissociation, replating, and suspension culture of NPs in low-attachment 96-well plates. These kidney organoids were shown to contain segmented nephrons that expressed markers of podocytes, proximal tubules, loops of Henle, and late distal tubules in an organized and continuous arrangement. Given the claim that this protocol generated an NP production with high specificity, the final structures were not surprisingly devoid of any collecting duct–like structures. Renal interstitial and endothelial compartments were also not reported in organoids generated using this method (46, 47).

A second protocol from the same group reported the generation of nephron structures after culture of hPSC as spheroids generated after "sandwiching" dissociated hPSCs between two layers of Matrigel. During this phase of culture, cells maintained pluripotent marker expression. Differentiation was subsequently initiated after CHIR99021 administration for 1.5 days, followed by a transition to medium containing B27 supplement for an additional 16 days (48). During this period, spheroid cultures underwent a stochastic transition into tubular organoids containing nephrons that expressed markers characteristic of proximal tubules and podocytes. In this instance, very much like that discussed in the initial report from Takasato et al. (42), the nephrons form in small clusters across a field of other nonrenal cell types. No characterization of the nonrenal elements was described. Although the majority of organoids generated by this protocol also contained a recognizable endothelial cell population, no evidence of UB or its derivatives was observed.

More recently, Taguchi & Nishinakamura (49) described a logical strategy for generating kidney organoids that contain both UB and NP compartments, again based on their previous work demonstrating that these lineages arise from distinct regions of the IM (anterior and posterior, respectively). In order to achieve the presence of derivatives of both anterior and posterior IM, they generated each separately and then performed coculture experiments. They reasoned that the use of optimized differentiation protocols to derive UB and NP in parallel, which are then combined by aggregation, could permit the generation of "higher-order" kidney organoids containing a nephrogenic niche capable of both branching morphogenesis and sustained nephrogenesis (49). This required the development of a specific differentiation approach for the anterior IM to give rise to nephric duct precursors. This was achieved again via an initial induction of primitive streak using activin A and BMP4, then BMP4 and CHIR followed by suppression of transforming growth factor and BMP signaling. Anterior IM was generated via the addition of retinoic acid, FGF9, and higher CHIR, after which fluorescence-activated cell sorting was used to isolate $CXCR4^{+}CKIT^{+}$ nephric duct cells. Subsequent culture included the addition of GDNF, FGF1, FGF9, and a Rho-kinase inhibitor, Y27632. These presumptive UB cultures were mixed with NP cultures and renal stroma isolated from mice, resulting in branching ureteric trees surrounded by forming nephrons. However, although the results were spectacular after coculture of UB and NP lineages derived from mouse PSCs, a sustainable nephrogenic niche could not be achieved with hPSC-derived UB and NP cells. The authors suggest this may be due to the lack of a suitable human stromal cell population, which likely plays a critical role in establishing/maintaining a nephrogenic niche. Alternatively, either or both of the hPSC-derived UB and NP may not have been patterned to appropriately undergo inductive physiological interactions.

Approaches to Validating the Identity of Cells Within Kidney Organoids

It is now possible to validate the identity of renal cell types derived from hPSCs through direct comparison to data obtained from the transcriptional profiling of human fetal kidney tissue (35, 50). The use of genetically engineered lineage-specific reporter hPSC lines that permit the isolation and characterization of a cell type of interest can greatly facilitate such studies. Lineage-specific reporter iPSCs that mark critical stages or cellular endpoints during differentiation can also be used for studying fate decisions in differentiating hPSCs and/or improving differentiation outcomes. Indeed, the earlier protocols for deriving primitive streak and IM, the precursors of all renal cell types, were greatly facilitated by the use of MIXL1 and OSR1 reporter lines, respectively (51, 52). A number of more recent articles have reported the generation and use of other knockin iPSC reporter lines that mark specific cell types in the developing kidney, such as podocytes, NPs, and endothelial cells (summarized in **Table 1**). Moreover, the advent of highly efficient and simple gene-editing technologies, such as CRISPR/Cas9, has spurred rapid and widespread generation of

Table 1 Knockin human iPSC reporter lines for monitoring kidney differentiation

Line (gene:reporter)	Cell types marked (related to kidney)	Reported uses	References
MIXL1:EGFP	Posterior primitive streak	Establishing optimal conditions for initial stages of kidney differentiation	42, 51
OSR1:EGFP	Intermediate mesoderm	Establishing optimal conditions for initial stages of kidney differentiation	52
SIX2:EGFP	Nephron progenitors	Facilitating development of a medium that supports nephron progenitor expansion	32
NPHS1:EGFP	Podocytes	Visualizing podocyte formation in vitro and in vivo following transplantation in recipient mice	83
MAFB:mTagBFP2	Early nephron/podocytes	Multiphoton imaging of transplanted kidney organoids in recipient mice	67, 94
SOX17:mCherry	Endothelium	Multiphoton imaging of transplanted kidney organoids in recipient mice	67, 95

genetically engineered hPSCs by researchers across many different fields. This will greatly facilitate the development of superior protocols for deriving renal cell types for downstream applications, including disease modeling, drug screening, toxicology studies, and tissue engineering.

Remaining Challenges of Generating Kidney Tissues Using Directed Differentiation

Although substantial progress has recently been made with respect to the derivation of renal tissue from hPSCs, several key challenges still remain. These include the fidelity, accuracy, and reproducibility of these kidney differentiation protocols. Initial global profiling in comparison to fetal human tissues did align organoids most closely with kidney (44). However, what this does not elucidate is the degree to which there are off-target populations within these protocols. Although there is a great deal of similarity between the different published protocols, a variety of mesodermal endpoints might arise simultaneously, including gonad, mesonephros, and even paraxial or lateral plate mesodermal derivatives. Indeed, in protocols in which the only directional cue being applied is mesodermal patterning to primitive streak, there is a high chance of off-target cell types. This is obvious if one directly compares the approach being used in directed differentiation to heart, for example. Using antibodies to known cell types within the developing mouse kidney, a remarkable number of distinct cell types in appropriate conformations have been described in several protocols. As such, any given kidney organoid has between 4 and 10 cell types based on immunofluorescence alone. More recent single-cell data would suggest perhaps 15–16 distinct cell types, although not all can readily be identified as appropriately patterned to kidney (53). There is evidence of neural off-target populations in both the Takasato et al. and Morizane protocols (53, 54); however, our own analysis of the reproducibility and transferability of the protocol between distinct iPSC lines, including control and patient lines, suggests considerable robustness to the protocol (55). In all instances, the core features of nephrons, stroma, and vasculature are present. While the cellular heterogeneity is one of the appealing features of an organoid, it is also one of the most intractable and challenging. From a manufacturing perspective, this creates a significant challenge with respect to quality control. The utility of any specific cell type from within an organoid, or even the isolation of kidney without off-target cell types, represents a major challenge to any cell therapy or engineering application. Indeed, prior to the recent publications describing the directed differentiation to multicellular kidney organoids, a number of articles have reported the

specific formation of podocytes (56) or proximal tubule cells (57), although these have not been extensively compared to bona fide cell types at a global transcriptional level.

Applications for hPSC-Derived Kidney Organoids

Having identified protocols in which models of the developing human kidney can be generated, what remains to be determined is the utility of such structures. The field of nephrology may view this as a major step forward or a minor distraction from the study of the real organ. However, a capacity to generate human kidney tissue in vitro from human cell lines available from any individual does bring considerable promise. In the rest of this review, we discuss a number of the most topical applications for human kidney organoids; we also consider what current options exist and whether or how hPSC-derived renal tissue may provide an advantage. The three areas on which we focus include the screening of compounds for nephrotoxicant activity, the modeling of human kidney disease in vitro, and the generation of renal replacement tissue.

Nephrotoxic Injury and Current Approaches to Predictive Screening

Nephrotoxic injury remains a significant reason for the failure of new pharmaceuticals in human clinical trials while also being a significant side effect of many currently used drugs, including many chemotherapeutic agents. The kidney is a major target for drug-induced injury. This is due to its role in eliminating toxic nitrogenous metabolic waste from, and metabolizing pharmaceuticals within, the circulation. Pharmaceuticals and other toxins in the blood can initiate damage to all major segments of nephron, resulting in disturbances to the tight junctions of glomerular podocytes; microvasculature; proximal, distal, and collecting ducts; epithelium; and interstitial cells (**Figure 3*a***). The proximal tubules represent the segment of the nephron most vulnerable to nephrotoxic injury due to their involvement in the reabsorption of glomerular filtrate and the active secretion of drug metabolites into the urine via solute transporters (both organic anionic and cationic). Impairment to this transporter function leads to the accumulation of noxious substances within the tubular cells, resulting in either acute or chronic kidney disease (CKD). Antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), immunosuppressants, and cytotoxic anticancer agents represent the major classes of drugs resulting in tubular nephrotoxicity and acute kidney injury (AKI) (58). Detection of AKI has been hampered by the lack of injury biomarkers, whereas in vitro and in vivo preclinical screening for nephrotoxicity has been poorly predictive. As a result, there is a need for the development of novel, accurate models for screening of drugs for nephrotoxic activity.

To date, modeling kidney disease and screening of drugs for nephrotoxicity have been performed using isolated primary cells, immortalized cell lines, or in vivo rodent models. These methods have substantial limitations in terms of specificity, reproducibility, and accuracy. Although rodent models provide an intact organism with appropriate physiological parameters, these remain poor predictors of toxic injury in humans due to species differences in the expression of transporters and metabolic enzymes. Many drugs are transported into the renal epithelium via either organic anionic transporter (OAT) or organic cationic transporter (OCT) located at the basolateral surface and via multidrug and toxin extrusion proteins (MATEs) at the apical cell membrane of the proximal tubules. Humans predominantly express OCT2, whereas mouse models express higher levels of OCT1. Similarly, MATE2-K is expressed in humans but is absent in rodents (**Figure 3*b***). Expression of these transporters is also variable between different rodent strains and between gender and species (59), further complicating the predictability of pharmacokinetic profiles between humans and rodents. Despite this interspecies variability, rodents remain

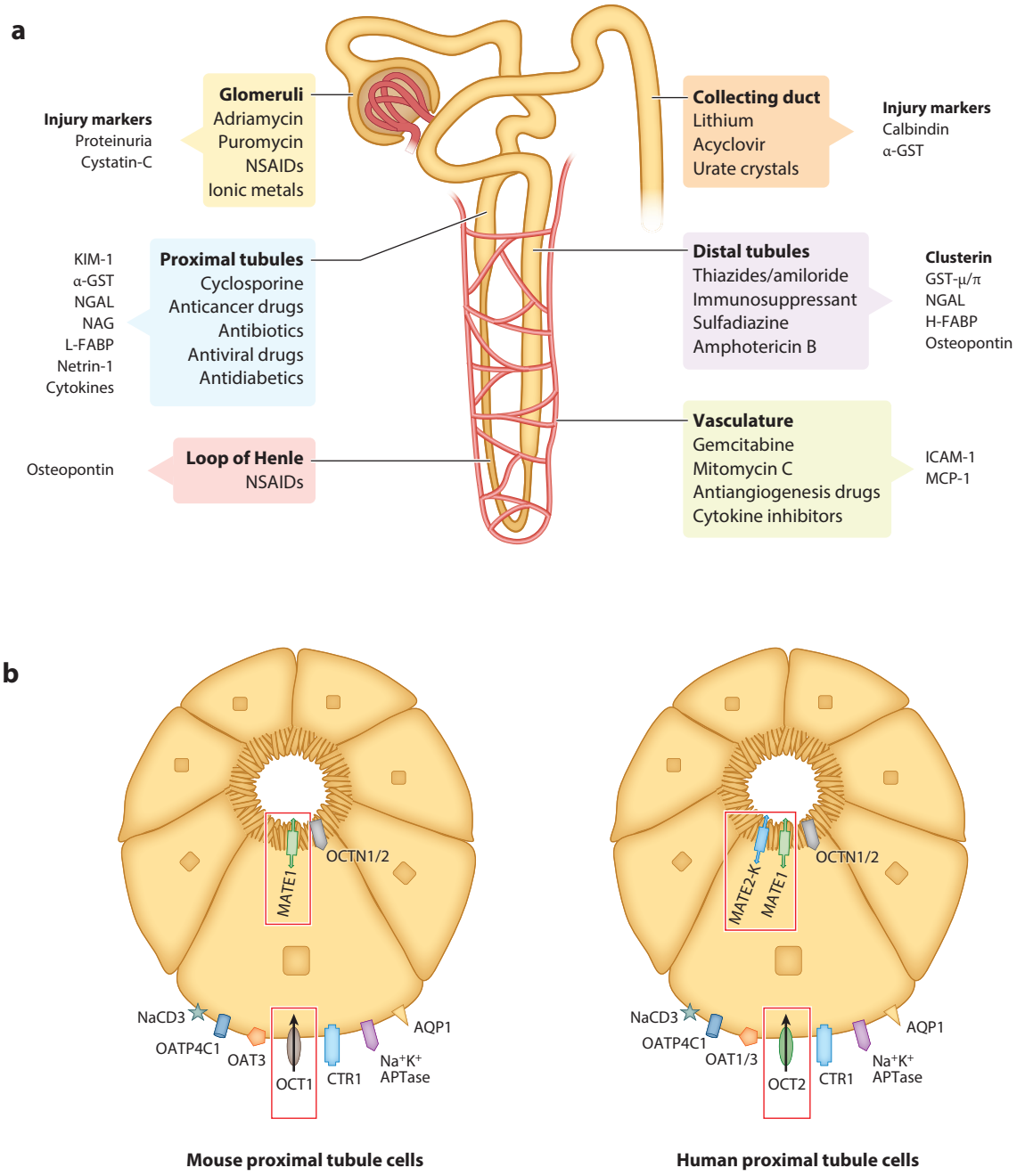

Figure 3

Segment-specific targets of nephrotoxicants and markers of injury in a comparison between mouse and human. (*a*) Diagram showing which segments of the nephrons, collecting duct, or vasculature are affected by specific nephrotoxic agents and what markers of injury response are provided by which nephron segment. (*b*) Species differences in apical and basolateral transporters within the proximal tubules of human and mouse. Differences in transporter proteins between mouse and human proximal tubules are shown in red boxes.

the only physiological models for the preclinical renal safety testing of pharmaceuticals in both mature adult kidney and immature fetal kidney (60, 61).

Researchers had hoped that in vitro cell systems would be able to provide more rapid safety evaluation of pharmaceutical molecules. To this end, many studies have used either human kidney cell lines or isolated primary kidney cells from cadaveric renal tissue cultured in vitro in 2D or 3D formats using complex microfluidic chambers. The most commonly employed immortalized cell lines are derived from proximal tubular epithelium and include HEK-293 (62, 63), HK-2, RPTEC/TERT1, and ciPTECS. Even though these cell lines express some of the major solute carrier transporters, they fail to show physiological response to nephrotoxic drugs in terms of induction of kidney injury molecule KIM-1. This further limits the use of these models for proof-of-concept studies.

WILL DRUG SCREENING BE POSSIBLE USING STEM CELL–DERIVED KIDNEY TISSUE?

As described previously, the directed differentiation of hPSCs to kidney organoids may represent a new approach for predictive toxicity and efficacy screening. As these are derived from human cells, there is potential for organoids to have a more appropriate suite of transporters compared to rodent models. The presence of nephrons formed in 3D within a surrounding stromal population may also provide a more physiological set of responses than an isolated cell type cultured in 2D. However, the fact that organoids are complex multicellular structures represents a challenge with respect to reproducibility and quality control. The key question is whether the tubular epithelium and vasculature within an hPSC-derived organoid is an accurate model of the postnatal human kidney. The epithelium of the proximal nephron segments present within kidney organoids that were generated using current protocols displays a lumen and polarized epithelium. Immunofluorescence staining shows the presence of appropriate apical markers such as CUBULIN, MEGALIN, and FcRN (**Figure 2*e***) and the presence of vasculature (44). Several kidney organoid studies reported evidence that drug toxicity testing is possible using kidney organoids from PSCs (32, 41, 44, 46, 48). This has included the treatment of kidney organoids with either cisplatin or gentamycin, which resulted in proximal tubular cell death or the expression of KIM-1 (44, 46, 48), although KIM-1 induction was minimal (47). Indeed, Li et al. (32) reported the generation of organoids via nephron induction of primary cultured NPs in which they observed massive cell death and caspase 3–specific induction within component proximal tubule cells in response to gentamycin, but the doses were very high.

However, as noted previously, hPSC-derived organoids most closely match trimester 1 human kidney, with the nephrons structurally reflective of the capillary-loop stage of nephron patterning. Current reports have not extensively characterized the relative expression of the key solute transporters, such as OAT1, OCT2, or MATEs, in comparison to primary or immortalized lines. Takasato et al. (44) performed expression profiling across the differentiation time course of this protocol. Notably, expression of these key nephrotoxicant targets was very low. It is likely that differentiation protocols will need to be extended and/or altered to maximize proximal tubular segmentation and maturation for such models to be useful in predictive testing. There are also reports in which hPSCs are apparently directly differentiated toward targeted cell types, including proximal tubule and podocyte (64). Kandaswamy et al. (64) developed a rapid single-step protocol by treating hPSCs with BMP2 and BMP7 to generate a >90% pure population of human proximal tubular-like cells. They report that the use of these cells facilitated the accurate screening of drugs for their nephrotoxicity (64, 65). They also express specific tight junction proteins including zonula occludens-1 (ZO-1) and E-cadherin (ECAD), water and glucose transporters such as

SGLT2, GLUT5, and AQP1, and major ion transporters including OAT1 and OCT2. These cells responded to specific nephrotoxic drugs by expressing inflammatory cytokines including interleukin (IL)-6, IL-8, and reactive oxygen species, with this inflammatory effect able to be reversed by using specific channel inhibitors (64). Another group directly reprogrammed mouse fibroblasts to induced renal epithelial cells (iRECs) by enforced reexpression of key transcriptional factors *Emx2*, *Hnf1b*, *Hnf4a*, and *Pax8* (66). These iRECs showed a similar mRNA expression profile to primary renal epithelial cells and were shown to respond to cisplatin-induced nephrotoxicity. This effect was thought to be mediated via OCT2 and resulted in increased expression of KIM-1 (66). The same group also showed evidence for iREC reprogramming using human cells.

As mentioned above, organoids cultured in vitro lack a functional circulatory network and urinary filtrate, which presents additional challenges for their use in nephrotoxic screening. Appropriate transporter expression and/or apical and basolateral patterning may limit the capacity to evaluate compounds that would normally enter the proximal tubular epithelium from the apical surface. Indeed, the absence of a closely opposed peritubular capillary bed will also prove an access challenge to the testing of compounds that target basolaterally positioned transporters, as these will have to reach their target via free diffusion across substantial numbers of cell diameters. By contrast, although the 2D culture of immortalized cell lines shows suboptimal transporter expression, the toxins can be directly applied to either the apical or basolateral surface. We have shown that transplantation of kidney organoids under the renal capsule not only results in the formation of glomerular and peritubular capillaries, but it substantially improves tubular ultrastructure suggestive of significant maturation (67). This includes the formation of an open lumen with a mature brush border. It is possible that such transplanted human organoids may act as human avatars in a similar fashion to patient-derived xenografts. Although this would not prove a high-throughput approach, it may present a more accurate preclinical test of nephrotoxicity (**Figure 4**).

Can We Model Human Kidney Disease Using Stem Cell–Derived Tissue?

CKD describes a group of heterogeneous disorders that result in persistent abnormal kidney structure and function (68). Its occurrence is common, and CKD often coexists with other medical conditions; however, there remains a limited understanding of the pathobiology of disease development. Our understanding of the genetic causes of kidney disease is continuously advancing, with ongoing improvements in both diagnostics and disease modeling allowing us to tease out the specific mechanisms of disease manifestation and progression. Until recently, research into kidney disease pathobiology was primarily performed using immortalized cell lines or animal models, as the acquisition of patient-derived tissue for primary cell culture is often limited. As for nephrotoxicity screening, in vitro immortalized cell lines allow specific analysis of the cell of interest, whereas in vivo mouse models provide the additional benefit of a functional whole organ with associated neighboring cell types within the context of a living organism. This offers the opportunity to investigate both the kidney disease phenotype and any additional nonrenal phenotypes that may also be associated with the disorder of interest. However, it is well understood that despite the conservation of gene expression observed between humans and mice, there are also distinct differences that are of crucial importance (69, 70), particularly when attempting to model human disease.

Kidney organoids derived from patient iPSCs provide a genetically identical model of the patient's disorder in a multicellular, 3D format. The advantages of such a system over 2D iPSC-derived cell types are that organoids provide the opportunity for cellular cross talk, with both cell–cell and cell–matrix interactions facilitating appropriate cellular conformation (71, 72). The use of kidney organoids in the modeling of genetic kidney disease was first reported in 2015.

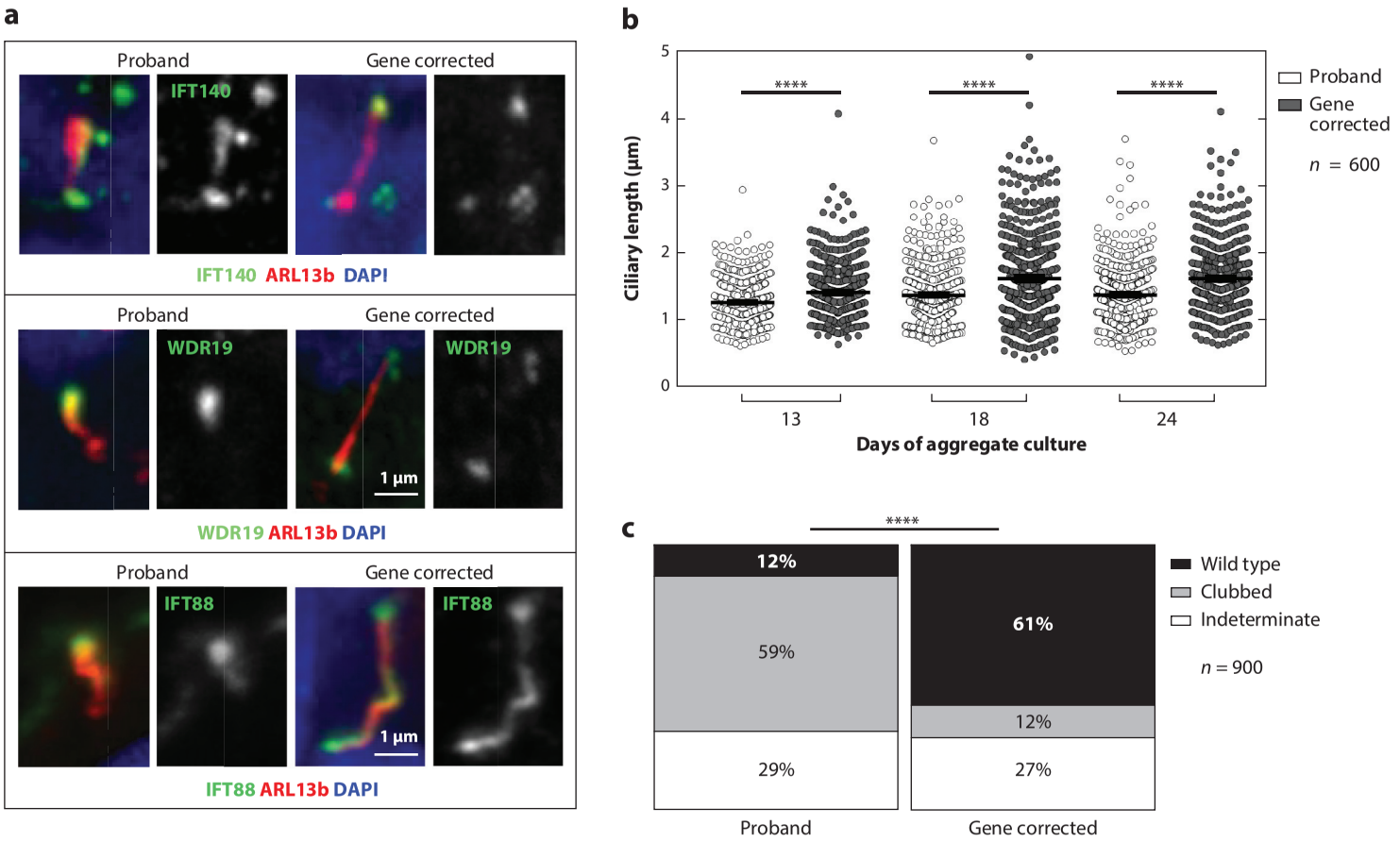

Figure 4

Application of organoids to patient-specific disease modeling. (*a*) High-resolution imaging of tubular epithelial apical cilia. Cilia were imaged within organoids generated from a proband with compound heterozygous mutations in *IFT140* and an isogenic gene-edited control in which a single point mutation has been gene corrected. Images show the subcilial localization of ARL13b and IFT140, showing the presence of shortened cilia with bulbous tips in the proband, which resolve after gene correction (77). (*b*) Quantification of cilia length across organoid culture in proband and isogeneic gene-corrected organoids (77). (*c*) Quantification of cilia morphology on epithelial cells within proband and isogenic gene-corrected kidney organoids (77). Figure adapted with permission from Reference 77. Copyright 2018, Elsevier.

Modeling was performed using iPSC lines generated by CRISPR/Cas9–mediated gene editing to induce gene knockout of *PKD1* or *PKD2* (48). The *PKD1* and *PKD2* genes are mutated in 85% and 15%, respectively, of all affected families with autosomal dominant polycystic kidney disease (ADPKD), the most frequently inherited nephropathy (73). *PKD1* and *PKD2* encode the proteins polycystin 1 and polycystin 2, both of which are components of the primary cilia of renal cells (74). Kidney organoids generated from these homozygous knockout lines demonstrated epithelial cystic dilations following extended culture in a small percentage (~6%) of organoids (48). Following on from this study, the same group improved the efficiency of cystogenesis achieved using this protocol by reducing organoid adherence and hence changing the microenvironment of the culture (75). This resulted in approximately 75% of PKD organoids forming cysts, highlighting the importance of optimal culture conditions to reveal specific disease phenotypes. Although these kidney organoids successfully demonstrated a disease phenotype in line with the development of ADPKD (48), it is worth noting that disease-causing variants reported in ADPKD patients are significantly less severe than the biallelic truncations generated in the iPSCs used in this study. Consequently, this study did not represent a model in which patient genotypes are accurately reflected. Indeed, ADPKD is dominant at the level of the patient but recessive at the level of the cell; hence, an iPSC derived from a patient will only carry a single mutation with a second hit required for disease onset. It remains to be seen whether progression to a recessive state can occur in an organoid state where this event may not occur for decades within the patient. Conversely, ARPKD (autosomal recessive PKD) presents early in life and may be more amenable to patient iPSC–based organoid modeling.

Although gene-edited lines have provided insight into the development of certain disease phenotypes, the use of patient iPSCs is more preferable, as they are more likely to reflect the disease state associated with both the identified genetic variant and other potentially unidentified interactors. The genetic basis of more than 50% of heritable kidney disease remains unknown (76). The use of patient-derived kidney organoids may also assist in the identification of novel gene variants and enable the study of the underlying pathogenic mechanism for both known and novel gene variants. A recent publication has shown the validity of patient-derived iPSCs for kidney organoid–based disease modeling of the heritable ciliopathic cystic kidney disease nephronophthisis (77). Patient-specific iPSCs were generated from an individual with known compound heterozygous mutations in *IFT140*$^{-/-}$. Matched, isogenic control iPSCs were also derived following CRISPR/Cas9–mediated gene correction of one allele. Patient organoids demonstrated a primary ciliary dysmorphology in conjunction with a downregulation of gene expression profiles associated with cell adhesion, apicobasal polarity, and dynein motor assembly. The observed bulbous ciliary tip characteristic of an IFT140 defect (78) was not present in kidney organoids generated using the isogenic gene–corrected iPSC line. This proof-of-concept study shows that patient iPSCs can be used to recapitulate a previously reported phenotype and that function can be restored following CRISPR/Cas9–mediated gene repair. This also confirms the utility of kidney organoids in disease modeling applications.

A distinct advantage of iPSC-derived organoids in the context of disease modeling is the ability to derive certain cell types that are particularly difficult to isolate from human subjects. A prime example is the glomerular podocyte. Not only is it difficult to acquire primary patient-derived human podocytes, but podocyte morphology is well known to be poorly preserved in 2D culture (79, 80). Therefore, validation of novel disease-associated mutations is most often performed in animal models that may not always replicate the human condition (81). A number of studies have reported the generation of podocytes from pluripotent stem cells, either via differentiation specifically to podocytes or within an organoid setting, with characterization of these podocyte-like cells often performed using 2D culture (56, 82, 83).

The study by Kim et al. (84) utilized CRISPR/Cas9–mediated gene editing to generate a knockout of *PODXL*. *PODXL* is a candidate gene for focal segmental glomerulosclerosis (85), though a *PODXL*-null patient was also recently reported with a novel syndromic type of congenital nephrotic syndrome (86), highlighting the essential function of *PODXL* in mammalian kidney development. *PODXL*$^{-/-}$ organoids displayed altered localization of the podocyte proteins podocalyxin (encoded by *PODXL*), synaptopodin, and ZO-1 in association with a reduction in the cell spacing between adjacent podocytes and a loss of microvillus formation (84). These findings were consistent with a previous *Podxl*$^{-/-}$ mouse model that implicated podocalyxin as a regulator of podocyte junction organization (87). Although this represents a proof-of-principle for the potential use of iPSC-based models in the study of human kidney disease, it should be noted that the transcriptional analysis of this mutation was performed in undifferentiated hPSCs and not in the podocytes generated from this line. However, the in vitro modeling of podocytopathies is likely to be highly feasible using patient-derived iPSCs. Indeed, in the field of idiopathic nephrotic syndrome, there has long been interest in the potential existence of a circulating factor(s) in both patients with steroid-sensitive nephrotic syndrome and those who screen negative for known steroid-resistant nephrotic syndrome genes (88). It is speculated that these individuals in fact have a form of circulating factor disease that initiates the rapid recurrence of disease within hours post-transplant (89). Access to patient serum may facilitate a response to such a circulating factor that could stratify patients according to risk of recurrence post-transplantation.

Although progress is being made in the field of disease modeling using iPSC-derived tissue, there remain a number of significant and ongoing challenges. As discussed, iPSC models are currently comparable to the level of tissue maturation seen in fetal or, at best, newborn tissues. Therefore, disorders with early- or antenatal-onset phenotypes, such as congenital nephrotic syndrome, represent better disease modeling candidates than late-onset diseases (**Table 2**). For example, to model immune-mediated disorders, a circulatory system or culture with the appropriate patient-derived immune effectors would be required. However, to monitor the presence in serum of anti-glomerular basement membrane antibodies, the use of organoid-derived glomeruli may prove useful. It is also currently unlikely that this approach can be applied to congenital anomalies of the kidney and urinary tract (CAKUT). CAKUT frequently involve structural anomalies such as duplex ureter, ureteropelvic junction obstruction, and vesicoureteric reflux. The lack of higher-order structure, including ureter, pelvis, and even medulla, makes current kidney organoids unsuitable to assist in modeling CAKUT. As noted above, the accurate modeling of kidney disease using hPSCs will require the development of improved methodologies to advance the maturation status of the iPSC-derived tissues produced. While recent data suggest considerable maturation after transplantation (67, 90), this would not be amenable to high content compound screening.

In conclusion, there are now several examples of the use of engineered or patient-derived hPSC modeling of genetic kidney disease. As the accuracy and maturity of these organoid protocols improve, they will likely be useful for the validation of disease association for novel variants of unknown significance and also for the screening of compounds to ameliorate phenotype.

Is Renal Replacement Therapy Possible Using Stem Cell–Derived Kidney Cells?

For over 70 years, renal failure has been treated with dialysis or organ transplantation. But with only 1 in 4 patients receiving a donor transplant and with the low quality of life and high morbidity associated with dialysis, there is an urgent need to find alternative treatment options. One possibility might be to deliver cellular therapy or engineer replacement renal tissue using kidney cell types generated from hPSCs. Indeed, these are not novel concepts, as there have been numerous prior approaches to the development of cellular solutions for kidney disease. These

Table 2 Kidney diseases suitable for iPSC-based modeling

Kidney disease type	Disease examples	Suited to iPSC models?	Comments
Cystic	Polycystic kidney disease (AD/AR)	Yes (48)	CRISPR/Cas9 gene–edited *PKD1*$^{-/-}$ and *PKD2*$^{-/-}$ iPSCs
	Autosomal dominant tubulointerstitial kidney disease	Yes	No data
Glomerular	Glomerulonephritis (e.g., IgA nephropathy)	No	NA
	Minimal change disease	No	NA
	Glomerulosclerosis (e.g., FSGS)	No	NA
	Membranous nephropathy	No	NA
	Diabetic nephropathy	Potential	Diabetic milieu required Proof of concept performed in diabetic cardiomyopathy (96)
	Alport syndrome	Potential	Further GBM maturation required
	Pierson syndrome	Potential	Further GBM maturation required
Tubular	Tubulointerstitial nephritis	No	NA
	Reflux nephropathy	No	NA
	Renal tubular acidosis	Yes	No data
Nephronophthisis	Nonsyndromic infantile nephronophthisis and other related ciliopathies, such as Mainzner-Saldino syndrome, Senor-Loken syndrome	Yes (77)	Patient-derived iPSCs harbor mutations in *IFT140*

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; FSGS, focal segmental glomerulosclerosis; GBM, glomerular basement membrane; iPSC, induced pluripotent stem cell; NA, not applicable.

have included additive bioprinting (91), recellularization of decellularized scaffolds (56), seeding of primary kidney cell isolates on hollow fibers for use as renal assist devices alongside dialysis (92), and the xenotransplantation of fetal kidney (14, 93). All approaches to date have had to rely upon cadaveric donor material or immortalized cell lines. The capacity to generate human kidney cell types from hPSCs will change what may be possible.

There remain three major hurdles to success in using kidney organoids for renal replacement: scale, structure, and function. The current in vitro formats for growing hPSC-derived kidney tissue are not amenable to the generation of a large 3D organ. Indeed, each protocol appears to form a limited number of nephrons depending on the configuration of the cultures, with nephrons forming simultaneously rather than as an ongoing process. With respect to structure, the current protocols available for generating human kidney tissue are structurally inappropriate. Takasato et al. (44) reported the presence of a tubular segment identified as collecting duct, but because these structures are generated after dissociation and reaggregation, the resulting structures do not necessarily have a single united epithelial network existing with a ureter. Taguchi & Nishinakamura (49) recently showed a capacity to create higher-order kidney organoids by separately generating ureteric epithelium/nephric duct and combining this with nephrogenic mesenchyme and murine kidney stroma. While this substantially improved the higher-order structure of the resulting organoids, this was not successful using hPSC-derived cell types.

It has also proven extremely challenging to derive cells with appropriate levels of functional maturation. The evidence to date suggests that the nephrons present in almost all kidney organoid

protocols are immature and, indeed, they exhibit a transcriptional profile that is most similar to that of a trimester 1 human kidney (44). This may in part be a challenge of time in culture, as the human kidney begins to form at week 5 as the UB arises. However, nephrogenesis continues to week 36 in humans, with final functional maturation of some tubular cell types not occurring until after birth (11, 12). It is therefore ambitious to imagine that such functional maturation can occur in a matter of 15 to 25 days, as per the protocols described to date. What is also not available to a forming nephron within an organoid is a blood supply or urinary filtrate flow. It is not inconceivable that differentiation cues arise as a result of the initiation of filtrate formation, which begins in each nephron as soon as the glomerulus is vascularized. Sharmin et al. (83) have demonstrated that hPSC-derived podocytes can mature into vascularized glomeruli with appropriate basement membrane and slit diagram formation when transplanted under the kidney capsule of a recipient mouse. Additionally, whole kidney organoids transplanted into recipient mice are also capable of drawing in host-derived vasculature that forms connections with endothelial networks preexisting in the organoids (67). In response to a patent circulation, such transplanted organoids showed evidence for substantial glomerular, tubular, and vascular maturation compared with those cultured in vitro. It remains promising, therefore, that with host vascularization comes substantial maturation in transplanted organoids. Although these initial findings are encouraging, they support the need for vascular flow for functional maturation.

CONCLUSION

In summary, decades of research into the molecular and cellular regulation of kidney morphogenesis in rodent models, particularly the mouse, have provided both an atlas of the mammalian kidney and a roadmap for recapitulating kidney morphogenesis. This information has been used to guide the development of protocols for the directed differentiation of hPSCs to models of the developing human kidney. The capacity to create a wide variety of human renal cell types in vitro provides the opportunity for patient-specific disease modeling, drug screening, cellular therapy, and bioengineered replacement renal tissue. However, there remains a large gap between the current state of play and many of these desired endpoints. The use of these models for drug toxicity or efficacy screening will require evidence of improved levels of maturation. Although proof of concept has been provided for the modeling of early onset genetic kidney disease, these models are unlikely to accurately represent even genetic disease resulting from prolonged insult, such as diabetic nephrotic syndrome, or an immunological challenge, such as glomerulonephritis. Finally, to generate renal replacement tissue for the treatment of chronic kidney disease will require improvements in scale, structure, and function. Despite these challenges, this system can provide a window into early developmental processes in the human kidney that have previously been unavailable for study. The use of patient-derived lines, the generation of isogenic control lines using CRISPR/Cas9–mediated gene editing, and the development of additional reporter and mutant iPSC tools may prove a valuable approach to understanding the regulation of nephrogenesis in the human context.

DISCLOSURE STATEMENT

M.H.L. holds intellectual property around the generation of kidney organoids from hPSCs and has consulted for and received funding from Organovo Inc. L.J.H., S.E.H., and S.V.K. are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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