

From human pluripotent stem cells to functional kidney organoids and models of renal disease

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In health, the mature human kidney contains on average 1.4 million nephrons (1). Each of these epithelial tubes begins in the cortex of the organ with a glomerulus that ultrafilters blood, clearing water and small molecules but retaining large proteins and blood cells. The two kidneys receive around 20% of the cardiac output, a high pressure and high volume arterial flow needed to generate daily the 200 litres of ultrafiltrate. The nephron tubule segments modify this ultrafiltrate, for example reclaiming some sodium and water. The nascent urine then flows into collecting ducts that executes concentration and further acidification. The branched collecting ducts converge towards the papilla where urine, around one to two litres a day, enters the renal pelvis and then the ureters, in which it is propelled towards the urinary bladder by peristaltic waves.

Kidney diseases are common and sometimes lead to end-stage disease with kidneys that fail to sustain life. Indeed, around 1–2 million people are being treated around the world with long-term dialysis or have received kidney transplants (2). Such treatments, however, are not always available in all countries, with at least a million people dying annually from untreated end-stage kidney disease (2). There are therefore urgent needs to understand the kidney disease mechanisms, with a view to designing treatments that slow progression of these entities, and also to find therapies that will replace or complement conventional dialysis and transplantation. It is increasingly appreciated that many kidney diseases have monogenic bases (3,4), and that, especially in children, the origins of kidney disease

are found in perturbed development and differentiation in the fetus (4–6). The human metanephric kidney, that will grow to become the mature organ, originates at 5 weeks gestation, with its first layer of glomeruli formed at eight weeks and new layers of nephrons generated in its outer cortex until 34 weeks of gestation (7).

There has been a long history of developmental biologists studying kidney development, mostly in mice. The focus has been to understand the biology of several key anatomical events within the metanephros that forms from the interaction of two types of tissue that each derive from intermediate mesoderm, the ureteric bud and the metanephric mesenchyme. The questions have centred on: how does the ureteric bud, a branch of the mesonephric duct, originate and then branch serially to form collecting ducts? and how does the metanephric mesenchyme become induced to undergo a transition to epithelium and thus form nephrons? The early investigators recognized the fact that the intact rudiment could be cultured *ex vivo* where it formed a small kidney, whereas if either the bud or the mesenchyme was cultured in isolation each structure would fail to differentiate and then die (8). Studies in mutant mice have clarified that the bud and mesenchyme release growth factors that generally act in a paracrine manner to nurture their neighbouring tissues (9). Furthermore, extracellular matrix molecules are required for metanephric survival and differentiation (10,11), and the expression of both growth factors and matrix molecules are regulated by transcription factors, themselves often linked in a hierarchical network (9).

Although kidney anatomy in the mouse is generally

similar to that in the human, it is not identical (12,13) and, moreover, each murine kidney contains only around 10,000 nephrons (14). Apart from the obvious question of scale, there are other important differences in kidney development between the two species. First, the mouse genome lacks homologues of certain genes present in humans. One such is *KAL-1* that encodes a basement membrane protein called anosmin which coats the surface of the ureteric bud (15,16). People who carry *KAL-1* mutations can have renal agenesis, absent kidneys and ureters (16). Second, mutant mouse models do not always exactly mimic human disease. A good example is provided by intragenic variants or whole gene deletions of hepatocyte nuclear factor 1B (*HNF1B*), a transcription factor prominently expressed in metanephric tubules. Such mutations cause human disease in the heterozygous state (17), yet mice with only one deleted allele are healthy and, even when both alleles are mutated in metanephric tissues (18,19), the murine kidney malformation lacks key histological features of human renal dysplasia such as tubules surrounded by smooth muscle collars and islands of cartilage (5). Moreover, the mouse models apparently lack the sequence of prenatal overgrowth followed by spontaneous involution characteristic of human multi-cystic dysplastic kidneys (20). Finally, recent analyses have emphasized that mouse and human nephron precursors do not have identical transcriptomes (12).

There is therefore a clear need to both understand normal and abnormal kidney development, using species-specific models. It is here that pluripotent stem cell (PSC) technology is showing great promise. The last few years have seen several research groups report protocols to drive human PSCs (hPSCs) to become metanephric kidney precursors via primitive streak and then intermediate mesoderm phenotypes (21-24). These strategies involve exposing hPSCs to precisely timed sequences of chemicals, for example to enhance WNT signalling, and growth factors, for example fibroblast growth factor 9 (21), a protocol that was then independently shown to be reproducible and effective using several clinical grade wild-type hPSC lines (25).

A recent study by Taguchi and Nishinakamura (26) builds on previous observations by the same laboratory (23). These investigators used a strategy they called the 'reverse induction approach' in which they first carefully defined the requirements for differentiation of components of wild-type mouse metanephric kidney rudiments from mesodermal precursors, and then applied this knowledge to

direct differentiation of mouse and then human PSCs into either ureteric bud or nephron lineages. Differentiation of cells into each of these lineages required different levels of WNT activity, and ureteric bud cell differentiation required exposure to retinoic acid and glial cell line-derived neurotrophic factor. During these studies, they also made the novel observation that, at least in mice, the ureteric bud and the nephron precursors arose *in vivo* from separate tissue compartments, respectively anterior and posterior intermediate mesoderm (23,26).

The first studies with hPSCs followed cells over several weeks in 2-dimensional cell culture, generating immature nephrons and collecting ducts (21). These protocols have been refined and continue to be extended to give more 'kidney-like' results. For example, Takasato and colleagues (27) built on their 2-dimensional protocol to generate kidney organoids. They dissociated hPSCs-derived renal precursors early in their differentiation, exposed them to a pulse of CHIR 99021, a GSK3 β inhibitor, and then plated them onto transwell organ culture membranes. There, the cells aggregated and differentiated into 3-dimensional 'organoids' containing nephrons, with immature glomeruli, proximal and distal tubules, as well as collecting duct units. This strategy advanced nephron development, but such organ culture protocols result in glomeruli that lack the full range of expected mature proteins, for example those required to make the mature glomerular basement membrane (25,27) that is required for size-selective ultrafiltration of blood. Furthermore, although some capillaries form in these organoids (25,27), almost all are located between tubules and these rarely invade glomerular tufts, as they should during normal development *in vivo*. This is associated with a lack of vascular endothelial growth factor A (VEGFA) (25), a critical angiogenic factor normally made by glomerular epithelia called podocytes (28). Naturally, there is no blood supply in any of the above organ culture protocols. In other experiments, hPSC-derived kidney precursor cells were implanted either under the skin (25) or beneath the kidney capsule (29) of immune deficient mice. In these contexts, life-like glomeruli were formed that contained capillary loops, with a molecularly and ultrastructurally mature glomerular basement membrane between endothelia and podocytes that themselves expressed VEGFA. Moreover, these glomeruli are supplied by blood (25,29), with evidence of at least a low level of ultrafiltration into nephron tubules that formed in implants (25).

Although superficially impressive, even such *in vivo* hPSCs-derived renal organoids are still far from precisely resembling a whole human kidney. First, each organoid contains around one hundred nephrons (25), while a native human kidney contains over a million (1). Moreover, each organoid is up to one centimetre long, while the average length of a mature human kidney is 12 centimetres. Second, they are fed by small vessels rather than having a renal artery. Third, their internal organization lacks the cortical-medullary patterning required to generate concentrated urine. Fourth, the organoids do not have a renal pelvis or ureter, so that any urine-like substance formed would merely diffuse into surrounding tissues. In future, harnessing tissue engineering approaches, for example to enhance blood supply (30) and kidney medullary maturation (31), and knowledge of the molecules that drive ureter development (32) may lead to more realistic kidney organoids. If such barriers can be overcome, then a next step would be to test whether implanted human kidney organoids can generate enough urine to prolong the life of animals without any native kidney function, as has been demonstrated to be feasible after implantation of rat metanephric kidneys onto the omentum of anephric rats (33). Indeed, similar transplantation approaches have been used with human metanephroi (34).

Finally, hPSC and kidney differentiation protocols are now being utilized to model human genetic kidney diseases. Here, mutant cells, generated either from wild-type PSCs by gene editing or directly from patients by making induced PSCs, are being induced to differentiate into kidney tissues. Examples include: mutant *podocalyxin* cells that form abnormal glomerular epithelia, a model of podocyte disease (35); mutant *PKD1* or *PKD2* cells that form cysts, models of polycystic kidney disease (36); mutant *IFT140* cells that form dysmorphic tubules, a model for the early onset kidney degenerative disease called nephronophthisis (37); and induced PSCs from Lowe syndrome caused by mutations of *OCRL1*, where the derived kidney cells have defects in primary cilia and protein exit from the Golgi complex (38). In future, these hPSC-centric approaches may be made more informative and realistic by implanting mutant kidney precursor cells to study their fates, with a view to designing novel treatments. Given that there are many types of monogenic kidney diseases (3,4), and the phenotypes of many of these may not be precisely replicated in mutant mouse models, this line of investigation is expected to increase exponentially over the next decade.

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Footnote

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