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Recreating kidney progenitors from pluripotent cells

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Abstract

Access to human pluripotent cells theoretically provides a renewable source of cells that can give rise to any required cell type for use in cellular therapy or bioengineering. However, successfully directing this differentiation remains challenging for most desired endpoints cell type, including renal cells. This challenge is compounded by the difficulty in identifying the required cell type *in vitro* and the multitude of renal cell types required to build a kidney. Here we review our understanding of how the embryo goes about specifying the cells of the kidney and the progress to date in adapting this knowledge for the recreation of nephron progenitors and their mature derivatives from pluripotent cells.

Keywords

Kidney development; embryonic stem cell; directed differentiation; kidney progenitor

Introduction

The kidney is a complex organ with a highly constrained architecture and more than 20 distinct mature cellular phenotypes, all of which are required for this organ to regulate fluid balance, nitrogenous waste removal, acid-base homeostasis, haematocrit and bone density. This cellular and structural complexity represents a significant challenge with respect to repair or prolongation of function. All of the 200,000 to 1.8 million nephrons present in a human kidney (1) are formed *in utero*, however the progenitor population from which these nephrons are formed does not persist into postnatal life (2). Therefore, whether renal disease presents at birth or later in life, prolongation of renal function followed by replacement in the form of dialysis or transplantation is the only option available to the patient.

While preferable, only a portion of end stage renal patients will receive a transplant due to limited availability and the requirement for immunological matching. However, there have been several recent breakthroughs in the field of bioengineering that may ultimately represent feasible approaches to the recreation of functional kidneys or kidney-equivalents. These include bioprinting and recellularization of tissue scaffolds. Bioprinting is the process in which cells are suspended in a biogel and robotically ‘printed’ into three dimensional

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structures for the replacement of tissues (3,4). This has proven successful for tissues such as the bladder, pinna of the ear and blood vessels (5,6). While currently limited by printer resolution and methods for the culture of large complex organs, it is hoped that bioprinting may eventually result in the recreation of adult organs including kidney (4). In contrast, recellularisation involves the use of the organ itself as a scaffold into which new cells can be placed (7). Researchers recently reported the successful decellularisation of a human kidney via a process of detergent perfusion, with the resulting organ maintaining an intact extracellular scaffold (8). As a proof of concept, a decellularised rat kidney scaffold was successfully repopulated with both an intact endothelium and a tubular epithelium via cellular infusion under negative pressure. The resulting organ was successfully transplanted into a host animal without major haemorrhage and with some evidence of appropriate cellular function. The cells used for repopulation of the tubular epithelium were harvested from sacrificed neonatal rat kidney, an inappropriate cell source for human recipients.

Both of these advances cannot be realised without a source of human cells. While possible as a source of scaffolds, reliance on access to cadaveric human material for renal cells is not feasible. One obvious solution would be the use of pluripotent stem cells, including either human embryonic stem cells (hESCs) or human induced pluripotent cell (hiPSC). (Figure 1). By definition, a pluripotent cell has the ability to form any mature cell type (9). Significant progress has been made in the direct differentiation of hESC/iPSC to specific somatic cell phenotypes, including neurons, cardiomyocytes, pancreatic and hematopoietic cells (10-13). If a direct differentiation protocol were developed for the generation of renal cell types, these might not only be of value for bioengineering but also for cellular therapies or as tools for nephrotoxicity screening. Indeed, the ability to generate a pluripotent state from an adult somatic cell (hiPSC) (14,15), and to edit the genome of the resulting cell line (16), heralds the prospect of cellular therapies to overcome inherited renal diseases including Alport syndrome, Finnish nephropathy and possibly even polycystic kidney disease.

The directed differentiation of pluripotent cells to a given endpoint is of interest to many fields. Two main approaches have been taken. Unbiased high throughput chemical screens make no assumptions about the signalling mechanisms required. This approach has been successfully applied to screen for compounds able to maintain pluripotency or promote differentiation (17, 18). The other approach is to use our understanding of the differentiation steps used by the embryo to move from the pluripotent state to a specific tissue cell type. This has been highly successful in the directed differentiation of pluripotent cells to hematopoietic, neural and cardiomyocytic endpoints. It is also the only approach that has been reported for the generation of renal cells. Here we will review the normal embryological processes involved in kidney organogenesis, discuss how this knowledge has been used as a framework for the differentiation of pluripotent cells to renal cell types and address the remaining challenges (Figure 1).

Pluripotency: the potential to start over

An embryonic stem cell is a pluripotent stem cell which is able to give rise to all three germ layers (<http://stemcells.nih.gov/info/basics/pages/basics1.aspx>). The gold standard assay for testing pluripotency is the spontaneous formation of teratomas, an assay frequently

performed for ES cells of any species via injection into the testis of mice (19). A more compelling evidence of pluripotency is the contribution of ES cells to the formation of all tissue types after reintroduction into a blastocyst. Germline transmission to form a clone indicates a capacity to even form gametes (20). The use of hESCs for germline transmission, referred to as reproductive cloning, is illegal in most countries. However, the use of such cells to generate specific cell types for cellular therapies opens the door for novel approaches to regenerative medicine.

The first hESCs were isolated almost 15 years ago (21). As self-renewing stem cells, such pluripotent cells may afford an ability to generate large numbers of the desired cell type for transplantation. However, the derivation of such pluripotent cell lines from the human blastocyst sparked considerable ethical debate internationally. Their use also raised the challenge of immunological rejection. In a landmark discovery, Takahashi et al demonstrated that a fully differentiated somatic cell (initially a mouse fibroblast) could be 'reprogrammed' to a pluripotent state via the enforced expression of four key transcription factors, *Oct4*, *Sox2*, *Klf4* and *c-Myc* (15). This proved to be transferable to human (16) and the generation of *bona fide* pluripotent cells has been demonstrated to be feasible from a wide array of somatic cell types (skin, blood, adipocytes) via a similarly wide array of gene delivery systems, including episomal systems that facilitate the generation of transgene-free and virus-free iPSCs (9).

Much research has focussed on the propagation and expansion of pluripotent cells without differentiation. There are a number of morphological differences between mouse and human ESCs. hESCs form flat colonies with features similar to epiblast stem cells and can be maintained in a pluripotent state in the presence of FGF2 and ActivinA. mESCs form reflective, raised colonies and require the addition of LIF and BMP4 for pluripotency (22,23). Despite these distinctions, both appear to differentiate in accordance with what we understand of normal embryology. Protocols for ESCs differentiation most commonly include monolayer culture, either on matrix (collagen, matrigel) or cellular feeder layer (usually mitotically inactivated murine embryonic fibroblasts (MEFs)), or via the formation of embryoid bodies (EBs) (19). An EB is formed via aggregation of a cluster of ESCs cultivated in bulk suspension within dishes coated with a non-adhesive material. The resulting EB undergoes spontaneous differentiation into all germ layers. Exposing EBs to distinct extrinsic factors results in differentiation that involves both the cell-autonomous response of cells within the EB and cell-cell interactions in three dimensions, as occurs during early embryogenesis. On the other hand, monolayer culture places the ESCs along a 2D surface. While this may limit the influence of neighbouring cells on differentiation, the use of highly specific culture conditions (growth factors, concentration and timing) may produce more robust and uniform differentiation to certain type of lineage.

The path from inner cell mass to mammalian kidney

The path from inner cell mass cell to kidney passes through primitive streak to definitive mesoderm and intermediate mesoderm (IM) with both the ureteric bud (UB) and metanephric mesenchyme (MM) being derived from IM. The decades of embryological research investigating the pathways involved in these processes cannot be comprehensively

covered in this review. Below is a summary of the critical fate decisions required to reach kidney with a brief description of the role of growth factor families that have subsequently been employed by the field to recapitulate this process *in vitro*.

Primitive streak:

In order to direct the differentiation of pluripotent stem cells, understanding of embryogenesis and reproducing the *in vivo* condition is crucial. In the embryo itself, it is the inner cell mass (ICM) that represents the pluripotent cell population able to give rise to all cell lineages. The initial patterning event differentiating the ICM into the three primary germ layers (ectoderm, mesoderm and endoderm) is called gastrulation. Gastrulation begins with the generation of the primitive streak (PS) which is divided into anterior and posterior ends based on differential gene expression (Figure 2). While the anterior PS forms endoderm, giving rise to tissues including the gut, liver and lung, the posterior PS develops into definitive mesoderm, which ultimately patterns to form tissues including the heart, muscles, blood, bone, kidneys and gonad.

The BMP/Activin/Nodal gradient along the dorsoventral axis of the embryo induces and patterns these two germ layers (24,25). Activation of Activin receptors leads to phosphorylation and nuclear translocation of heterodimeric Smad proteins that activate the *Mixl1* promoter (26), a marker of PS. The expression of other markers, including *Brachyury* (*T*) (27) and *Eomes* mark the forming mesoderm while sustained *Mixl1* and *Sox17* expression marks the endoderm (13, 28). Specification of anterior PS is driven by synergistic Activin/Nodal and Wnt/ β -catenin signaling synergistically (29) whereas BMP signalling is considered critical in inducing mesodermal cell fates (30). Mice deficient in BMP4 (31), or the BMP receptors type I and II, failed to develop mesoderm (32,33), whereas blockade of BMP signaling in hESCs completely abolished mesoderm generation. Conversely, addition of BMP4 induced formation of a *MIXL1*⁺ mesendoderm (primitive streak) from hESC which were subsequently able to give rise to mesodermally-derived hematopoietic precursors (13).

Intermediate mesoderm:

Having reached posterior primitive streak / definitive mesoderm, this tissue is further patterned via dorsal-ventral gradients into at least four major populations: notochord (a transient 'embryonic backbone'), paraxial mesoderm (PM; future somites ie. progenitors of certain muscles and other connective tissues), intermediate mesoderm (IM; the precursor to the kidneys), and lateral plate mesoderm (LPM; includes progenitors of the heart, blood, and vascular cells). This differentiation is again patterned via growth factor gradients, as determined from studies using a variety of embryological models, including *Xenopus*, chick and mouse. In the chick, BMP4 is expressed in the LPM where it functions in an autocrine manner while the BMP antagonist, Noggin, produced by the spinal cord and notochord maintain PM (34,35). Fate commitment of the IM is also controlled by the dose-dependent activation of the BMP signalling cascade along this embryonic dorso-ventral axis (34,36,37). In addition to BMP, retinoic acid (RA) is also known to regulate the body plan of the embryo along the anterior-posterior (A-P) axis. Reduced RA signalling in the posterior embryo is critical for the appropriate pattern of Hox gene expression (38,39). These Hox genes confer

regional identity with Hox11 paralogs making the presumptive kidney (40). However, some level of RA in the trunk mesoderm is necessary for nephric duct formation (41). RA emanating from the paraxial mesoderm has also been suggested to be critical for the initial specification of renal progenitor cells (42,43).

Metanephros:

The IM gives rise to the urogenital tract, comprising the kidneys and gonads. In fact, three distinct ‘kidney’ fields are formed in a specific temporospatial pattern, starting from the cranially located pronephros, then the mesonephros and finally the caudally located metanephros. It is the metanephros that forms that final postnatal kidney. For all three of these structures, organogenesis involves the interaction between the nephric duct (also called the Wolffian duct or mesonephric duct) and the adjacent nephric cord, the block of mesoderm that runs the length of the nephric duct. As well as its earlier role in dorso-ventral patterning of the embryo, RA also appears to have an effect on induction of kidney from IM. Treatment of *Xenopus* embryos with RA and Activin A induced pronephric differentiation (44). In mouse, ectopic expression of RA increases the size of the developing kidney, while blocking the pathway prevents kidney specification (43,45).

Formation of the nephric duct via a mesenchymal to epithelial transition (MET) within the IM is the earliest morphologic evidence of renal development. Formation of the metanephros is initiated via the secretion of glial-derived neurotrophic factor (GDNF) by MM adjacent to the caudal end of the nephric duct. This GDNF signal is transduced at the Ret receptor expressed in the nephric duct, mediating outgrowth of a *Ret*⁺ UB. Once the UB has invaded the MM, it undergoes continuous dichotomous branching. The MM condenses around each ureteric tip to form the condensed or cap mesenchyme (CM) which in turn drives continued branching via GDNF secretion. Reciprocally, distinct signals from the ureteric tip drive maintenance/self-renewal and differentiation of the CM. It is the signal to differentiate, via a mesenchyme to epithelial transition (MET), which results in the formation of the nephrons which represent the basic functional and structural units of the kidney (reviewed in 46). Reciprocal signalling between these two compartments continues until the completion of nephrogenesis. The signal from the ureteric tip regarded as promoting MET / nephron formation is Wnt9b, mediated via canonical signalling, which in turn upregulates Wnt4 to drive Ca-mediated non-canonical signalling (46). The maintenance of the CM can be supported via FGF2 and BMP7 in explant culture (47), however FGF2 is unlikely to be the FGF ligand critical for this role *in vivo* (48). Indeed, the combination of FGF2 and EGF or TGF α has also been proposed to sustain CM in culture (49) and more recently FGF9 expression in the ureteric tip has been attributed a major role in survival of the CM (50). BMP7 appears to induce CM differentiation via canonical Smad1/5 signalling (51), but also supports CM proliferation via JNK-MAPK signalling (52).

Mileposts of success

The successful generation of any given embryonic population requires an intimate understanding of the gene expression changes anticipated and the genes that mark specific endpoint states. While the PS expresses *Mixl1*, the expression of *Brachyury* (*T*) (27) and

Eomes identifies the forming mesoderm while sustained *Mixl1* and *Sox17* expression marks the endoderm (13). One of the first specific markers upregulated in the IM of the mouse embryo is *Osr1* (or Odd-skipped related-1; *Odd1*) (53). The homeobox gene, *Lhx1* (previously known as *Lim1*), is also induced in the developing IM, however it is initially expressed both in LPM and IM before becoming more restricted to IM (54). Hence it may be the coexpression of *Osr1*, *Lhx1* and *Pax2* that is most specific to IM. In contrast, LPM is characterised by the expression of *Foxf1* while PM expresses genes including *Paraxis* and *Tbx6* (55-57).

A number of genes are known to be required for the induction of MM from IM. *Osr1* acts upstream of genes implicated in metanephros induction, including *Eya1* and *WT1*, thereby promoting MM formation and survival (58). The MM also expresses *Six1*, *Sall1* and *Hox11* orthologs including *Hoxa11*, *Hoxc11* and *Hoxd11* (40). Fate mapping experiments showed that *Osr1*+ MM gives rise to not only the CM but also the stromal cells, the vasculature and the mesangium. Another *Osr1* target is the transcription factor, *Pax2* (59), which functions redundantly with *Pax8* and is involved in nephric duct formation and extension. *Pax2* is also expressed in the MM. The nephric duct is also marked by *Lhx1*, *Ret*, *Slit2*, *Hoxb7* and *Gata3* expression, along with other markers (reviewed in 46).

While the CM continues to express many of these genes, the co-expression of *Six2*, *WT1* and *Cited1* is characteristic of the self-renewing CM state (60), whereas the onset of differentiation of CM to the early nephron marks the onset of expression of *Fgf8*, *Wnt4*, *Ffgr11*, *Cdh4*, *Cdh6* and *Lhx1* (61). This is followed by a series of morphological patterning and segmentation events that lead to the generation of distinct mature renal cell types including the proximal and distal tubular epithelium, loops of Henle and parietal (lining the Bowman's capsule) and visceral (podocytes) epithelium. While again few of these cells express genes located at no other time or place in embryogenesis, there are a large number of segment-restricted genes described for each cell type. Recent studies have defined gene expression signatures of different nephrons segments across developmental time and space (62-64) (see Figure 3). Studies of freshly isolated or immortalised renal cell types have also identified markers of the mature differentiated cell state, particularly for cell types such as podocytes (including *WT1*, *synaptopodin*, *nephrin*) proximal tubule (including *Aqp1*, *Lrp2*, *Slc3a1*) and collecting duct (*Aqp2* in principal cells; *pendrin* and *AE1* in intercalated cells) (see Figure 3). Such mature cells also show characteristic functional attributes, such as water transport, amino acid transport or acid-base regulation, that can also assist in defining their identity. Hence the tools are in place for assessing a successful outcome from directed differentiation to kidney.

Directed differentiation of mouse ES cells to kidney

The preceding literature describing factors known to drive kidney development has been used to guide most attempts at direct ESC differentiation to kidney. Table 1 summarises the approaches taken using mouse ESCs. While most studies investigating the differentiation of mESC to kidney derivatives have relied on an understanding of embryology, early studies drew on spontaneous differentiation. Yamamoto et al (65) examined teratomas for evidence of kidney differentiation, while Steenhard et al (66) injected undifferentiated mESC into

developing kidney showing integration into proximal tubules. The first specific attempt to generate kidney from mESC drew on the evidence that Activin A and RA are key growth factors for intermediate mesoderm differentiation. The addition of these growth factors together with BMP7 to EB cultures resulted in the induction of *Pax2*⁺ cells that could integrate into proximal tubules in an embryonic kidney (67). In most subsequent attempts, some combination of these three factors has been added to mESC-derived EBs (68-72). The assessment of success has varied between studies, and has included evidence for *Pax2*⁺ or *Aqp*⁺ cells, as determined by gene expression of presence of protein, and/or integration into developing murine kidneys (see Table 1). However, neither of these genes identifies a specific cell type within the kidney and both are also expressed in many other tissues. Indeed, none of these studies provided clear evidence of the formation of nephron progenitor cells with the potential to differentiate into all components of the nephron. This reflected the lack of known CM markers at the time and the complexity of the differentiation process within EBs, the latter making them relatively intractable for monitoring outcomes or the regulation of cell fate induction towards the desired phenotype in a stepwise manner. Other studies performed stepwise differentiation under monolayer culture conditions (73,74), showing evidence for gene expression changes consistent with differentiation through definitive mesoderm and IM to MM. Nishikawa et al (73) used a successive combination of ActivinA, BMP4, LiCl and RA to induce *Brachyury* (*T*)-expressing mesoderm followed by *Pax2*⁺ intermediate mesoderm. The subsequent addition of conditioned media from a ureteric bud cell line (CMUB-1) induced the metanephric mesenchymal genes *GDNF*, *WT1* and *Cdh11*. This suggested that the secretion of unknown factors from the UB cells contributed to MM development, highlighting the critical nature of reciprocal interactions between the UB and the MM in kidney differentiation. However, none of these studies included analyses of *Six2*. It is very likely that additional factors are required for the optimal generation and subsequent support of a cap mesenchymal state. As noted, recent studies have implicated FGF9 (50), low levels of canonical Wnt signaling (75) and inhibition of pSMAD1/5/8 signaling (51) in CM maintenance, suggesting further options for the optimization of this stage.

While there has been significant attention paid to the CM as the nephron progenitor population, the kidney contains at least two other progenitor populations; the ureteric progenitors which give rise to the collecting ducts / ureter and the stromally-located vascular progenitors (46). Little attention has been paid to the generation of the ureteric progenitor population from mESCs. GDNF-Ret signaling is regarded as essential for the support of this compartment during development. Hence, success might be identified as the formation of a *Ret*⁺ epithelial population, presumably in response to the addition of GDNF. While *in vitro* culture conditions for mouse UB have not been well established, rat primary UB cells can be maintained with TGF α , EGF and low concentrations FBS (1-2%) (76). *Ret* expression in the UB has been reported to rely upon expression of the RA-synthesizing enzyme retinaldehyde dehydrogenase (*Raldh2*) in the adjacent stroma and freshly isolated mouse UB can remain *Ret*⁺ *in vitro* only in the presence of RA (77). To date, these conditions have not been investigated for the purposes of mESC differentiation into ureteric progenitors.

Progress on forming kidney from human pluripotent cells

In comparison to mESC, there have been fewer reports of attempts to direct the differentiation of human pluripotent cells to kidney (Table 1). Lin et al (78) approached the problem by reducing the concentration of serum to encourage spontaneous mesoderm differentiation and then fractionating EB cultures based on their expression of three markers; presence of CD24 and podocalyxin, both proposed to mark MM (79), and absence of GCTM2, a cell surface marker of pluripotency. The *CD24⁺Podocalyxin⁺GCTM2⁻* fraction contained *WT1⁺PAX2⁺* cells and displayed an expression profile more similar to embryonic kidney than other fractions. This suggests that hESC are able to form a nephrogenic IM. This is supported by Mae et al (80) who report the directed differentiation of hESCs and iPSCs into IM using a combination of Activin A and the GSK-3 β inhibitor, CHIR. Subsequent addition of BMP7 and CHIR resulted in evidence of an *OSR1⁺* IM and further differentiation suggested isolated instances of mature kidney cell types, however this was not extensively analysed. As noted previously, in mouse *Osr1* is known to be expressed in the MM of the kidney but also prior to that in IM and, to a lesser extent, LPM. Indeed, *Osr1* marks trunk mesoderm and is relatively broadly expressed in cells in culture, including mesenchymal stem cells (81). Hence, while the hESC differentiated to an *OSR1⁺* state may include MM, further analyses are required to assess whether a robust CM was induced. Narayanan et al (82) showed hESCs could spontaneously differentiate into *AQP1⁺* cells at 12% efficiency after 20 days monolayer culture on Matrigel in a renal epithelial supportive media. This efficiency was improved to more than 30% by the addition of BMP2 and BMP7. These authors compared the expression pattern and functional properties of the resulting differentiated hESCs with primary human proximal tubule cells. They report a similar expression profile and functional evidence for response to parathyroid hormone, γ -glutamyl transferase (γ GT) activity, ammonia production and water transport. Finally, Song et al (83) took a single step approach to the directed differentiation of human iPSC to mature kidney cell types. iPSC-derived EBs were cultivated with Activin A, BMP7 and RA for 20 days, forming cells with a podocytic morphology and positive for *SYNPO* expression. Moreover, these cells actively endocytosed albumin uptake in a similar fashion to a primary podocyte cell line.

The challenge of defining success

While these studies are beginning to show evidence of differentiation towards potential renal endpoints, a major imperative for the field is to define and identify success. *In vitro*, the identification of a CM/nephron progenitor population is challenged by the lack of any one definitive gene or protein unique to this cell type. Gene expression analysis must involve multiple markers and accompanying co-immunoreactivity for more than one protein within the same cell is required to support any claim of a successful phenotype. More importantly, functional evidence that a CM/nephron progenitor state has been reached must be provided and this will involve demonstration of nephron formation. Present evidence for this does not extend beyond *in vitro* epithelialisation or poorly characterised evidence for contribution to an embryonic explant. Some studies only provide data on the relative levels of gene expression of one of more renal cell types within what is a mixed culture.

The generation of a mature cell type with characteristic morphology and functional properties would appear to be an easier target than differentiation to a progenitor. However, in contrast to endpoints such as neurons or cardiomyocytes, properties such as the capacity to endocytose albumin or express a water channel are not unique to podocytes or proximal tubule cells. *In vivo*, functional ‘proof of concept’ has relied upon evidence for integration into embryonic or neonatal explant cultures. Few studies have carefully identified the phenotypes of the introduced cells and even fewer studies present comparisons with the introduction of undifferentiated pluripotent cells into the same tissue. The field has also not settled on what cell types need to be generated for what purposes. The advent of iPSC opens the door for gene editing to correct inherited mutations within patient-derived lines (16) (Figure 1). Podocyte differentiation of such lines might allow cellular therapies for conditions such as Alport syndrome or Finnish nephropathy. The proof of differentiation to this endpoint would be evidence for function in the recipient adult organ, however delivery of the resulting cells remains a major obstacle. A much broader repertoire of renal cell types would be required for the seeding of a decellularized renal scaffold or the bioprinting of an adult organ. To achieve this, the specificity and efficiency of differentiation approaches must improve, together with the tools used to assess success and to purify / isolate the desired cellular endpoints.

Despite these major hurdles, progress towards the generation of renal cells from pluripotent cell sources is gaining momentum. The advances required to turn the possible into the plausible will include further insights into the specification, maintenance and differentiation of renal cellular compartments. Hence, we continue to need to learn from the embryo itself.

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References

1. Bertram JF, Douglas-Denton RN, Diouf B, Hughson MD, Hoy WE. (2011) Human nephron number: implications for health and disease. *Pediatr Nephrol* 26(9):1529–33. [PubMed: 21604189]
2. Rumballe BA, Georgas KM, Combes A, Ju A, Gilbert T, Little MH. (2011) Nephron formation adopts a novel spatial topology at cessation of nephrogenesis. *DevBiol* 360(1):110–122.
3. Chang CC, Boland ED, Williams SK, Hoying JB (2011) Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. *J Biomed Mater Res B Appl Biomater* 98(1): 160–70. [PubMed: 21504055]
4. Mironov V, Drake C, Wen X. (2006) Research project: Charleston Bioengineered Kidney Project. *Biotechnol J* 1(9):903–905. [PubMed: 16986103]
5. Skardal A, Zhang JX, Prestwich GD (2010) Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates. *Biomaterials* 31: 6173–6181. [PubMed: 20546891]
6. Reiffel AJ, Kafka C, Hernandez KA, Popa S, Perez JL, Zhou S, Pramanik S, Brown BN, Ryu WS, Bonassar LJ, Spector JA (2013) High-fidelity tissue engineering of patient-specific auricles for reconstruction of pediatric microtia and other auricular deformities. *PLoS One* 8(2):e56506. [PubMed: 23437148]
7. Badylak SF (2007) The extracellular matrix as a biologic scaffold material. *Biomaterials* 28(25): 3587–3593. [PubMed: 17524477]

8. Song JJ, Guyette JP, Gilpin SE, Gonzalez G, Vacanti JP, Ott HC (2013) Regeneration and experimental orthotopic transplantation of a bioengineered kidney. *Nat Med* 19(5):646–651. [PubMed: 23584091]
9. Okita K, Yamanaka S (2011) Induced pluripotent stem cells: opportunities and challenges. *Philos Trans R Soc Lond B Biol Sci* 366(1575):2198–2207. [PubMed: 21727125]
10. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L (2009) Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 27(3):275–280. [PubMed: 19252484]
11. Yang L, Soonpaa MH, Adler ED, Roepke TK, Kattman SJ, Kennedy M, Henckaerts E, Bonham K, Abbott GW, Linden RM, Field LJ, Keller GM (2008) Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population. *Nature* 453(7194):524–528. [PubMed: 18432194]
12. Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, Lam K, Peng LF, Schreiber SL, Rubin LL, Melton D (2009) A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 4(4):258–265. [PubMed: 19287398]
13. Davis RP, Ng ES, Costa M, Mossman AK, Sourris K, Elefanty AG, Stanley EG (2007) Targeting a GFP reporter gene to the MIXL1 locus of human embryonic stem cells identifies human primitive streak-like cells and enables isolation of primitive hematopoietic precursors. *Blood* 111(4):1876–1884. [PubMed: 18032708]
14. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 126(4):663–676. [PubMed: 16904174]
15. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 131(5): 861–872. [PubMed: 18035408]
16. Collin J, Lako M (2011) Concise review: putting a finger on stem cell biology: zinc finger nuclease-driven targeted genetic editing in human pluripotent stem cells. *Stem Cells* 29(7):1021–1033 [PubMed: 21544904]
17. Sachinidis A, Sotiriadou I, Seelig B, Berkessel A, Hescheler J (2008) A chemical genetics approach for specific differentiation of stem cells to somatic cells: a new promising therapeutical approach. *Comb Chem High Throughput Screen*. 11(1):70–82. [PubMed: 18220544]
18. Zhu S, Wurdak H, Schultz PG (2010) Directed embryonic stem cell differentiation with small molecules. *Future Med Chem* 2(6):965–973 [PubMed: 21426114]
19. Daley GQ (2003) From embryos to embryoid bodies: generating blood from embryonic stem cells. *Ann N Y Acad Sci* 996:122–131. [PubMed: 12799290]
20. Bradley A, Evans M, Kaufman MH, Robertson E (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* 309(5965):255–256. [PubMed: 6717601]
21. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. *Science*. 282(5391):1145–1147. [PubMed: 9804556]
22. Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, Howlett SK, Clarkson A, Ahrlund-Richter L, Pedersen RA, Vallier L (2007) Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature* 448(7150):191–195. [PubMed: 17597762]
23. Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, McKay RD (2007) New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature* 448(7150):196–199. [PubMed: 17597760]
24. Schier AF (2003) Nodal signaling in vertebrate development. *Ann Rev Cell Devel Biol* 19:589–621 [PubMed: 14570583]
25. Shen MM (2007) Nodal signaling: developmental roles and regulation. *Development* 134: 1023–1034 [PubMed: 17287255]
26. Pereira LA, Wong MS, Mei Lim S, Stanley EG, Elefanty AG (2012) The Mix family of homeobox genes--key regulators of mesendoderm formation during vertebrate development. *Dev Biol* 367:163–177 [PubMed: 22580160]

27. Wilson V, Manson L, Skarnes WC, Beddington RS (1995) The T gene is necessary for normal mesodermal morphogenetic cell movements during gastrulation. *Development* 121: 877–886. [PubMed: 7720590]
28. Hart AH, Hartley L, Sourris K, Stadler ES, Li R, Stanley EG, Tam PP, Elefanty AG, Robb L (2002) Mixl1 is required for axial mesendoderm morphogenesis and patterning in the murine embryo. *Development* 129(15):3597–3608. [PubMed: 12117810]
29. Sumi T, Tsuneyoshi N, Nakatsuji N, Suemori H (2008) Defining early lineage specification of human embryonic stem cells by the orchestrated balance of canonical Wnt/beta-catenin, Activin/Nodal and BMP signaling. *Development* 135(17):2969–2979. [PubMed: 18667462]
30. Zhang P, Li J, Tan Z, Wang C, Liu T, Chen L, Yong J, Jiang W, Sun X, Du L, Ding M, Deng H (2008) Short-term BMP-4 treatment initiates mesoderm induction in human embryonic stem cells. *Blood* 111:1933–1941 [PubMed: 18042803]
31. Winnier G, Blessing M, Labosky PA, Hogan BL (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev* 9: 2105–2116. [PubMed: 7657163]
32. Kimelman D (2006) Mesoderm induction: from caps to chips. *Nat Rev Genet* 7:360–372. [PubMed: 16619051]
33. Beppu H, Kawabata M, Hamamoto T, Chytil A, Minowa O, Noda T, Miyazono K (2000) BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev Biol* 221:249–258 [PubMed: 10772805]
34. James RG, Schultheiss TM (2003) Patterning of the avian intermediate mesoderm by lateral plate and axial tissues. *Dev Biol* 253:109–124 [PubMed: 12490201]
35. Wijgerde M, Karp S, McMahon J, McMahon AP (2005) Noggin antagonism of BMP4 signaling controls development of the axial skeleton in the mouse. *Dev Biol* 286(1):149–157. [PubMed: 16122729]
36. James R, Schultheiss T (2005) Bmp signaling promotes intermediate mesoderm gene expression in a dose-dependent, cell-autonomous and translation-dependent manner. *Dev Biol* 288:113–125. [PubMed: 16243309]
37. Obara-Ishihara T, Kuhlman J, Niswander L, Herzlinger D (1999) The surface ectoderm is essential for nephric duct formation in intermediate mesoderm. *Development* 126(6):1103–1108. [PubMed: 10021330]
38. Sakai T, Larsen M, Yamada KM (2003) Fibronectin requirement in branching morphogenesis. *Nature* 423(6942):876–881. [PubMed: 12815434]
39. Abu-Abed S, Dollé P, Metzger D, Beckett B, Chambon P, Petkovich M (2001) The retinoic acid-metabolizing enzyme, CYP26A1, is essential for normal hindbrain patterning, vertebral identity, and development of posterior structures. *Genes Dev* 15(2):226–240. [PubMed: 11157778]
40. Wellik DM, Hawkes PJ, Capecchi MR (2002) Hox11 paralogous genes are essential for metanephric kidney induction. *Genes Dev* 16(11):1423–1432. [PubMed: 12050119]
41. Niederreither K, Subbarayan V, Dollé P, Chambon P (1999) Embryonic retinoic acid synthesis is essential for early mouse post-implantation development. *Nat Genet* 21(4):444–448. [PubMed: 10192400]
42. Serluca FC, Fishman MC (2001). Pre-pattern in the pronephric kidney field of zebrafish. *Development* 128:2233–2241 [PubMed: 11493543]
43. Cartry J, Nichane M, Ribes V, Colas A, Riou JF, Pieler T, Dollé P, Bellefroid EJ, Umbhauer M (2006) Retinoic acid signalling is required for specification of pronephric cell fate. *Dev Biol* 299:35–51 [PubMed: 16979153]
44. Osafune K, Nishinakamura R, Komazaki S, Asashima M (2002) *In vitro* induction of the pronephric duct in *Xenopus* explants. *Dev Growth Differ* 44:161–167 [PubMed: 11940102]
45. Wingert RA, Davidson AJ (2008) The zebrafish pronephros: a model to study nephron segmentation. *Kidney Int* 73:1120–1127 [PubMed: 18322540]
46. Little MH, McMahon AP (2012) Mammalian kidney development: principles, progress and projections. *Cold Spring Harb Perspect Biol* doi: 10.1101/cshperspect.a008300

47. Dudley AT, Godin RE, Robertson EJ (1999) Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. *Genes Dev* 13(12):1601–1613. [PubMed: 10385628]
48. Barasch J, Qiao J, McWilliams G, Chen D, Oliver JA, Herzlinger D (1997) Ureteric bud cells secrete multiple factors, including bFGF, which rescue renal progenitors from apoptosis. *Am J Physiol* 273:F757–F767. [PubMed: 9374839]
49. Brown AC, Adams D, de Caestecker M, Yang X, Friesel R, Oxburgh L (2011) FGF/EGF signaling regulates the renewal of early nephron progenitors during embryonic development. *Development* 138(23):5099–5112 [PubMed: 22031548]
50. Barak H, Huh SH, Chen S, Jeanpierre C, Martinovic J, Parisot M, Bole-Feysot C, Nitschké P, Salomon R, Antignac C, Ornitz DM, Kopan R (2013) FGF9 and FGF20 maintain the stemness of nephron progenitors in mice and man. *Dev Cell* 22(6):1191–1207.
51. Brown AC, Muthukrishnan SD, Guay JA, Adams DC, Schafer DA, Fetting JL, Oxburgh L (2013) Role for compartmentalization in nephron progenitor differentiation. *Proc Natl Acad Sci U S A* 110(12):4640–4645 [PubMed: 23487745]
52. Blank U, Brown A, Adams DC, Karolak MJ, Oxburgh L (2009) BMP7 promotes proliferation of nephron progenitor cells via a JNK-dependent mechanism. *Development* 136(21):3557–3566. [PubMed: 19793891]
53. So PL, Danielian PS (1999) Cloning and expression analysis of a mouse gene related to *Drosophila* odd-skipped. *Mech Dev* 84:157–160 [PubMed: 10473132]
54. Cirio MC, Hui Z, Haldin CE, Cosentino CC, Stuckenholtz C, Chen X, Hong SK, Dawid IB, Hukriede NA (2011) *Lhx1* is required for specification of the renal progenitor cell field. *PloS One* 6:e18858 [PubMed: 21526205]
55. Burgess R, Cserjesi P, Ligon KL, Olson EN (1995) Paraxis: a basic helix-loop-helix protein expressed in paraxial mesoderm and developing somites. *Dev Biol* 168(2):296–306. [PubMed: 7729571]
56. Chapman DL, Agulnik I, Hancock S, Silver LM, Papaioannou VE (1996) *Tbx6*, a mouse T-Box gene implicated in paraxial mesoderm formation at gastrulation. *Dev Biol* 180(2):534–542. [PubMed: 8954725]
57. Mahlapuu M, Ormestad M, Enerbäck S, Carlsson P (2001) The forkhead transcription factor *Foxf1* is required for differentiation of extra-embryonic and lateral plate mesoderm. *Development* 128(2):155–166. [PubMed: 11124112]
58. James RG, Kamei CN, Wang Q, Jiang R, Schultheiss TM (2006) Odd-skipped related 1 is required for development of the metanephric kidney and regulates formation and differentiation of kidney precursor cells. *Development* 133:2995–3004 [PubMed: 16790474]
59. Dressler GR, Deutsch U, Chowdhury K, Nornes HO, Gruss P (1990) *Pax2*, a new murine paired-box-containing gene and its expression in the developing excretory system. *Development* 109:787–795 [PubMed: 1977574]
60. Mugford JW, Yu J, Kobayashi A, McMahon AP (2009) High-resolution gene expression analysis of the developing mouse kidney defines novel cellular compartments within the nephron progenitor population. *Dev Biol* 333(2):312–323. [PubMed: 19591821]
61. Georgas KM, Rumballe BA, Valerius MT, Chiu HS, Thiagarajan RD, Lesieur E, Aronow BJ, Brunskill EW, Combes AN, Tang D, Taylor D, Grimmond SM, Potter SS, McMahon AP, Little MH. (2009) Analysis of early nephron patterning reveals a role for distal RV proliferation in fusion to the ureteric tip via a cap mesenchyme-derived connecting segment. *Dev Biol* 332(2):273–286. [PubMed: 19501082]
62. Thiagarajan R, Georgas K, Rumballe B, Lesieur E, Chiu H, Taylor D, Tang D, Grimmond SM, Little MH (2011) Identification of anchor genes during kidney development defines ontological relationships, molecular subcompartments and regulatory pathways. *PLoS ONE* 6(2):e17286. [PubMed: 21386911]
63. Brunskill EW, Georgas K, Rumballe B, Little MH, Potter SS (2011) Defining the molecular character of the developing and adult kidney podocyte. *PLoS One* 6(9):e24640. [PubMed: 21931791]

64. Georgas K, Rumballe B, Wilkinson L, Chiu HS, Lesieur E, Gilbert T, Little MH (2008) Use of dual section mRNA in situ hybridisation/immunohistochemistry to clarify gene expression patterns during the early stages of nephron development in the embryo and in the mature nephron of the adult mouse kidney. *Histochem Cell Biol* 130(5):927–942. [PubMed: 18618131]
65. Yamamoto M, Cui L, Johkura K, Asanuma K, Okouchi Y, Ogiwara N, Sasaki K (2005) Branching ducts similar to mesonephric ducts or ureteric buds in teratomas originating from mouse embryonic stem cells. *Am J Physiol Renal Physiol* 290(1):F52–60. [PubMed: 16106040]
66. Steenhard B, Isom K, Cazcarro P, Dunmore J, Godwin A, St John P, Abrahamson D (2005) Integration of embryonic stem cells in metanephric kidney organ culture. *J Am Soc Nephrol* 16:1623–1631. [PubMed: 15872079]
67. Kim D, Dressler GR (2005) Nephrogenic factors promote differentiation of mouse embryonic stem cells into renal epithelia. *J Am Soc Nephrol* 16(12):3527–3534. [PubMed: 16267156]
68. Kobayashi T, Tanaka H, Kuwana H, Inoshita S, Teraoka H, Sasaki S, Terada Y (2005) Wnt4-transformed mouse embryonic stem cells differentiate into renal tubular cells. *Biochem Biophys Res Commun* 336:585–595. [PubMed: 16140269]
69. Bruce S, Rea R, Steptoe A, Busslinger M, Bertram J, Perkins A (2007) *In vitro* differentiation of murine embryonic stem cells toward a renal lineage. *Differentiation* 75:337–349. [PubMed: 17286599]
70. Vigneau C, Polgar K, Striker G, Elliott J, Hyink D, Weber O, Fehling HJ, Keller G, Burrow C, Wilson P (2007) 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. [PubMed: 17475814]
71. Morizane R, Monkawa T, Itoh H (2009) Differentiation of murine embryonic stem and induced pluripotent stem cells to renal lineage in vitro. *Biochem Biophys Res Commun* 390:1334–1339. [PubMed: 19883625]
72. Ren X, Zhang J, Gong X, Niu X, Zhang X, Chen P, Zhang X (2010) Differentiation of murine embryonic stem cells toward renal lineages by conditioned medium from ureteric bud cells in vitro. *Acta Biochim Biophys Sin (Shanghai)*. 42(7):464–471. [PubMed: 20705585]
73. Nishikawa M, Yanagawa N, Kojima N, Yuri S, Hauser PV, Jo OD, Yanagawa N (2012) Stepwise renal lineage differentiation of mouse embryonic stem cells tracing in vivo development. *Biochem Biophys Res Commun* 417(2):897–902. [PubMed: 22209845]
74. Mae S, Shirasawa S, Yoshie S, Sato F, Kanoh Y, Ichikawa H, Yokoyama T, Yue F, Tomotsune D, Sasaki K (2010) Combination of small molecules enhances differentiation of mouse embryonic stem cells into intermediate mesoderm through BMP7-positive cells. *Biochem Biophys Res Commun* 393(4):877–882. [PubMed: 20171952]
75. Karner CM, Das A, Ma Z, Self M, Chen C, Lum L, Oliver G, Carroll TJ (2011) Canonical Wnt9b signaling balances progenitor cell expansion and differentiation during kidney development. *Development* 138(7):1247–1257. [PubMed: 21350016]
76. Perantoni AO (2003) The ureteric bud. Tissue-culture approaches to branching morphogenesis and inductive signaling. *Methods Mol Med* 86(0):179–192. [PubMed: 12886769]
77. Rosselot C, Spraggon L, Chia I, Batourina E, Riccio P, Lu B, Niederreither K, Dolle P, Duester G, Chambon P, Costantini F, Gilbert T, Molotkov A, Mendelsohn C (2011) Non-cell-autonomous retinoid signaling is crucial for renal development. *Development* 137(2):283–292.
78. Lin SA, Kolle G, Grimmond SM, Zhou Q, Doust E, Little MH, Aronow B, Ricardo SD, Pera MF, Bertram JF, Laslett AL (2010) 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(10):1637–1648. [PubMed: 20143954]
79. Challen GA, Martinez G, Davis M, Teasdale R, Grimmond S, Little MH. (2004) Identifying the molecular phenotype of renal progenitor cells. *J Am Soc Nephrol* 15(9):2344–2357.
80. Mae S, Shono A, Shiota F, Yasuno T, Kajiwarra M, Gotoda-Nishimura N, Arai S, Sato-Otubo A, Toyoda T, Takahashi K, Nakayama N, Cowan CA, Aoi T, Ogawa S, McMahon AP, Yamanaka S, Osafune K (2013) Monitoring and robust induction of nephrogenic intermediate mesoderm from human pluripotent stem cells. *Nat Commun* 4:1367. [PubMed: 23340407]

81. Lusi M, Li J, Ineson J, Christensen ME, Rice A, Little MH (2010) Isolation of clonogenic, long-term self renewing embryonic renal stem cells. *Stem Cell Research* 5(1):23–39. [PubMed: 20434421]
82. Narayanan K, Schumacher KM, Tasnim F, Kandasamy K, Schumacher A, Ni M, Gao S, Gopalan B, Zink D, Ying JY (2013) Human embryonic stem cells differentiate into functional renal proximal tubular-like cells. *Kidney Int* 83(4):593–603 [PubMed: 23389418]
83. Song B, Smink AM, Jones CV, Callaghan JM, Firth SD, Bernard CA, Laslett AL, Kerr PG, Ricardo SD (2012) The directed differentiation of human iPS cells into kidney podocytes. *PLoS One*. 7(9):e46453. [PubMed: 23029522]
84. Georgas KM, Chiu HS, Lesieur E, Rumballe BA, Little MH (2011) Expression of metanephric nephron-patterning genes in differentiating mesonephric tubules. *Dev Dyn* 240(6):1600–1612. [PubMed: 21491542]

Figure 1. Possible renal options for directed differentiation of pluripotent cells.

This schematic demonstrates the derivation of iPSC from adult somatic fibroblasts followed by the directed differentiation to mature kidney cells. The resulting cells might be useful for nephrotoxicity screening, disease modelling *in vitro*, or the generation of renal cells for use in bioengineering or cellular therapy. Gene editing to correct inherited mutations may allow the reintroduction of autologous normal cells to the original cell donor.

Figure 2. Embryonic differentiation from inner cell mass to kidney. Illustrated are the developmental decisions required during embryonic differentiation to both nephron progenitor and ureteric bud progenitor states (left) together with marker genes that would assist in the identification of intermediate endpoints (right). ICM, inner cell mass; Mesen, mesendoderm; Mes, mesoderm; Endo, endoderm; IM, intermediate mesoderm; LPM, lateral plate mesoderm; PM, paraxial mesoderm; MM, metanephric mesenchyme; NP, nephron progenitor / cap mesenchyme; WD, Wolffian duct; UB, ureteric bud.

Figure 3. Temporal gene expression patterns during renal development from posterior primitive streak to mature renal cell types.

This framework illustrates the steps involved in forming renal cell types from the IM stage illustrating gene expression by compartment / cell type across development, as well as the interplay between the UB and NP populations that allows nephron formation via a mesenchyme to epithelial (Mes to Epith) transition. It also shows the spectrum of different mature epithelial cell types generated from the renal vesicle or from the UB after this event and illustrates markers used to identify stages of differentiation as well as mature cell types. IM, intermediate mesoderm; ND, nephric duct; UB, ureteric bud; U Tip, ureteric tip; U Stalk, ureteric stalk / branch; PC, Principal cell; IC, intercalated cell; MM, metanephric mesenchyme; CM, cap mesenchyme (nephron progenitor); RV, renal vesicle (Stage 1 nephron); DT, distal tubule; PT, proximal tubule; POD, podocyte. Note that many of the genes involved in MET in the mesonephros are shared with that of the metanephros, however no CM or UB form (84). The mesonephric mesenchyme is also lacking expression of Hoxa11/d11.

Table 1.

Overview of directed differentiation mouse and human embryonic stem cells to renal cell types.

Ref.	Year	Starting cell	Differentiation method	Growth factors	Time	Endpoint	Assays used
Mouse ESCs	65	mESCs	Teratoma formation in the mouse retroperitoneum		2–3w	Pax2+/DBA+ tubules	IF
	66	ROSA26 mESCs	Injection into E12.5 kidney.		3–5d	Integration into NA+/K+ ATPase+ tubules	IF
	67	ROSA26 mESCs	EB formation	10% FCS-LIF 10% FCS + Activin/BMP7/RA	2–5 d 5–7 d	Intermediate mesoderm	<i>Pax2</i> , <i>Wt1</i> , <i>Lhx1</i> in EBs by RT-PCR. <i>Pax2</i> + by IF. Injection into E12.5 kidney showed the integration into LTA+ PT
	68	Wnt4 expressing mESCs	EB formation	15% FCS-LIF 15% FCS + Activin/HGF	2d 20d	AQP2+ cells in EBs	3D culture showed tubule formations using Wnt4-ESCs
	69	<i>Pax2</i> -GFP mESCs	EB formation	Serum free + LIF/BMP4	16d	Pax2+ tubules in EBs	RT-PCR and IHC of EBs
	70	<i>T</i> -GFP mESCs	EB formation	Serum free-LIF + Activin	4d	Mesoderm	<i>T</i> -GFP+ cells are injected into P0 kidney then integrated into AQP1+ tubules.
	71	mESCs miPSCs	EB formation	10% FCS 10% FCS + GDNF or BMP7 or Activin	3d 15d	Unknown	<i>Wt1</i> / <i>PAX2</i> upregulated by GDNF or BMP7. <i>KSP</i> upregulated by Activin
	74	mESCs	Monolayer	Serum free + JAK inhibitor/PI3K inhibitor/RhoA inhibitor	8d	Intermediate mesoderm	IF showed <i>Osr1</i> +/ <i>Pax2</i> +
	73	mESCs	Monolayer	10% FCS + Activin + BMP4 + LiCl + RA MM-CM or UB-CM	2d 2d 2d 2d 4–8d	UB markers ON by MM-CM. MM markers ON by UB-CM.	RT-PCR showed, <i>T</i> for PS, <i>Pax2</i> / <i>Lhx1</i> for IM, <i>GDNF</i> / <i>Wt1</i> / <i>Cdh11</i> for MM and <i>Hoxb7</i> / <i>Wnt11</i> / <i>C-ret</i> for UB.
	72	mESCs	EB formation	15% FCS-LIF 15% FCS + Activin/RA UB-CM	2d 6d 10d	Renal lineage marker+ cells	IF of EBs showed <i>WT1</i> +, <i>Pax2</i> +, <i>Pod1</i> + or <i>DBA</i> + cells
	78	hESCs	Monolayer spontaneous differentiation followed by FACS by CD24+, Podocalyxin+ and GCTM2–	On MEF 20% FCS 5% FCS	2d 12d	<i>WT1</i> +/ <i>PAX2</i> + intermediate mesoderm cells	IF showed <i>WT1</i> +/ <i>PAX2</i> +. Microarray showed renal genes upregulated.

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Ref.	Year	Starting cell	Differentiation method	Growth factors	Time	Endpoint	Assays used
80	2013	hiPSCs	Monolayer or EB formation	Serum free +ActivA/CHIR BMP7/CHIR	2d 8d	Intermediate mesoderm	IF showed renal markers. Integration assay into mouse kidney.
82	2013	hESCs	Monolayer	0.5%FCS+BMP2+BMP7	20d	AQP+ cells	In vitro functional assay and integration assay for PT.
83	2012	hiPSCs	EB formation	2.5%FCS+ActivA/BMP7/RA, plated down	3d/7d	Podocyte	IF and Functional assay.