

The Ever-Expanding Kidney Repair Shop

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When entering the kidney repair shop, a sign on the first floor directs you to the replacement parts warehouse. There, you can find unlimited and ready to use cell supplies: podocytes, parietal epithelia, proximal and distal tubules, and collecting duct cells. More complex renal structures are on the second floor. There, the most recent and exciting display was constructed by Sharmin et al.,1 showing glomeruli that were generated from induced human pluripotent stem cells (iPSCs).

The renal potential of human pluripotent stem cells was shown previously.2 When human embryonic stem cells were injected into immunodeficient mice, they generated teratoma. Inspection of the teratoma disclosed areas of embryonic glomeruli and renal tubules adjacent to nonrenal tissues.2 It took some time for this uncontrolled in vitro potential to be translated into highly directed and controlled in vitro differentiation of pluripotent stem cells toward renal lineages.3,4 The understanding of how the mammalian kidney develops in vivo laid the foundation for deciphering the inductive signals required for the in vitro directed renal differentiation protocols from pluripotent stem cells. For instance, Six2+Cited1+ nephron progenitors residing in the nephrogenic cortex were shown to give rise to all types of nephron epithelia.5 Therefore, derivation of nephron epithelial progenitors from pluripotent stem cells represents an attractive intermediate step in the differentiation protocol from which renal tissue and nephron-like structures can be further induced in culture or mature after in vivo grafting into mice. Nishinakamura and colleagues6 further translated knowledge of early kidney development into an efficient differentiation protocol; they were able to separate anterior from posterior intermediate mesoderm in the early stages of renal differentiation.3 Although a transcription factor T (also known as Brachyury) is mainly expressed in the immature mesoderm of the primitive streak at E7.5, Nishinakamura and colleagues3 found that the precursors of nephron progenitors remained as T+ immature cells at the posterior end of E8.5 embryos. These cells convert to Osr1+ posterior intermediate mesoderm at E9.5, whereas the ureteric bud precursors convert from T+ to Osr1+ anterior intermediate mesoderm 1 day earlier (E8.5). On the basis of these findings, Nishinakamura and colleagues3 were able to establish a protocol to induce nephron progenitors from T+ cells of E8.5 embryos and subsequently, succeeded in inducing nephron progenitors from mouse embryonic stem cells and human induced pluripotent stem cells (iPSCs). The induced nephron progenitor aggregates readily formed three-dimensional primordial glomeruli and renal tubules on Wnt stimulation in vitro, similar to those spontaneously observed in teratomas.2 Importantly, like other recent in vitro renal differentiation protocols,6 Nishinakamura and colleagues3 use iPSCs generated according to the Yamanaka method as the starting material, potentially affording patient–derived autologous cells rather than allogeneic cells for transplant and the possibility of personalized renal medicine.

In this study, Sharmin et al.1 used their elegant differentiation protocol to perform in-depth inspection of the human iPSC–derived three-dimensional primordial glomeruli that they had generated.3 Sharmin et al.1 did so by inserting the GFP gene into the nephrin (NPHS1) locus of human iPSCs and visualizing the GFP–tagged podocyte formation in vitro. Sharmin et al.3 went on to show that the podocytes in vitro have the typical transcriptional and morphologic features of those in vivo. On grafting of the iPSC–derived human glomeruli under the renal capsule of immunodeficient mice, Sharmin et al.1 showed that these induced glomeruli could be vascularized with the mouse vasculature, forming chimeric glomeruli with human epithelia and mouse endothelium. Even more remarkable, the iPSC–derived podocytes adjacent to the host–derived vasculature formed complex cellular processes between which slit diaphragm–like structures could be observed.1 Thus, iPSC–derived avascularized fetal glomeruli grown in vitro became fully vascularized after in vivo transplantation in mice, showing for the first time a connection of host vasculature to iPSC–derived kidney tissues and pushing the field...
forward toward possible generation of functional iPSC–derived miniature organs in vivo.

Why were complete glomerular structures not generated in vitro, and why did vascularization depend on host vessels growing into the grafted material? In the metanephric kidney, epithelial (Six2+/Cited1+), endothelial (Flk1+/Scl/Tal1+), and stromal (Foxd1+) cellular lineages are within strict lineage boundaries, and therefore, a differentiation protocol aimed at enriching Six2+/Cited1+ nephron epithelial progenitors will likely not include endothelial and stromal progenitors required to generate glomerular, endothelial, and mesangial cells. Moreover, complicating the concomitant in vitro derivation of vascular progenitors along with epithelial/stromal counterparts is a possible lack of a direct shared precursor for the former with the latter. Accordingly, a human iPSC–derived cell suspension enriched for nephron progenitors and depleted for endothelial progenitors will give rise to avascularized glomeruli in vitro, and no donor–derived glomerular vessels will be observed after in vivo grafting. In contrast, when using graft material, which harbors all metanephric cell lineages, glomerulogenesis with donor-derived vessels can occur; for instance, grafts of early human metanephric kidneys, prior to glomerular formation, which contain epithelial, stromal and endothelial progenitors in their niches and that maintain proper progenitor cell interactions can generate on transplantation in immunodeficient mice fully vascularized glomeruli with donor human vessels. Similarly, cell suspensions of embryonic kidneys are likely to contain all metanephric progenitor cell types, because they were shown to reaggregate in vitro and generated vascularized glomeruli of donor origin when grafted in vivo. Importantly, with the aim of creating human vasculature in iPSC–derived fetal glomeruli, Sharmin et al. attempted to exogenously add a mixture of nonrenal human cells known to readily generate vessels to their induced cell suspension. Nevertheless, the latter failed to vascularize the iPSC–derived glomerular structures. It remains to be determined whether human vascularization of iPSC glomeruli specifically requires the derivation of the cells responsible for glomerular vascularization in development (e.g., embryonic renal angioblasts residing in close proximity to nephron progenitors). In this regard, recent exciting attempts aimed at generating human iPSC–derived renal primordia using the three–dimensional organoid and spheroid culture previously used for somatic kidney cell growth show nephrogenesis in an array of early nephron structures. These included glomerular epithelial structures likely harboring early mesangial cells and surrounded by endothelial cells. Therefore, iPSC–derived renal organoids may encompass a broader repertoire of metanephric cellular lineages, providing an advantage over induction protocols using two–dimensional cell growth. In any event, in accordance with the work by Sharmin et al., grafting of iPSC–derived renal organoids may enhance maturation and importantly, glomerular vascularization.

So are these new replacement parts in the kidney repair shop for display only? Currently, yes. The in vitro maturation process generates renal structures mostly equivalent to those found in first trimester human fetal kidney. For renal bioengineering and growing kidneys, it is noteworthy to mention that in vivo organogenesis of grafts of first trimester human fetal kidney led, at best, to formation of miniature kidneys producing dilute urine 2 months after transplantation, far away from what is expected from an adult kidney. Therefore, essential tasks to create urine–producing functional miniature kidneys would be to extend renal maturation in vitro as much as possible and achieve induction and incorporation of ureteric bud–derived collecting ducts for possible urine drainage. For cell therapy aimed at repair of diseased kidneys (rather than creating whole new ones), there have been some reports on the beneficial effects of iPSC–derived nephron progenitor cell formulations in models of AKI. A comparison of the latter with tissue–specific somatic progenitors, such as expanded human nephron progenitors derived from fetal kidney, including analysis in CKD models, will be of importance. In the case of in vivo manipulation, retention of iPSCs at the nephron progenitor state and lack of maturation have potential to carry increased risk for tumorigenicity, because these cells may serve as precursors for human Wilms tumors.

It seems that other applications aside from renal regenerative medicine would benefit instantly from our ability to assemble nephron tubular and glomerular structures from human iPSCs as documented by Nishinakamura and colleagues. Human disease models of inherited and congenital kidney diseases, nephrotoxicity assays, and drug screens are examples of extended use. Our ability to use iPSC derived kidney cells and tissues for understanding disease mechanisms and related therapeutic targets (short term) and growing kidneys (long term) will determine whether the kidney repair shop will continue to expand and flourish.

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DISCLOSURES

None.

REFERENCES

induced pluripotent stem cell–derived podocytes mature into vascu-

2. Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waksman MA, Iatkovicz-Eldor J, Thomson JA: Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for pro-


13. Harari-Steinberg O, Mutsuyanin S, Omer D, Gnakte Y, Gershon R, Pri-

14. Pode-Shakked N, Shukrun R, Mark-Danieli M, Tsvetkov P, Bahar S, Pri-
