Producing Purer Podocytes

Benjamin S. Freedman
Division of Nephrology, Kidney Research Institute, and Institute for Stem Cell and Regenerative Medicine, Department of Medicine, and Department of Pathology, University of Washington School of Medicine, Seattle, Washington

doi: https://doi.org/10.1681/ASN.2018101045

Podocytes are highly specialized cells that play a central role in many renal disorders. For many years, podocyte research has been stymied by an inability to culture these cells in a well differentiated state. Recently, this has begun to change - kidney organoids have been generated containing podocytes with differentiated features.1 The presence of podocytes in organoids has enabled new mechanistic insights into glomerular development and disease processes.2 Organoids, however, also contain many cell types other than podocytes, which might obscure molecular details associated with this unique cell population. In this issue of the Journal of the American Society of Nephrology, Yoshimura et al.3 address this problem by tweaking organoid differentiation to produce a podocyte population with a high degree of purity (90%).

To discover signals that enrich for podocytes during kidney development, the authors start with mouse nephrin progenitor cells that have been genetically engineered to express a fluorescent reporter protein when they differentiate into podocytes. Using these cells as a screening tool, the timing and concentration of a differentiation-inducing kinase inhibitor, CHIR99021, is optimized to maximize podocyte yield. Additional screening of additives that affect key signaling pathways reveals that treatment of the cultures at the renal vesicle stage with SB431542, an inhibitor of TGF-β, suppresses the development of nonpodocyte lineages, resulting in purer podocytes.

To test whether this approach might also work in humans, the authors perform a corresponding analysis of human podocyte differentiation from induced pluripotent stem (iPS) cells, which are similarly engineered to express a podocyte-specific fluorescent reporter. Starting at the nephrin progenitor stage, a low dose of CHIR99021 with retinoic acid followed by inhibition of TGF-β and Wnt signaling pathways is shown to produce a near-homogenous culture of human podocytes. Each iPS cell is calculated to generate approximately 30 podocytes, making this a very efficient method. Because human podocytes are a rare commodity, the ability to generate these cells efficiently from iPS cells opens the door to many future applications.

Importantly, the podocytes produced using this optimized protocol appear to be very similar to those typically found in kidney organoids. They form large aggregates of tightly clustered epithelial cells joined together by basal junctions containing slit diaphragm components, which are a hallmark of podocytes in organoid cultures. Analysis of global gene expression indicates that the induced podocytes more closely resemble adult human podocytes than do immortalized podocyte cell lines, which lack these well differentiated features. This contrasts with other recent podocyte differentiation protocols that produce flatter, fibroblast-like cells, which resemble immortalized podocyte cell lines.4 How to resolve these disparate podocyte morphologies in vitro remains to be determined, ideally by directly comparing the differentiation protocols side by side.

Although the purity of the podocytes produced with the new protocol is greatly increased over kidney organoids, their actual maturation state does not seem to be substantially improved. Thus, although these cells express slit diaphragm proteins and form basal junctions, they do not form bona fide foot processes, and they arrest at a stage of development typical of early glomerular capillary loops.2 Also, a specific interaction does not arise between these podocytes and endothelial cells to reconstruct the glomerular filter. Indeed, RNA sequencing analysis of induced podocytes reveals a conspicuous absence of extracellular matrix components, such as the α3-subunit of collagen IV, that are typically associated with glomerular basement membranes.3 Why this should be is not yet clear, but it seems likely that improvements to the matrix microenvironment may be key to generating more physiologically relevant podocyte structures.

Mechanistically, enrichment of podocytes via inhibition of TGF-β seems to result from negative selection against other lineages typically arising in organoids. Because we do not yet understand well the signals that induce nephrin progenitor cells to differentiate into different types of epithelial cells, these findings raise several interesting questions. What exactly happens to the nonpodocyte progenitor cells when TGF-β is inhibited? Can other lineages, such as proximal tubules, be selected for by supplying opposing signals? Is there a correlate role in vivo for TGF-β in patterning nephron segments? Can the relative populations of nephron cell types be shifted in vivo, and if so, what would that look like?
Interestingly, when treated with puromycin aminonucleoside, induced podocytes downregulate slit diaphragm components, such as would be expected of podocytes in vivo. Thus, these cultures can reveal molecular changes associated with podocyte disease states in bulk populations. This may be a useful property in understanding the effect of mutations in gene products, such as podocalyxin, which produce morphologic phenotypes in organoid podocytes. Bulk podocyte differentiation may also facilitate the adaptation of genetic phenotypes into high-throughput screening formats to identify regulatory small molecules. It may furthermore be possible to perform genome-wide screens in culture using this system to identify genes that are critical for podocyte differentiation or health. Such experiments may, in turn, lead to conceptual breakthroughs that manifest as new therapies.

Notably, podocytes in vivo are postmitotic and can only partially be replaced by other cells. Therefore, these cells constitute a valuable resource, particularly in patients with glomerular diseases whose podocytes are vulnerable to depletion. Because IPS cells can, in theory, be derived from anyone, this study raises the possibility of bulk generation of autologous, patient-derived podocytes for clinical applications. Whether these might provide a possible source for cell replacement therapy in the future is an interesting question for future investigation.

DISCLOSURES

B.S.F. is an inventor on patent applications related to kidney organoid generation and its uses. The Freedman laboratory is supported by NIH Awards R01DK117914, K01DK102826, and UG3TR002158; Regular Award from the United States-Israel Binational Science Foundation; Allen Institute Translational Science Grant; Institute for Stem Cell and Regenerative Medicine Innovation Pilot Award; gift from Northwest Kidney Centers to the Kidney Research Institute; and University of Washington School of Medicine start-up funds.

REFERENCES


Readmissions Metrics in Hemodialysis: Do the Specifics Matter?

Taimur Dad and Daniel E. Weiner

William B. Schwartz Division of Nephrology, Tufts Medical Center, Boston, Massachusetts


doi: https://doi.org/10.1681/ASN.2018101033

The 2010 Affordable Care Act required establishment of the Hospital Readmissions Reduction Program by 2012 in the United States. Under this mandate, which aimed to improve quality by reducing hospital readmissions, the Centers for Medicare and Medicaid Services (CMS; the largest single payer in the United States) developed payment adjustment factors to reduce hospital reimbursement for unplanned readmissions that occurred within 30 days of an index discharge for a set of diagnoses. Endorsed by the National Quality Forum, a quality metric clearinghouse, these measures subsequently were adopted by most other payers in the United States. However, although clearly undesirable for patient experience and quality of life, whether 30-day readmission rates truly reflect the quality of care delivered by a hospital remains controversial.

Readmissions among Medicare beneficiaries are costly and are particularly common among patients on hemodialysis in the United States, with 35% of index discharges followed by an unplanned readmission within 30 days. These readmissions are often for causes not related to the initial admission (Figure 1). This readmission rate exceeds readmission rates for other chronic conditions, such as congestive heart failure or chronic obstructive pulmonary disease. Multiple factors are associated with an increased likelihood of readmission, only some of which may be modifiable by hospitals and dialysis facilities. These risk factors include extremes of age, the presence and severity of both medical and psychologic comorbid conditions, longer index hospitalization and need for mechanical ventilation during the index hospitalization, and more admissions over the preceding 6 months. Limited data suggest that early evaluation and intervention in the dialysis facility may reduce the overall risk of readmission.

Unplanned hospital readmission rates were incorporated into the ESRD Quality Incentive Program in the United States in 2015, such that worse performance on the standardized readmission ratio (SRR) metric could place a dialysis facility

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

Correspondence: Dr. Daniel E. Weiner, William B. Schwartz Division of Nephrology, Tufts Medical Center, 800 Washington Street, Box #391, Boston, MA 02111. Email: dweiner@tuftsmedicalcenter.org

Copyright © 2019 by the American Society of Nephrology