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COMMENTARY

Podocyte Regeneration

Who Can Become a Podocyte?

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During evolution from sea water to dry land, our kidneys became more and more sophisticated and developed from an aglomerular secretory tubule into a complex mammalian glomerular-nephron unit.¹ The increased complexity and the arrangement of individual nephrons in a dedicated three-dimensional architecture within the organ matrix came at the price of reduced regenerative capacity after injury.² Hence, although nature endowed us with approximately twice as many nephrons as we actually need to keep our internal environment in balance, the incidence of chronic kidney disease is steadily increasing.^{2,3} Glomerular podocytes especially appear to be the weak link when injury occurs.⁴ These highly specialized, postmitotic cells are exposed to a whole array of mechanical, metabolic, and immunologic stressors.⁵ There are only two anticipated ways for podocyte loss to be mitigated: hypertrophy of the remaining podocytes to cover denuded capillaries or regeneration of lost podocytes from a renal progenitor pool.^{6,7} The latter possibility has received considerable interest during the last decade, and two potential podocyte progenitor cell types could be identified in humans and rodents.^{8–10} Regeneration from the bone marrow compartment has so far mainly been seen in mouse models with a damaged glomerular basement membrane, with negative findings in rats and limited findings in humans.^{10–13} Podocyte transdifferentiation from parietal epithelial cells (PECs) has been reported by several groups in humans and mice. Strikingly, PEC progenitors not only share the same metanephric mesenchymal ancestors as podocytes but are also located within the Bowman capsule, which brings them close to the glomerular tuft.

In this issue of *The American Journal of Pathology*, Pippin et al¹⁴ present a potential third podocyte progenitor cell niche: renin lineage cells (RLCs) of the juxtaglomerular

apparatus (Figure 1). In their article entitled “Cells of Renin Lineage Are Progenitors of Podocytes and Parietal Epithelial Cells in Experimental Glomerular Disease,” they provide evidence that during experimental focal segmental glomerular sclerosis (FSGS) cells of the renin lineage repopulate both podocytes on the glomerular tuft and PECs lining the Bowman capsule. By using several distinct renin promoter-driven Cre and reporter mouse lines, they genetically fate mapped RLCs in healthy and FSGS adult mice. In this disease model, they found a significant increase of tagged RLCs in these two compartments by co-labeling them with known marker proteins of podocytes and PECs, respectively.

Because of the merit and persistence of the authors, this exciting and novel cell population is now a possible renal progenitor population. Despite the considerable effort and initial characterization, a few key issues regarding progenicity of RLCs and podocyte turnover as a whole still remain elusive. Renin cells reside outside the glomerulus in the preglomerular vascular wall and hence have to breach the parietal and/or the glomerular basement membrane to become a PEC or podocyte, respectively. Although Pippin et al¹⁴ correctly state that a subset of RLCs are pericytes of

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Figure 1 Potential podocyte progenitor niches. The article by Pippin et al¹⁴ in this issue of the *AJP* adds a third potential progenitor niche to the existing PEC and bone marrow niches.^{8,10} Podocyte proliferation has so far only been seen in crescent formation and HIV-associated nephropathy but could potentially also serve as a means to form new podocytes.^{17,18} (1) Parietal epithelial cells (PEC) to podocyte differentiation, (2) bone marrow to podocyte differentiation, (3) renin lineage cells (RLC) to podocyte and PEC differentiation, and (4) podocyte proliferation. BM, bone marrow; E, endothelial cell; JG, juxtaglomerular cell; MD, macula densa; P, podocyte.

peritubular capillaries and possess migratory potential, it is currently unclear whether this also applies to preglomerular capillary RLCs. Serial sections at different time points, probably with an electron microscopy traceable marker, will be needed to spatially and temporally resolve this issue. At first sight, the apparent distance of RLCs from both PECs and podocytes seems to be an obstacle to the progenitor properties of RLCs. However, considering the eventual destructive nature of intraglomerular disease, it might be advantageous to have a glomerular epithelial cell progenitor pool sitting outside Bowman space, which will not be affected by any intraglomerular damage.

Another obstacle is that RLCs are of mesenchymal origin and have to undergo mesenchymal-epithelial transition before transdifferentiating into PECs or podocytes. During kidney development the metanephric mesenchyme condenses around a branch of the ureteric bud and receives inductive signals, which include Wnt9b among others. These signals induce a pretubular aggregate adjacent to the distal side of each ureteric bud branch, which under the influence of Wnt4 undergoes a mesenchymal to epithelial transformation and forms the renal vesicle.^{15,16} It remains to be determined whether similar cues are operating during adulthood when RLCs become epithelial cells of parietal or glomerular origin or whether there are different cellular programs that govern this cell type switch. In this context, it will be interesting to delineate the exact sequence of transdifferentiation. On the basis of the findings by Pippin et al,¹⁴ RLCs seem to be able to replenish both podocytes and parietal cells. However, it remains unclear whether this occurs in parallel or sequentially with RLCs transdifferentiating into PECs that then give rise to podocytes.

At least during renal development such differentiation of subsets of parietal cells into podocytes is known to be present.⁸ Using the *Ren1cCreER* × *Rs-tdTomato-R* mouse strain, Pippin et al¹⁴ found that after adult tamoxifen induction RLCs appear on the glomerular tuft, staining positive for podocin and nephrin, two known podocyte slit diaphragm markers. Further studies will have to prove to what extent these cells are being integrated into the complex three-dimensional podocyte network with primary and secondary foot processes.

What amount of regeneration can we expect at all? Using the FSGS model, Pippin et al¹⁴ reported a podocyte loss of approximately 25% to 30%, which translates into a loss of three to four podocytes per glomerular cross-section. In contrast, they detected approximately 0.7 to 1.4 RLCs per glomerulus, suggesting that only one in 50 lost podocytes was replaced from this progenitor niche. A decisive question would be, how much regeneration do we have overall. Because their model seems to be progressive, the amount of regeneration does not match podocyte loss. This, so far, seems to be the case in all reports of podocyte regeneration. In a seminal article, Wiggins and colleagues⁴ determined that glomeruli can only withstand a podocyte loss of up to 20%. If injury is above this threshold, glomeruli inevitably become sclerotic. Therefore, it will be interesting to determine whether PEC and podocyte renewal from RLCs will also occur with less injury or whether RLCs only translocate into the glomerulus as a lemming-like rush before definite sclerosis occurs.

A drawback of most reports on podocyte regeneration is their lack of providing quantitative data on podocyte loss, podocyte renewal overall, and podocyte renewal from the

specific niche studied. With the elaborated mouse models available today, these lineage tracing experiments seem overdue to clarify the myths on podocyte regeneration and to better judge the potential translational relevance of these results.

In summary, although important questions remain to be addressed in follow-up studies, the article by Pippin et al¹⁴ in this issue of the *AJP* adds an exciting new player to the regenerative podocyte roulette, opening a new arena of kidney research. Future studies will have to elucidate the exact role of each potential progenitor niche for podocyte and PEC renewal and will also have to clarify how relevant these processes are to sustain human renal function.

References

1. Smith HW: Evolution of the kidney: lectures on the kidney. Edited by Khan SL, Lawrence, University of Kansas Press, 1943, pp 1–23
2. Kriz W: Glomerular diseases: podocyte hypertrophy mismatch and glomerular disease. *Nat Rev Nephrol* 2012, 8:618–619
3. Khwaja A, El Kossi M, Floege J, El Nahas M: The management of CKD: a look into the future. *Kidney Int* 2007, 72:1316–1323
4. Wharram BL, Goyal M, Wiggins JE, Sanden SK, Hussain S, Filipiak WE, Saunders TL, Dysko RC, Kohn K, Holzman LB, Wiggins RC: Podocyte depletion causes glomerulosclerosis: diphtheria toxin-induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *J Am Soc Nephrol* 2005, 16: 2941–2952
5. Nelson PJ, Alpers CE: Glomerular disease in 2011: new clues to environmental influences in glomerular disease. *Nat Rev Nephrol* 2012, 8:65–66
6. Wiggins JE, Goyal M, Sanden SK, Wharram BL, Shedden KA, Misk DE, Kuick RD, Wiggins RC: Podocyte hypertrophy, “adaptation,” and “decompensation” associated with glomerular enlargement and glomerulosclerosis in the aging rat: prevention by calorie restriction. *J Am Soc Nephrol* 2005, 16:2953–2966
7. Romagnani P, Lasagni L, Remuzzi G: Renal progenitors: an evolutionary conserved strategy for kidney regeneration. *Nat Rev Nephrol* 2013, 9:137–146
8. Appel D, Kershaw DB, Smeets B, Yuan G, Fuss A, Frye B, Elger M, Kriz W, Floege J, Moeller MJ: Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol* 2009, 20:333–343
9. Ronconi E, Sagrinati C, Angelotti ML, Lazzeri E, Mazzinghi B, Ballerini L, Parente E, Becherucci F, Gacci M, Carini M, Maggi E, Serio M, Vannelli GB, Lasagni L, Romagnani S, Romagnani P: Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol* 2009, 20:322–332
10. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R: Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. *Proc Natl Acad Sci U S A* 2006, 103:7321–7326
11. Meyer-Schwesinger C, Lange C, Brocker V, Agustian P, Lehmann U, Raabe A, Brinkmeyer M, Kobayashi E, Schiffer M, Busche G, Kreipe HH, Thaiss F, Becker JU: Bone marrow-derived progenitor cells do not contribute to podocyte turnover in the puromycin aminoglycoside and renal ablation models in rats. *Am J Pathol* 2011, 178:494–499
12. Becker JU, Hoerning A, Schmid KW, Hoyer PF: Immigrating progenitor cells contribute to human podocyte turnover. *Kidney Int* 2007, 72:1468–1473
13. Poulson R, Forbes SJ, Hodivala-Dilke K, Ryan E, Wyles S, Navaratnasah S, Jeffery R, Hunt T, Alison M, Cook T, Pusey C, Wright NA: Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol* 2001, 195:229–235
14. Pippin JW, Glenn ST, Buitrago S, Coffman TM, Duffield JS, Shankland SJ: Cells of renin lineage are progenitors of podocytes and parietal epithelial cells in experimental glomerular disease. *Am J Pathol* 2013, 183:542–557
15. Carroll TJ, Park JS, Hayashi S, Majumdar A, McMahon AP: Wnt9b plays a central role in the regulation of mesenchymal to epithelial transitions underlying organogenesis of the mammalian urogenital system. *Developmental Cell* 2005, 9:283–292
16. Stark K, Vainio S, Vassileva G, McMahon AP: Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* 1994, 372:679–683
17. Shkreli M, Sarin KY, Pech MF, Papeta N, Chang W, Brockman SA, Cheung P, Lee E, Kuhnert F, Olson JL, Kuo CJ, Gharavi AG, D’Agati VD, Artandi SE: Reversible cell-cycle entry in adult kidney podocytes through regulated control of telomerase and Wnt signaling. *Nat Med* 2012, 18:111–119
18. Moeller MJ, Soofi A, Hartmann I, Le Hir M, Wiggins R, Kriz W, Holzman LB: Podocytes populate cellular crescents in a murine model of inflammatory glomerulonephritis. *J Am Soc Nephrol* 2004, 15:61–67