Parietal Epithelial Cells Regenerate Podocytes

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In response to acute damage, such as poisons, some fish display an effective neo-nephrogenic potential in adulthood whereby new nephrons form from a residual progenitor pool.[1] In contrast, the response of kidney parenchyma in mammals seems restricted to proliferation of surviving tubule cells without new nephron production. Nephron formation is restricted to organogenesis in mammals because the progenitor population is exhausted before birth in humans and shortly afterward in mice.[2] This scenario severely restricts the renal restoration of functional capacity after damage in mammals. So how does the adult kidney replenish cells lost through damage, aging, or, as occurs for podocytes, ongoing regular loss of viable cells into the urine?

Although epithelial progenitor cells may exist in adult kidney, as shown for several other organs, the evidence remains equivocal. Simple proliferation may be sufficient to maintain most tubular segments; however, highly specialized epithelial cells of the glomerulus, the podocytes, are hardly ever observed to divide yet remain crucial for renewal and maintenance of renal function. As a result of their close developmental relatedness, there are almost no markers that discriminate parietal from visceral epithelium during renal development, yet there is clear specialization of the visceral epithelium into podocytes as the glomerulus matures. Adult glomeruli often possess cells within the lining of Bowman's capsule that bear podocyte markers,[3] leading to the proposal that bridging between the tuft and capsule contributes to pathology such as glomerular crescent formation[4]; however, why parietal cells resembling podocytes are centered on the vascular pole[5] has been challenging to explain.

As described in this issue of *JASN*, Appel *et al.*[6] used electron microscopy and immunofluorescence to observe parietal cells, intermediate peripolar cells, and podocyte phenotypes as cells "progressed" onto the tuft. They then used an elegant transgenic approach to mark permanently parietal cells (and their progeny) and monitor the fate of the parietal epithelium. This approach exploited a serendipitous observation that a specific region of the promoter of the podocalyxin-like gene (a protein normally expressed in podocytes and parietal cells among others) specifically drove marker gene expression to the parietal (but not visceral) epithelium. In this way, they were able to show that the parietal cells do indeed migrate onto the vascular tuft and differentiate into podocytes.

Also in this issue of *JASN*, Ronconi *et al.*[7] extend their previous research characterizing a progenitor population in human nephrons.[8] They identified cells around the urinary pole of the Bowman's capsule that coexpressed two previously proposed "stemness" markers of progenitor cells, CD133 (using the antibody capable of enriching for hematopoietic stem cells) and CD24. Ironically, murine CD24 had been proposed as a marker of renal stem cells on the basis of its early expression in the metanephric mesenchyme, but this protein is not equivalent to human CD24.[2] Confocal fluorescence imaging of human kidney tissues stained for stemness markers (CD24 and CD133) and markers of differentiated podocytes revealed the lining of Bowman's capsule to be formed by heterogeneous cells that follow a fairly strict spatial organization. Progressing from the urinary pole to the surface of the glomerular tuft, there was loss of stemness markers and gain of markers indicating commitment to a podocyte phenotype.

To study this commitment further, these authors isolated $CD24^+CD133^+$ cells from dissociated kidney and sorted them according to their expression of podocalyxin-like (PODXL) protein, before expanding them clonally in culture. Clones expressing all three markers seem committed to producing only podocytes and grow relatively poorly, whereas $CD24^+CD133^+PODXL^-$ cells show a dual potential as they generate podocytes or tubular epithelium (bearing appropriate differentiation markers) when cultured in selective media. A remarkable property specific to $CD24^+CD133^+PODXL^-$ cells is their integration into damaged glomeruli and tubules after injecting them into SCID mice that ameliorates the proteinuria and histologic damage from previous exposure to adriamycin. Approximately 11% of all podocytes and 7.5% of proximal tubule cells in regenerating mouse kidney were of exogenous origin, so the benefits gained may have resulted from

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true integration and transdifferentiation from a common bi-potential precursor.

How do these new studies complement what we understand already, and how might they alter future expectations? Some morphologic clues supporting a relationship between podocytes and parietal cells exists from previous scanning electron microscopy studies. In atubular glomeruli and glomerular cysts in which the vascular tuft became atrophied, parietal cells were replaced by podocytes, and some bosselated cells of intermediate morphology were seen[9]; however, the dynamic view now made possible by lineage tracing is key evidence for the existence of a flux of cells to compensate for wear and tear of the podocytes.[6] It will be exciting indeed to see whether the precursors shown to have bi-potential can contribute more broadly to regeneration.[7]

Hughson *et al.*[10] first described a phenomenon in human "end stage" kidneys after long-term dialysis whereby cells of Bowman's capsule seemed to proliferate and take on a more "embryonal" phenotype. Termed embryonal hyperplasia of the Bowman's capsule epithelium (EHBCE), this was proposed to involve de-differentiation to a more primitive state. Ogata *et al.*[11] further characterized EHBCE in association with obsolescent glomeruli in long-term dialysis patients and concluded, on the basis of ultrastructure, that these cells resembled anlage of the glomerular epithelium. This view was reiterated from a molecular perspective by Fukuzawa *et al.*,[12] who found reactivation of *WT1* and *PAX2* coexpression in EHBCE in two dialysis patients with mutated *WT1*. In light of the new observations from Appel *et al.*[6] and Ronconi *et al.*,[7] we might ask whether EHBCE is an abnormal manifestation of a normal reparative response—with accumulation of the progenitors of both parietal cells and podocytes.

Have we now found the only way podocytes are replenished? Other highly specialized cells, the Purkinje cells of the brain, seem to rejuvenate by fusion with macrophages from the blood stream, without a requirement for the structural disruption of mitosis.[13] In addition, others[14] proposed that in response to chronic injury, both the parietal and visceral epithelium of the glomerulus reverts to a more primitive macrophage-like phenotype that displays an inflammatory response. Some evidence for the incorporation of bone marrow–derived cells into podocytes has been reported in a mouse model of Alport syndrome, a process that would require the incoming cells to traverse the glomerular basement membrane.[15,16] Although this may occur rarely, even with a genetic selective pressure, the concept of a progenitor pool contiguous with the podocytic side of the glomerular basement membrane seems more feasible for regular turnover. The question of whether this population also contributes substantially to turnover of proximal tubule cells and, if so, how far along the tubules is as yet unknown.

Bi-directional flux of cells from a renal progenitor pool would resemble that proposed many years ago for the stomach.[17] Furthermore, the dependence of specific epithelial cell fates on β-catenin/Wnt, Notch, and Hedgehog signaling pathways in the epidermis and hair follicle[18] leads one to speculate that modulating such signaling in the kidney would also alter the balance of cell types produced. Hence, even if no new nephrons can form, it may prove possible to encourage the formation of new podocytes, hopefully in the right place.

The two articles in this issue[6,7] suggest there is normally a turnover of podocytes, emanating from the tubular neck region and flowing through the parietal epithelium and onward to the tip of the glomerular tuft; if so, then it is clear that in states of chronic renal damage, this reparative mechanism fails. The ability of harvested Bowman's capsule progenitors to assist repair after adriamycin in mice is therefore an exciting finding,[7] because it opens up the possibility of seeding immunologically compatible precursors to promote functional repair.

DISCLOSURES

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REFERENCES

1. Salice CJ, Rokous JS, Kane AS, Reimschuessel R: New nephrons development in goldfish (*Carassius auratus*) kidneys following repeated gentamicin-induced nephrotoxicosis. *Comp Med* 51: 56–59, 2001
2. Hopkins C, Li J, Rae F, Little MH: Stem cell options for kidney disease. *J Pathol* 217: 265–281, 2009
3. Bariety J, Mandet C, Hill GS, Bruneval P: Parietal podocytes in normal human glomeruli. *J Am Soc Nephrol* 17: 2770–2780, 2006
4. Thorner PS, Ho M, Eremina V, Sado Y, Quaggin S: Podocytes contribute to the formation of glomerular crescents. *J Am Soc Nephrol* 19: 495–502, 2008
5. Gibson IW, Downie I, Downie TT, Han SW, More IA, Lindop GB: The parietal podocyte: A study of the vascular pole of the human glomerulus. *Kidney Int* 41: 211–214, 1992
6. Appel D, Kershaw D, 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* 20: 333–343, 2009
7. Ronconi E, Sagrinati C, Angelotti ML, Lazzer 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* 20: 322–332, 2009
8. Sagrinati C, Netti GS, Mazzinghi B, Lazzeri E, Liotta F, Frosali F, Ronconi E, Meini C, Gacci M, Squecco R, Carini M, Gesualdo L, Francini F, Maggi E, Annunziato F, Lasagni L, Serio M, Romagnani S, Romagnani P: Isolation and characterization of multipotent progenitor cells from the Bowman's capsule of adult human kidneys. *J Am Soc Nephrol* 17: 2443–2456, 2006
9. Gibson IW, Downie TT, More IA, Lindop GB: Atubular glomeruli and glomerular cysts: A possible pathway for nephron loss in the human kidney? *J Pathol* 179: 421–426, 1996

10. Hughson MD, McManus JF, Hennigar GR: Studies on 'end-stage' kidneys. II. Embryonal hyperplasia of Bowman's capsular epithelium. *Am J Pathol* 91: 71–84, 1978
11. Ogata K, Hajikano H, Sakaguchi H: So-called embryonal hyperplasia of Bowman's capsule epithelium: An immunohistochemical and ultrastructural study. *Virchows Arch A Pathol Anat Histopathol* 41: 143–147, 1991
12. Fukuzawa R, Eccles MR, Ikeda M, Hata J: Embryonal hyperplasia of Bowman's capsule epithelium in patients with WT1 mutations. *Pediatr Nephrol* 18: 9–13, 2003
13. Johansson CB, Youssef S, Koleckar K, Holbrook C, Doyonnas R, Corbel SY, Steinman L, Rossi FM, Blau HM: Extensive fusion of haematopoietic cells with Purkinje neurons in response to chronic inflammation. *Nat Cell Biol* 10: 575–583, 2008
14. Barisoni L, Nelson PJ: Collapsing glomerulopathy: An inflammatory podocytopathy? *Curr Opin Nephrol Hypertens* 16: 192–195, 2007
15. Prodromidi EI, Poulsom R, Jeffery R, Roufosse CA, Pollard PJ, Pusey CD, Cook HT: Bone marrow-derived cells contribute to podocyte regeneration and amelioration of renal disease in a mouse model of Alport syndrome. *Stem Cells* 24: 2448–2455, 2006
16. 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* 103: 7321–7326, 2006
17. Karam SM: Dynamics of epithelial cells in the corpus of the mouse stomach. IV. Bidirectional migration of parietal cells ending in their gradual degeneration and loss. *Anat Rec* 23: 314–332, 1993
18. Ambler CA, Määttä A: Epidermal stem cells: Location, potential and contribution to cancer. *J Pathol* 217: 206–216, 2009

See related articles, "Recruitment of Podocytes from Glomerular Parietal Epithelial Cells," on pages 333–343, and "Regeneration of Glomerular Podocytes by Human Renal Progenitors," on pages 322–332.

Plasmin and Sodium Retention in Nephrotic Syndrome

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Epithelial Na^+ channels (ENaC) are found within the most distal aspects of the nephron, where they serve as a final arbitrator of the reabsorption of filtered Na^+. This process has a critical role in the regulation of extracellular fluid volume and BP. Edema-forming states, including cirrhosis, heart failure, and nephrotic syndrome, are associated with enhanced renal Na^+ absorption. Aldosterone has a role in renal Na^+ retention in these disorders; however, Na^+ retention in nephrotic syndrome as a result of activation of Na^+ absorptive processes in the distal nephron may occur by aldosterone-independent mechanisms.[1,2]

A number of factors that activate ENaC have been described, including cleavage of ENaC subunits by proteases.[3] The α and γ subunits are cleaved by proteases in specific regions within their extracellular domains. By cleaving subunits at least twice, inhibitory tracts are released and channels are activated.[4,5] ENaC is moderately activated when cleaved by furin, a protease that resides in the trans-Golgi network, as the α subunit is cleaved twice by furin releasing an inhibitory tract. In contrast, the γ subunit is cleaved only once by furin. A second cleavage event distal to the γ subunit furin site is needed to activate the channel fully.[5–7] Studies by Svenningsen *et al.*[8] in this issue of *JASN*, as well as recent work from our group,[9] provide evidence that plasmin may function as the second protease that cleaves the γ subunit and activates ENaC in the setting of nephrotic syndrome.

Both plaminogen and plasmin are present in nephrotic urine,[8–10] suggesting that plasminogen is filtered by a damaged glomerulus. Plasminogen is cleaved to its active form, plasmin, by various proteases, including urokinase. The presence of urokinase within the tubular lumen of the nephron facilitates the processing of filtered plasminogen to an active form.[8,11,12] Plasmin joins a growing list of proteases that cleave the γ subunit at sites distal to the furin cleavage site and activates ENaC in association with release of an inhibitory tract.[3,5,13]

These observations provide new insights regarding a mechanism for renal Na^+ retention in nephrotic syndrome. They also raise a number of questions that will need to be addressed in future studies. Because amiloride inhibits both ENaC and urokinase, is it effective in ameliorating renal Na^+ retention and volume expansion in nephrotic syndrome in humans? If plasmin is the activation culprit, then are renal Na^+ retention and volume expansion in nephrotic syndrome prevented by plasmin inhibitors or by a lack of plasminogen expression (plasminogen knockout mouse model)? Although nephrotic syndrome occurs in the setting of various disorders, is the presence of plaminogen and plasmin in the urine a common finding, or is it restricted to subsets of individuals with nephrotic syndrome? Are there other clinical disorders whereby disease-specific proteases cleave and activate ENaC? With regard to this last question, enhanced ENaC proteolysis may contribute to enhanced ENaC activity in the airways of individuals with cystic fibrosis.[14,15]

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