Glomerular parietal epithelial cells contribute to adult podocyte regeneration in experimental focal segmental glomerulosclerosis

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As adult podocytes cannot adequately proliferate following depletion in disease states, there has been interest in the potential role of progenitors in podocyte repair and regeneration. To determine whether parietal epithelial cells (PECs) can serve as adult podocyte progenitors following disease-induced podocyte depletion, PECs were permanently labeled in adult PEC-rtTA/LC1/R26 reporter mice. In normal mice, labeled PECs were confined to Bowman's capsule, whereas in disease (cytotoxic sheep anti-podocyte antibody) labeled PECs were found in the glomerular tuft in progressively higher numbers by days 7, 14, and 28. Early in disease, the majority of PECs in the tuft coexpressed CD44. By day 28, when podocyte numbers were significantly higher and disease severity was significantly lower, the majority of labeled PECs coexpressed podocyte proteins but not CD44. Neither labeled PECs on the tuft nor podocytes stained for the proliferation marker BrdU. The *de novo* expression of phospho-ERK colocalized to CD44 expressing PECs, but not to PECs expressing podocyte markers. Thus, in a mouse model of focal segmental glomerulosclerosis typified by abrupt podocyte depletion followed by regeneration, PECs undergo two phenotypic changes once they migrate to the glomerular tuft. Initially these cells are predominantly activated CD44 expressing cells coinciding with glomerulosclerosis, and later they predominantly exhibit a podocyte phenotype, which is likely reparative.

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Classic focal segmental glomerulosclerosis (FSGS), the leading cause of nephrotic syndrome in the US, is considered a disorder of adult terminally differentiated glomerular visceral epithelial cells, also called podocytes.[1] Classic FSGS is characterized by a depletion in podocytes,[1] and when podocyte number is depleted below a critical threshold glomerulosclerosis ensues.[2,3] In contrast to other kidney cells, adult podocytes are unable to adequately proliferate, and thus they cannot replenish their numbers following depletion in disease.[4] Thus, several groups are attempting to identify possible progenitors that might replace podocytes.

Several lines of evidence support that neighboring glomerular parietal epithelial cells (PECs) are attractive candidates to serve as podocyte progenitors.[5–10] In reporter mice, a subset of adolescent podocytes do arise from PECs.[5,11,12] In adults, a subset of cells lining Bowman's capsule coexpress both a podocyte and a PEC protein in normal rats, mice and humans, raising suspicion that PECs serve a podocyte progenitor role.[10,13,14] Furthermore, their number increases when diseased animals are given ACE inhibitors,[15] corticosteroids,[16] and retinoids[17] and when diabetic mice have their metabolic milieu improved.[18] A subset of human PECs coexpress progenitor markers.[19] When isolated and injected into mice with experimental FSGS, they engraft within the glomerular tuft, and begin to express markers, and acquire ultrastructural features of podocytes.[20] Recent studies showed that PECs adopt a podocyte phenotype when miR-193a levels are lowered.[21]

However, several studies have dampened enthusiasm for the notion that PECs serve as adult podocyte progenitors. Using reporter mice, Berger *et al.*,[12] Sakamoto *et al.*,[22] Hackl *et al.*,[23] and Miyazaki *et al.*[24] showed that in disease adult podocytes move from the tuft to Bowman's capsule and begin to coexpress PEC proteins, and they may even become PECs. Cell fate mapping studies of PECs have failed to show a progenitor niche in models of aging,[11,12] and glomerular hypertrophy following partial nephrectomy.[12] Studies also showed that the expression of podocyte-specific proteins in PECs might simply reflect alterations in protein degradation.[25]

Taken together, the controversial nature for the role for PECs as potential adult podocyte progenitors may depend on model systems used, species studied, and other factors. The purpose of the current study was to determine whether a subset of adults PECs that migrate to the glomerular tuft acquire features of podocytes, following an abrupt depletion in podocyte number that leads to glomerulosclerosis. Accordingly, an experimental model of FSGS characterized by an abrupt and substantial decline in podocyte number,[15–17,26,27] followed by a phase of regeneration, was induced in reporter mice, in which PECs were fate mapped following the induction of permanent labeling of adult PECs.

RESULTS

Expression of β-galactosidase reporter was induced in PECs in reporter mice

To determine whether glomerular PECs serve as possible adult podocyte progenitors in this FSGS model of abrupt and marked podocyte depletion, studies were conducted in PEC-reverse tetracycline-transactivator/LC1/Rosa26 reporter (R26R) (PEC-reporter) mice. As reported previously,[5,28] doxycycline induced the expression of β-galactosidase (β-gal) in PECs, measured by both enzymatic X-gal staining and by immunohistochemistry for β-gal (Supplementary Figure S1 online). In normal mice before disease induction (i.e., at baseline), both the enzymatic reaction and fluorescent staining for β-gal were restricted to cells along Bowman's capsule within the glomerulus (Supplementary Figure S1 online). Approximately 70% of PECs reported positive at baseline, similar to that previously reported.[5,28] As expected, mice that did not receive doxycycline administration, or mice without all the transgenes did not have labeled PECs (data not shown).

Glomerulosclerosis and albuminuria in PEC reporter mice

Experimental FSGS with a significant decrease in podocytes accompanied by glomerulosclerosis was successfully induced in PEC-reporter mice. Immunostaining for sheep immunoglobulin G (IgG) was performed to confirm deposition of the podocyte cytotoxic antibody, and examples are shown in Supplementary Figure S2 online. Despite antibody binding to all glomeruli, and as expected with focal glomerular diseases, an average of $22 \pm 5\%$ of glomeruli had evidence of glomerulosclerosis at day 7 (d7; $P < 0.00001$ vs. baseline; Figure 1). Glomerulosclerosis increased to involve $33 \pm 8\%$ of all glomeruli by d14 ($P < 0.00001$ vs. baseline). However, by d28, the number of glomeruli with evidence of disease was lower on average than d14, but it was not significant owing to the large variation between animals at this time point ($24 \pm 7\%$; $P = 0.06$ vs. d14). Subsequent analysis included all glomeruli, and was not limited to the subset with sclerosis.

Within individual glomeruli, disease severity, assessed by scoring 55 ± 5 gloms in each animal on a scale of 0–4,[26] was progressively higher (worse disease) at d7 (0.38 ± 0.12 vs. 0.03 ± 0.03, $P < 0.0001$vs. baseline) and d14 (1.00 ± 0.30, $P < 0.001$ vs. d7; Figure 1). Importantly, the sclerosis score was significantly lower by d28 (0.55 ± 0.12, $P < 0.01$ vs. d7 and d14).

Albuminuria was assessed in individual mice at all time points by measuring the urinary albumin-to-creatinine ratio. The albumin-to-creatinine ratio increased significantly at day 7 (335.3 ± 55.51 vs. 0.42 ± 0.16 at baseline, $P < 0.001$). By day 14, albuminuria was reduced significantly (159.5 ± 40, $P < 0.01$ vs. d7). There were no significant changes at day 21 (121.8 ± 15.93) and day 28 (143.7 ± 23.39). Taken together, proteinuria and glomerulosclerosis both increase early in disease, with significant improvements by day 28.

Podocytes regenerate in PEC reporter mice with FSGS independent of proliferation

Although there were no significant differences in glomerular tuft area at any time point in disease compared with baseline, unbiased stereology was used to measure the average number of podocytes per mm^2 of glomerular tuft, by counting the number of p57-positive cells/glomerular cross-section/tuft cross-sectional area, and the results are shown in Figure 1. Podocyte number was significantly lower at d7 (2159 ± 448 vs. 3355 ± 398, $P < 0.001$ vs. baseline, a 36% decrease) and at d14 (1712 ± 263 vs. 3355 ± 398, $P < 0.0001$ vs. baseline, a 49% decrease). The decrease in the average number of podocytes between d14 and d7 was not significant (1712 ± 263 vs. 2159 ± 448, a 21% decrease; $P > 0.05$ vs. d7, $P < 0.0001$ vs. baseline; Figure 1). However, the number of podocytes was 44% higher at d28 (2461 ± 452 vs. 1712 ± 263, $P < 0.01$ vs. d14).

Because of the potential concern that the decrease in p57 staining at d7 and 14 might simply reflect podocyte injury or dedifferentiation, and not actual loss of podocytes, costaining for the nuclear marker DAPI with both podocin and p57 was performed. As shown in Supplementary Figure S3 online, in areas of segmental reduction and/or absence of staining for podocin and p57, DAPI staining was also absent. This is consistent with actual depletion of podocytes. If injured podocytes had simply dedifferentiated, these areas would have still contained nuclei. These data show that (i) the decrease in podocyte number and podocyte markers was indeed owing to loss of podocytes and not simply a manifestation of podocyte injury or dedifferentiation; (ii) the higher number of podocytes observed at day 28 compared with earlier time points was owing to regeneration of podocytes and not simply repair or redifferentiation. To determine whether the higher number of podocytes at d28 was owing to proliferation of podocytes, 5-bromo-2-deoxyuridine and 5-fluoro-2'-deoxyuridine (BrdU) staining was performed (Supplementary Figure S4 online). BrdU was rarely detected in the glomerular tuft at any time point, suggesting that another source was responsible for the 44% increase in podocyte number at d28 (see below).

Taken together, these data were consistent with podocyte regeneration, and the improvement in glomerulosclerosis was consistent with repair.