The phenotypes of podocytes and parietal epithelial cells may overlap in diabetic nephropathy

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Reversal of diabetic nephropathy (DN) has been achieved in humans and mice, but only rarely and under special circumstances. As progression of DN is related to podocyte loss, reversal of DN requires restoration of podocytes. Here, we identified and quantified potential glomerular progenitor cells that could be a source for restored podocytes. DN was identified in 31 human renal biopsy cases and separated into morphologically early or advanced lesions. Markers of podocytes (WT-1, p57), parietal epithelial cells (PECs) (claudin-1), and cell proliferation (Ki-67) were identified by immunohistochemistry. Podocyte density was progressively reduced with DN. Cells marking as podocytes (p57) were present infrequently on Bowman's capsule in controls, but significantly increased in histologically early DN. Ki-67-expressing cells were identified on the glomerular tuft and Bowman's capsule in DN, but rarely in controls. Cells marking as PECs were present on the glomerular tuft, particularly in morphologically advanced DN. These findings show evidence of phenotypic plasticity in podocyte and PEC populations and are consistent with studies in the BTBR *ob/ob* murine model in which reversibility of DN occurs with podocytes potentially regenerating from PEC precursors. Thus, our findings support, but do not prove, that podocytes may regenerate from PEC progenitors in human DN. If so, progression of DN may represent a modifiable net balance between podocyte loss and regeneration.

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Reversal of morphologically advanced diabetic nephropathy (DN), although rarely reported, has been achieved in humans following long-term pancreas transplantation[1] and in the BTBR *ob/ob* diabetic mouse model.[2] Initiation and progression of DN is associated with podocyte injury and loss;[3–5] reversal of the structural and functional abnormalities of DN must require restoration of podocytes. However, it is well accepted that podocytes are terminally differentiated cells and generally do not replicate,[5,6] presenting a major obstacle to their restoration. Recent studies[2,7–12] have demonstrated the possibility of a progenitor cell in the parietal epithelial location that could serve as a source for podocytes lost in the course of DN, located in an anatomic niche along Bowman's capsule traditionally thought to be populated exclusively by parietal epithelial cells (PECs). Supporting the possibility of a podocyte progenitor cell are lineage tracing studies in adolescent mice showing recruitment of podocytes from PECs located on Bowman's capsule, and the presence of a transitional cell population at the vascular stalk with characteristics of both podocytes and PECs.[6,7,13–15] PECs located near the tubular pole in humans have been shown to co-express stem cell markers and have the potential to differentiate into renal and non-renal cells under various conditions;[10] upon injection of these human progenitor cells into mice, some were incorporated into glomerular structures and resulted in reduced proteinuria and chronic glomerular damage in a mouse model of adriamycin-induced nephropathy.[8] Recent studies of human PECs suggest that expression of microRNA-193a may mediate a transition from a PEC to podocyte phenotype.[16] Intriguing studies in mice have shown that cells of renin lineage can also take on immunophenotypic and morphologic characteristics of either PECs or podocytes, and may serve as a source of glomerular epithelial progenitor cells.[17–19] Alternately, recent studies by the groups of Moeller *et al.*, Nagata *et al.*, Peti-Peterdi *et al.*, Weins *et al.*, and others[20–26] suggest that podocytes may become PECs, but that PECs cannot necessarily take on the functional role of podocytes and only migrate to the glomerular tuft at sites of injury in order to mitigate the effects of podocyte loss. In one lineage tracing study,[27] adolescent mice had PEC-derived cells with features of fully differentiated podocytes, whereas adult mice displayed podocyte regenerative capacity after acute podocyte loss, but not during aging. Finally, in a murine model in which changes

of DN were reversed, there was *de novo* expression of a podocyte immunophenotype (presence of p57 and WT-1 proteins in cell nuclei) identified in numerous cells whose anatomic location on Bowman's capsule would normally identify them as PECs,[2] suggesting that PECs might be a source of restored podocytes in this model.

In this study, we reasoned that if podocytes may be derived from PECs and if morphologically advanced DN in humans has the potential for reversibility—as demonstrated by Fioretto *et al.*[1,28]—then perhaps the potential for restoration of podocytes lost in DN from PECs is always present and this may be an ongoing process, albeit at a low level that is unable to keep up with concurrent podocyte loss. Such a scenario implies that some degree of podocyte loss and restoration is a constant feature of DN, but one where progression of disease is characterized by a predominant process of podocyte loss. The potential for reversal of DN is then determined, at least in part, by changes in the balance of podocyte loss and restoration, and that therapeutic interventions that alter this balance in favor of podocyte restoration are a highly desired goal. As a first test of the relevance of this hypothesized scenario, we examined whether advancement of DN is associated with podocyte loss and with PEC changes consistent with acquisition of a podocyte immunophenotype.

RESULTS

We retrospectively identified 31 cases of DN in human renal biopsies that could be separated into morphologically early (class I, II) or advanced (class III, IV) lesions, corresponding to a recent classification of DN.[29] The median number of patent glomerular profiles per case was 13 (range 6–26) in controls, 12 (range 5–27) in early DN (range 5–27), and 6 (range 3–23) in advanced DN.

The number and density of both WT-1- and p57-expressing podocytes per glomerulus was progressively reduced in histologically early and advanced DN

The number of podocytes per glomerular profile as defined by WT-1 nuclear stain and position on the glomerular tuft was significantly reduced in DN. This reduction was progressive with severity of DN as follows: from controls to histologically early DN: 35% reduction ($P<0.001$); from early DN to advanced DN: 45% reduction ($P<0.001$); and from controls to advanced DN: 67% overall reduction in podocytes per glomerular profile ($P<0.001$; Figure 1). The average number of podocytes identified per glomerular tuft profile differed slightly with the two markers, with WT-1 generally highlighting more podocytes; however, each antibody demonstrated a concordant percentage of podocyte decrease (within 5% of each other) with advancement of DN (Figure 1). As this reduction in podocyte number per glomerular profile could be related to increased glomerular volume in DN, average podocyte number per glomerulus was calculated from glomerular volume and numerical density of podocytes per glomerular volume for each biopsy. The number of podocytes per glomerulus and numerical density of podocytes per glomerulus were both progressively decreased, while the mean glomerular volume was increased with advancement of DN (Figure 1). Thus, the average podocyte number per glomerulus was greater in control subjects (397 ± 98) vs. early DN (268 ± 73; $P=0.0012$) and vs. advanced DN (144 ± 52; $P<0.0001$), and in early vs. advanced DN ($P<0.001$).

The mean glomerular volume was greatest in advanced DN ($4{,}276{,}700 \pm 1{,}445{,}600\ \mu m^3$) vs. early DN ($3{,}208{,}600 \pm 1{,}462{,}900\ \mu m^3$; $P<0.0001$) and vs. control subjects ($2{,}319{,}000 \pm 862{,}000\ \mu m^3$; $P<0.0001$), and in early DN vs. control subjects ($P<0.0001$). The mean podocyte density progressively decreased from control subjects (181.5 ± 714 podocytes per $10^6\ \mu m^3$) vs. early DN (96.1 ± 55.8 podocytes per $10^6\ \mu m^3$; $P=0.002$) and vs. advanced DN (38.5 ± 21.4 podocytes per $10^6\ \mu m^3$; $P<0.0001$), and in early vs. advanced DN ($P=0.0002$). The mean podocyte nuclear diameter was increased in advanced DN ($8.446 \pm 0.455\ \mu m$) vs. early DN ($7.863 \pm 0.708\ \mu m$; $P=0.048$) and vs. control subjects ($7.182 \pm 0.599\ \mu m$; $P<0.0001$), and in early DN vs. control subjects ($P<0.001$).

Ki-67 expressing cells were identified on Bowman's capsule and the glomerular tuft in DN, but only rarely in controls

Ki-67 is a marker of late G2 phase of the cell cycle, but may be expressed by any cell outside the G0, and is used as a marker of proliferation.[30,31] The number of Ki-67-expressing PECs per glomerular profile was significantly increased in both early (median 0.4; range 0–2.4; $P<0.05$) and advanced DN (median 0.5; range 0–2.2; $P<0.01$) compared with control subjects (median 0.0; range 0–0.4). The number of Ki-67-expressing cells on the glomerular tuft profiles was significantly increased in histologically early DN (median 0.3; range 0–1.4) compared with controls (median 0.0; range 0–0.1; $P<0.001$). In cases of advanced DN, there was a lesser increase in Ki-67 immunoreactivity in the glomerular tufts (median 0.1; range 0–0.4) compared with controls, which did not achieve statistical significance (Figures 2 and 3). In tissue sections stained with PAS and further immunostained for Ki-67, the Ki-67-expressing cells on the glomerular tuft were predominantly endothelial and mesangial cells. No definitive Ki-67-expressing podocytes were present, although rare candidates were identified.

p57-expressing cells (marker of podocytes) on Bowman's capsule were significantly increased in early DN

Cells marking as podocytes were present in PEC locations and significantly increased in histologically early DN compared with controls ($P<0.01$). In cases of advanced DN, there was no significant increase of these cells compared with controls (Figures 2 and 3). p57-expressing cells were identified singly and occasionally consecutively along portions of Bowman's capsule. Relative to controls, p57-expressing cells were more concentrated in the region of the glomerular hilus in DN. Specifically, on average, p57-expressing cells near the vascular stalk (within 1/4th the diameter of Bowman's capsule) comprised 21% of all p57-expressing cells on Bowman's