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

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Abstract

Reversal of diabetic nephropathy (DN) has been achieved in humans and mice, but only rarely and under special circumstances. Since 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 (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.

Keywords

Podocyte; diabetic nephropathy

INTRODUCTION

Reversal of morphologically advanced diabetic nephropathy (DN), although rarely reported, has been achieved in humans following long term pancreas transplantation (1) and in the

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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 diabetic nephropathy, located in an anatomic niche along Bowman's capsule traditionally thought to be populated exclusively by PECs. Supporting the possibility of a podocyte progenitor cell are lineage tracing studies in adolescent mice showing recruitment of podocytes from parietal epithelial cells (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 diabetic nephropathy 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 diabetic nephropathy in human renal biopsies which 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 diabetic nephropathy

The number of podocytes per glomerular profile as defined by WT-1 nuclear stain and position on the glomerular tuft was significantly reduced in diabetic nephropathy. This reduction was progressive with severity of diabetic nephropathy 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). Since 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 diabetic nephropathy (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 ± 1,445,600 μm^3) *vs.* early DN (3,208,600 ± 1,462,900 μm^3; $p<0.0001$) and *vs.* control subjects (2,319,000 ± 862,000 μm^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/10^6 μm^3) *vs.* early DN (96.1 ± 55.8 podocytes/10^6 μm^3; $p=0.002$) and *vs.* advanced DN (38.5 ± 21.4 podocytes/10^6 μm^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 ± 0.455 μm) *vs.* early DN (7.863 ± 0.708 μm; $p=0.048$) and *vs.* control subjects (7.182 ± 0.599 μm; $p<0.0001$), 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 diabetic nephropathy, 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 to 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 to 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 to controls which did not achieve statistical significance (Figures 2, 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 diabetic nephropathy

Cells marking as podocytes were present in PEC locations and significantly increased in histologically early DN compared to controls ($p<0.01$). In cases of advanced DN, there was no significant increase of these cells compared with controls (Figures 2, 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 capsule in controls *vs*. 35% in early DN ($p=0.05$) and 29% in advanced DN respectively (did not reach statistical significance), with the remainder of cells distributed throughout Bowman's capsule. In addition, rare dual labeled p57/claudin-1 expressing cells on Bowman's capsule were identified in 3 cases of morphologically early DN (Figure 4).

Cells expressing claudin-1 (marker of PECs) were identified on the glomerular tuft and significantly increased in advanced diabetic nephropathy

Cells expressing claudin-1 were present on the glomerular tuft and were significantly increased in histologically advanced stages of DN compared with controls ($p<0.01$; **Figures 2, 3**). Claudin-1 expressing cells were sometimes present as caps of cells overlying segmentally sclerotic regions of the glomerular tufts (with and without capsular adhesions in the plane of section), which were found in cases of advanced DN. Claudin-1 expressing cells were sometimes present in other glomerular segments characterized histologically by primarily increased mesangial matrix (Figure 3). There was no co-expression of p57 and claudin-1 in cells on the glomerular tuft (Figure 4). The average number of claudin-1 expressing cells per glomerular tuft profile was not different between early DN and either controls or advanced DN. 85-92% of PECs were positive for claudin-1 in all biopsies.

Synaptopodin expression on Bowman's capsule was significantly increased with histologic progression of diabetic nephropathy

The extent of synaptopodin expression along the circumference of Bowman's capsule significantly increased from controls (mean 1.2% ± 4%) *vs.* early DN (mean 6.5% ± 14.1%; p=0.002) *vs.* advanced DN (mean 13.6% ± 22.8%; p=0.0001; early *vs.* advanced p=0.09). Similar to prior investigations (15), synaptopodin labeling was predominantly seen in the region of the vascular pole in continuity with visceral podocytes, but was also identified throughout Bowman's capsule and in areas of segmental adhesions (Figure 2).

DISCUSSION

In this study, we observed a progressive decrease in both podocyte number and number density in morphologically early to advanced diabetic nephropathy. Decreased WT-1 and p57 immunolabeling of podocytes confirms previous studies in DN, suggestive of podocyte detachment and loss occurring in patients with either type 1 or type II diabetes (3, 32). There was concurrent increase in podocyte nuclear size with advancement of DN, which correlates with prior investigations in DN (33). The novel findings of this study are that concurrent with podocyte loss in DN, there is cell cycle activation by cells situated on Bowman's capsule, that immunophenotypic changes occur in both podocytes and cells located in parietal epithelial cell locations, and that there are rare cells on Bowman's capsule expressing immunophenotypic markers of both parietal epithelial cells (i.e. claudin-1) and podocytes (i.e. p57). Our observation of increased podocyte nuclear volume is consistent with what was reported by Pagtalunan et al (33). The pathophysiologic significance of this finding is uncertain, but from a methodological point of view, this phenomenon could potentially lead to overestimation of numerical density in biopsies with larger nuclei. However, this caveat could only work against the hypothesis that podocytes are reduced in DN.

There are at least two mechanistic options to explain these observations. Combining the current observations with prior studies, one might propose that expression of claudin-1 on the glomerular tuft represents a sclerosing or capping response to glomerular injury/ podocyte loss which contributes to the injury process and/or represents a suboptimal reparative response. This pathologic pattern was present in some glomeruli in biopsies of advanced DN, and accounts for some of the increased claudin-1 expressing cells identified in this category. In this scenario, Ki-67 expressing cells lining Bowman's capsule are indicative of activated PECs which will migrate to the glomerular tuft via a segmental adhesion, and that p57 expression on Bowman's capsule is unrelated to podocyte regeneration. In support of this scenario, claudin-1 expression on the glomerular tuft was most prominent in cases of histologically advanced DN, and some of these claudin-1 expressing cells showed segmental attachment to Bowman's capsule in the histologic plane of section. In early DN, despite an increase in p57 expressing PECs, there was no significant increase in claudin-1 expression on the glomerular tuft, and no dual p57/claudin-1 labeled cells were identified on the glomerular tuft in controls or in any stage of DN. Thus our findings cannot necessarily support a hypothesis that claudin-1 positive cells on the glomerular tuft are derived from p57 expressing PEC progenitors. In isolation, the claudin-1

studies are consistent with recent investigations which demonstrate migration of PECs onto the glomerular tuft in association with advanced podocyte injury (20-25, 34), presumably in order to limit the physiological consequences of podocyte loss that leaves the glomerular filter uncovered.

However, this scenario alone does not adequately account for the significantly increased p57, Ki-67, and synaptopodin expressing cells on Bowman's capsule in diabetic nephropathy, nor the rare p57/claudin-1 double labeled cells in PEC regions. Cyclin dependent kinase inhibitor p57 is initially expressed during nephrogenesis in human fetal podocytes, and its expression is associated with loss of PAX2 expression, a transcription factor retained in PECs, distal tubules, and collecting ducts (15). p57 has been previously shown to be a sensitive and specific marker of podocytes in mice (2, 35-38) and in humans, where in one study of normal glomeruli (15), all visceral podocyte nuclei were positive for p57, while 75% of glomeruli had zero p57 labeling of cells in PEC regions. Thus, p57 is a sensitive and specific marker for podocytes, yet it may be expressed by rare cells in PEC locations in normal glomeruli as seen in this study. As a cyclin dependent kinase inhibitor, the possibility that some PECs acquire p57 expression due to their entering a phase of cellular senescence cannot be excluded (38-40). Arguing against this however, is the parallel increase in synaptopodin and Ki-67 expression PECs seen in DN. Thus the increased expression of p57, synaptopodin, and Ki-67 by PECs in DN and the presence of dual p57/claudin-1 expressing cells suggest an alternate response to injury consistent with mobilization of a PEC to podocyte regenerative process.

Our observations support a second scenario, which provides for the possibility of a podocyte progenitor cell, located on Bowman's capsule, which may transdifferentiate and migrate to the glomerular tuft, possibly via the vascular stalk as previously suggested (2, 7-10, 41). On Bowman's capsule, this progenitor cell may undergo cell cycle activation in response to podocyte loss in DN and transiently express both podocyte and PEC immunophenotypic markers as it migrates from the tubular pole to the vascular pole and onto the glomerular tuft. It is also possible such cells may “jump” directly from Bowman's capsule across the urinary space to the glomerular tuft, as has been suggested by studies of the collapsing variant of focal and segmental glomerulosclerosis (Palma Diaz M, et al. unpublished observations). Claudin-1 expressing cells on the glomerular tuft may represent migrated progenitor cells or PECs as a component of a sclerotic process (similar to first scenario). Thus these two models of injury and repair are not biologically mutually exclusive, and may represent different stages of, or types of response to, injury. The landmark observation that structurally advanced diabetic nephropathy can be reversible challenges theories which do not offer mechanisms for restoration of podocytes (1). This second scenario has the appealing feature of offering a mechanism for podocyte restoration that in turn is permissive or enabling of reversibility of DN.

Our study also poses important pathophysiologic questions that cannot be answered by the data at hand. One question is whether the proliferative (and potentially regenerative) capacity of PECs may differ in early versus late DN. We identified p57 expressing cells in dual labeled p57 and claudin-1 expressing cells along Bowman's capsules, predominately in early DN. The relative absence of these cells in later stages may reflect a reduced capacity

for repair in the advanced DN. We recognize that landmark studies of Fioretto, et. al., demonstrated morphologically advanced DN could be reversed, and hence that podocytes could still be restored from some unspecified source, but we also recognize that the process took between 5 and 10 years to be effected and so may reflect a very limited capacity for podocyte regeneration in late stages of DN. This possibility is further suggested by recent studies of Berger, et. al, which showed the potential for podocyte regeneration by PECs was confined to juvenile mice; PEC to podocyte transformation could not be detected in adult mice in their model system (20).

Some limitations of this study provide opportunities for further investigation. First, expression of a small set of proteins suggestive of immunophenotypic plasticity are not sufficient evidence of cellular transdifferentiation nor of a functional role for cells on the glomerular tuft, and need to be correlated with larger studies and patient outcomes. Second, investigations of some stem cell markers seen in renal progenitor cells (CD133+/CD24+) are limited to studies of frozen tissue, and cannot currently be performed in formalin-fixed tissue utilized for the present study (8, 42). In considering other biologic markers and comparing transdifferentiation with nephrogenesis, the developing kidney is known to have a variety of cell signaling molecules which affect podocyte vs. PEC differentiation decisions, including β-catenin (43), bone morphogenic proteins (44), and platelet-derived growth factors (45-47); these may or may not play a role in transdifferentiation, and are candidates for future study. The relationship between these cells and cells of renin lineage are not specifically addressed in this study. Although we do not have clinical information available for our study population, podocyte loss plays a major role in the progression of both type I and type II diabetic kidney disease (3, 32). Additionally, we grouped histologic class I and class II DN together as morphologically early diabetic nephropathy; however, these groupings represent a wide spectrum of disease. In our study, the morphologically early DN group had approximately 32% podocyte loss per glomerulus compared to controls. It has been suggested that a biologic “tipping point” of 20% podocyte loss leads to a cascade of injuries causing progression to segmental or global glomerulosclerosis (30, 31, 48, 49), but our study suggests lower podocyte densities may still allow for some degree of glomerular capillary preservation and presumably some degree of glomerular filtration, if not hyperfiltration. The seminal work by Fioretto et al (1) in patients with pancreas transplants suggests that even some nodular lesions of diabetic glomerulosclerosis can potentially be reversed. Many of our cases in the cohort of advanced DN were characterized by diffuse mesangial expansion and multiple Kimmelstiel-Wilson nodules, and a histology-based single time point cannot identify the “reversal point” for these lesions. The possibility of achieving podocyte restoration suggests that it may be valuable to look prospectively and longitudinally for podocyte and PEC plasticity in early and advanced stages of diabetic nephropathy.

In summary, these findings demonstrate activity and provide some evidence for phenotypic plasticity in podocytes and parietal epithelial cell populations. Taken together, they suggest that some low level of podocyte regeneration from a PEC niche may be an occult but ongoing process that, if sufficiently stimulated, could potentially enable repair of DN via podocyte restoration. Cell cycle activation and expression of the podocyte marker p57 in

PECs in human DN are findings consistent with preclinical studies in the BTBR *ob/ob* murine model of reversible DN, and support a hypothesis that podocytes may regenerate from progenitor cells on Bowman's capsule in human DN. If true, progression of disease representing an unfavorable balance between podocyte loss and regeneration could potentially be modified by new therapeutics for diabetic nephropathy that either promote podocyte restoration or ameliorate podocyte loss.

METHODS

This study was approved by the institutional review board (IRB) at the University of Washington. We retrospectively identified 31 cases of diabetic nephropathy in human renal biopsies which could be separated into morphologically early (class I, II) or advanced (class III, IV) lesions corresponding to recent classification (29), and 19 controls from time zero renal transplant biopsies. Immunohistochemistry was performed for markers of podocytes (WT-1, p57, synaptopodin), PECs (claudin-1) and cell proliferation (Ki-67). Glomerular profiles containing at least 3 distinct mesangial segments were evaluated and scored. Formalin-fixed paraffin embedded tissue was sectioned at 4 micrometers and immunohistochemistry was performed with antibodies specific for p57 (Santa Cruz Biotechnology, Santa Cruz CA), WT-1 (Santa Cruz Biotechnology), synaptopodin (Fitzgerald Industries International, Concord, MA) claudin-1 (Abcam, Cambridge MA), and Ki67 (clone SP6, LabVision Fremont CA) as previously described (2). Following deparaffinization and rehydration, the slides were subjected to heat mediated antigen retrieval in citrate buffer pH 6 and blocked with 2% normal horse serum. Primary antibodies were incubated overnight at 4C and detected with ImmPress HRP polymer reagent (Vector Laboratories, Burlingame CA) and 3,3’ diaminobenzidine. The slides were then counterstained with hematoxylin or with a standard periodic acid Schiff's stain, dehydrated and coverslipped. For double immunohistochemistry, slides were first incubated with anti-p57 antibody, detected with ImmPress anti-rabbit HRP polymer and developed with 3,3’ diaminobenzidine (brown reaction product), blocked with 3% hydrogen peroxide and then incubated with anti-claudin-1 followed by ImmPress anti-rabbit HRP polymer and developed with Vina Green substrate (Biocare Medical, Concord CA). Negative controls for all immunohistochemistry included using non immune IgG in place of primary antibodies.

Average glomerular volume estimation

Glomerular volume was estimated using the maximum profile area method (50). For all cases of DN and controls, each glomerulus was assessed in approximately 10 level sections to determine the greatest cross-sectional glomerular area. An intact glomerulus was chosen for measurement if both bordering level sections showed an apparent decrease in size, indicating that the chosen glomerular profile most likely represented the largest diameter cross-section. Glomeruli for which level sections did not contain these characteristics were excluded. An Olympus BX41 microscope and Leica DFC420 camera were used to take digital photomicrographs at 400x. Images were uploaded into Adobe Photoshop. The magnetic lasso tool was employed to connect the outer-most points on the periphery of glomerular profiles to define a polygon. The area of the polygon was subsequently measured using the Adobe Photoshop measuring tool. A standard calibration slide was used to

calibrate the software measuring tool. The glomerular diameter and volume was calculated from the maximal profile area measurements. The median number of glomeruli which could be measured in each case was 6 (range 2-12).

Quantification of podocyte nuclear size and density

To evaluate podocyte nuclear size and density of podocytes on the glomerular tuft, all slides stained with WT-1 were photographed. Photographs were then processed using ImagePro to measure the glomerular tuft area, podocyte nuclear number, and the mean podocyte nuclear diameter by measuring the long and short axis of each labeled podocyte and calculating a mean diameter for all intact glomeruli. Calculations described by Venkatareddy et al (51) and the Excel formulas provided in their Supplement were utilized to determine glomerular podocyte density normalized to glomerular volume.

Statistics

For each case, the locations and number of cells staining in the glomerulus for each immunohistochemical marker were recorded, and mean and median values and standard deviations were calculated. Statistical analyses were performed on GraphPad Prism using Mann-Whitney U tests for each marker; a p value less than 0.05 was considered significant. Glomerular volume data was calculated on Microsoft Excel, and statistically analyzed using two-tailed T-tests. Glomerular podocyte density, podocyte nuclear diameter, and mean podocyte number per glomerulus were analyzed with GraphPad Prism using Kruskal-Wallis nonparametric one-way analysis of variance test, followed by Mann-Whitney post-tests to compare groups.

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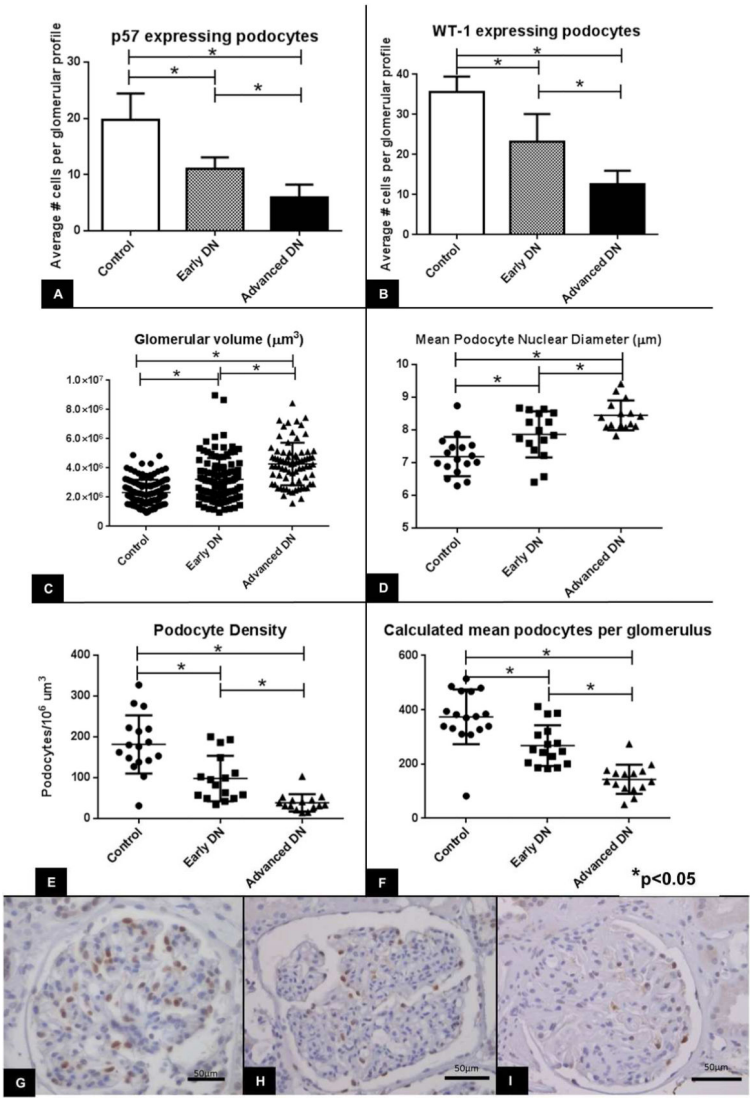

Figure 1.

A, B) The number of p57 and/or WT-1 expressing podocytes per glomerular profile was progressively and significantly reduced in histologically early and advanced diabetic nephropathy compared to controls; this was out of proportion to the increase in glomerular size in DN. Morphometric analysis of the glomerular tuft showed that with advancement of diabetic nephropathy, there is C) significant increase in mean glomerular volume, D) an increase in the mean podocyte nuclear diameter, E) progressive decrease in podocyte density, and F) a progressive decrease in the calculated mean podocytes per glomerulus (*p variable, all <0.05). G, H, I) p57 highlights progressively decreased podocytes on the glomerular tuft from controls (G) to early (H) to advanced DN (I) (400×).

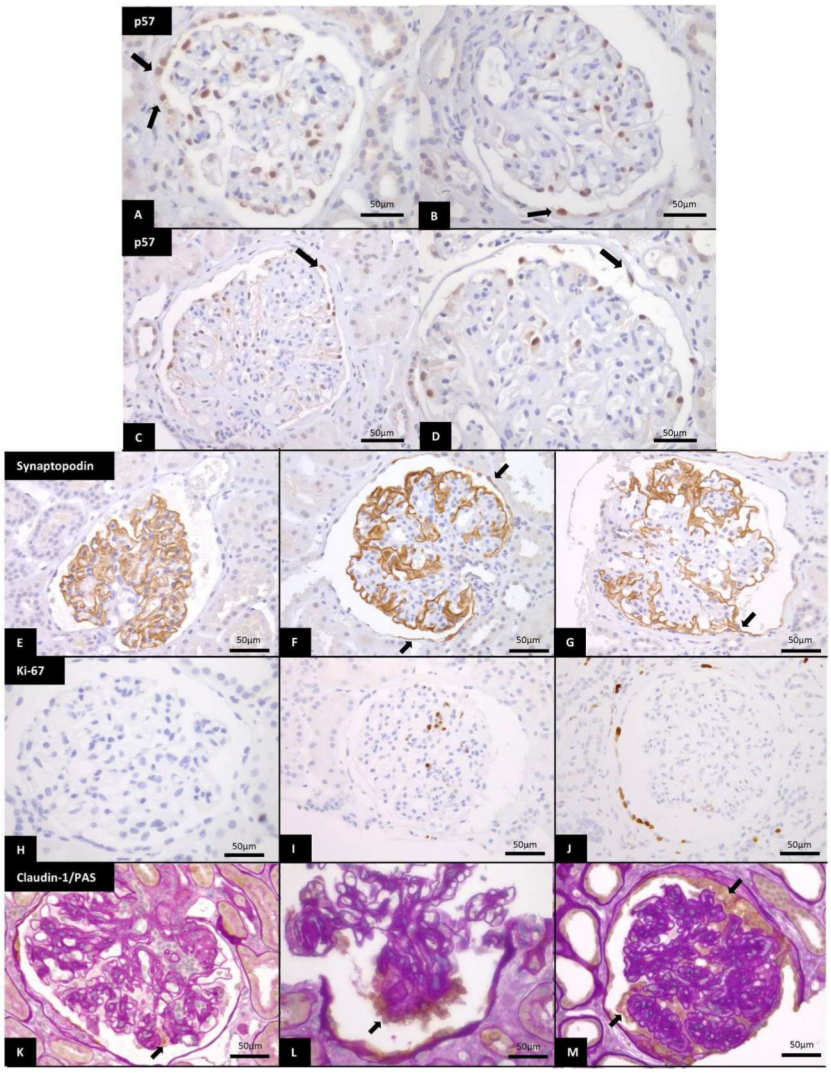

Figure 2.

A-D) Cells marking as podocytes were present in PEC locations and significantly increased in histologically early DN (A,B), with a non-significant increase in advanced DN (C,D) compared with controls (400×).

E-G) Synaptopodin highlighted a significantly increasing percentage of staining of cells lining Bowman's capsules from controls (E) to early (F) to advanced (DN), including areas of segmental adhesions (400×)

H-J) Ki-67 expressing cells were identified on the glomerular tuft and Bowman's capsule in morphologically early (I) and advanced (J) diabetic nephropathy, but only rarely in controls (H) (400×).

K-M) Claudin-1/PAS revealed claudin-1 positive cells in areas of increased mesangial matrix in early DN (K), in areas of “capping” of segmentally sclerotic regions (L), and having a variable glomerular distribution in advanced DN (M) (400×).

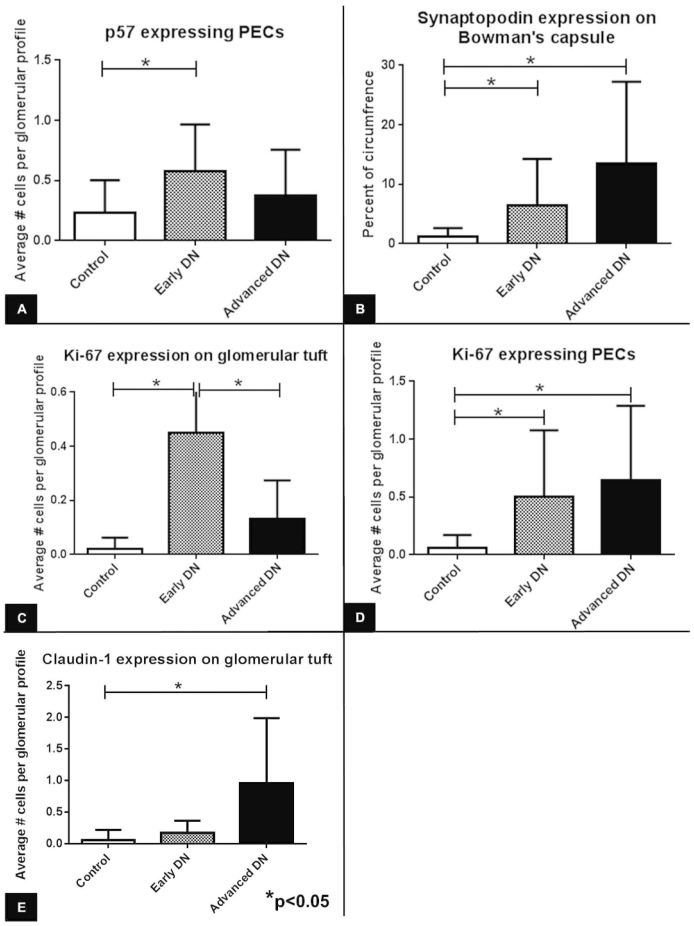

Figure 3.

A) p57 expressing cells (marker of podocytes) on Bowman's capsule were significantly increased in early diabetic nephropathy.

B) Synaptopodin expression on Bowman's capsule was significantly increased in both early and advanced DN.

C, D) The mean number of Ki-67 expressing cells on the glomerular tuft profiles was significantly increased in histologically early DN compared to controls; Ki-67 expressing PECs were significantly increased in both early and advanced DN compared to controls.

E) Cells immunoreactive for claudin-1 (marker of PECs) were identified on the glomerular tuft and significantly increased in advanced stages of DN.

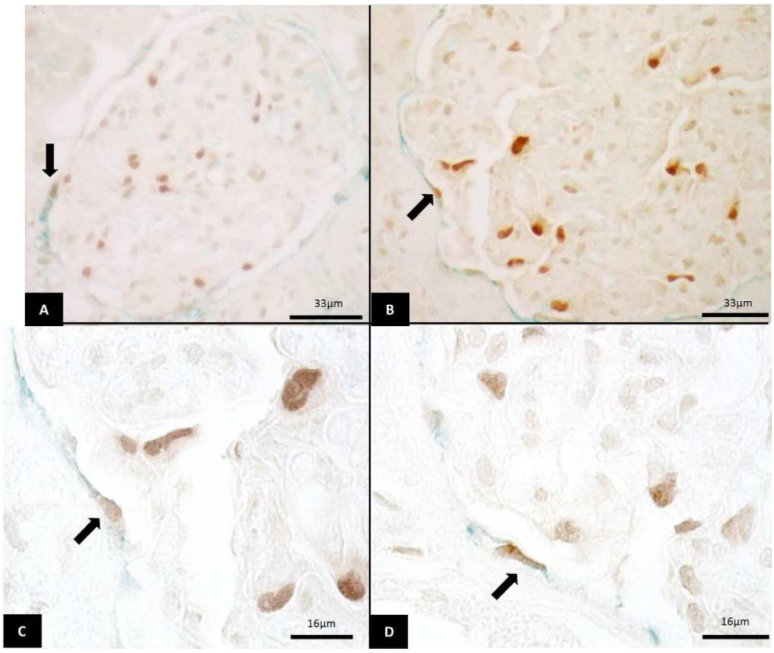

Figure 4.

A-D) Rare claudin-1/p57 dual expressing cells were identified on Bowman's capsule in 3 different cases of early diabetic nephropathy, but not in controls nor advanced DN (claudin-1 green cytoplasmic stain; p57 nuclear brown stain; A, B at 600×; C,D at 1250×).