Epithelial-Mesenchymal Transition and Podocyte Loss in Diabetic Kidney Disease

Developmentally, podocytes evolve from columnar epithelial cells linked together by an apical junction complex containing both tight junctions (TJs) and adherent junctions. As epithelial cells develop into podocytes, the apical TJs and adherent junctions migrate basally and ultimately morph into a single slit diaphragm (SD) that links foot processes from adjacent podocytes. Mature podocytes develop spindle-like foot processes, reorganize their actin cytoskeleton, and express vimentin and synaptopodin. A mature SD retains features of both the TJ and adherent junction. Immunostaining for the TJ-associated protein zonula occludens 1 (ZO-1) in adult rat kidney shows the greatest intensity at the insertion sites of the SD into the foot processes. Also, like the TJ, the SD functions as a barrier that imposes selective permeability between the blood and urinary space and serves as a signaling platform.

Podocyte injury and reduced podocyte density have been documented in patients with diabetic kidney disease and are among the strongest predictors of its progression. When a critical proportion of the total podocyte population is lost, the remaining cells are unable to compensate and glomerulosclerosis develops. Although the mechanism of podocyte loss is still being debated, detachment and apoptosis are both believed to contribute to the decrease in podocyte density. The diabetic milieu, including hyperglycemia, oxidative stress, and advanced glycation end products, decreases podocyte survival through multiple pathways, including decreased phosphorylation of survival factor AKT and activation of proapoptotic P38 mitogen-activated protein kinases. Activation of Notch in diabetic nephropathy alters cell-cycle signaling and induces apoptosis through the p53 pathway. Although apoptosis can be identified in experimental diabetic nephropathy, it has been challenging to identify apoptotic podocytes in human diabetic nephropathy. Several recent studies described urinary podocytes in both experimental and clinical diabetic nephropathy, suggesting that podocyte dropout might be caused by decreased podocyte adhesion. In support of this hypothesis, failure of attachment of podocytes to the glomerular basement membrane by integrin receptors and increased expression of antiadhesive proteins have been identified in diabetic nephropathy. However, many recent studies question whether these cells are truly glomerular epithelial cells or are of parietal epithelial origin. In addition, the majority (~90%) of podocytes that can be detected in urine are apoptotic. Therefore, the possibility arises that apoptosis and detachment contribute to the disease development together; they might even be linked, as cells that do not attach to a basement membrane die and apoptotic cells may detach from the glomerular basement membrane.

In this issue of the American Journal of Kidney Diseases, Yamaguchi et al propose a new mechanism for podocyte loss through an epithelial-to-mesenchymal transition (EMT). EMT is believed to signify a reversal of the developmental transition from metanephric mesenchyme to the epithelial phenotype characteristic of differentiated podocytes and tubular cells. EMT has been proposed to contribute to tubulointerstitial fibrosis and the progression of kidney disease. Transforming growth factor β (TGF-β) is a strong inducer of EMT of tubular epithelial cells cultured in vitro. Diabetic kidney disease is associated with increased expression of TGF-β in glomerular and tubular epithelial cells. In 2008, Li et al proposed that TGF-β induces the expression of snail, a transcriptional inducer of EMT. Downregulation of nephrin, ZO-1, and P-cadherin in cultured podocytes might be dependent on snail. Li et al also described the expression of fibroblast-specific protein 1 (FSP-1), a marker of EMT, in human diabetic nephropathy.
What is EMT? EMT is loosely defined by 3 major changes in cellular phenotype: (1) morphological changes from a cobblestone-like monolayer of epithelial cells with an apical-basal polarity to dispersed spindle-shaped mesenchymal cells with migratory protrusions, (2) changes in differentiation markers from cell-cell junction proteins and cytokeratin intermediate filaments to vimentin filaments and fibronectin, and (3) functional changes associated with the conversion of stationary cells to motile cells that can invade through the extracellular matrix (ECM). Not all 3 changes are invariably observed during an EMT; however, acquisition of the ability to migrate and invade extracellular matrix as single cells is considered a functional hallmark of the EMT program.

Is there a podocyte EMT? When podocytes are grown in cell culture, a cobblestone-like morphological state resembling that of epithelial cells can be observed; however, differentiated podocytes have many spindle-like protrusions. Similarly, podocytes in vivo have primary, secondary, and tertiary foot processes and express vimentin, giving them a more mesenchymal appearance at baseline. Therefore, podocytes cannot necessarily fulfill the general morphological change premise of EMT. Increased podocyte motility recently has been considered an important aspect of proteinuria. In addition, injured or diseased podocytes de novo express different, more mesenchymal-like markers. However, most importantly, podocytes do not fulfill the last and maybe the most important marker of EMT, the extracellular matrix invasion.

**Figure 1.** (Top panel) Arrow demarks the epithelial-mesenchymal transition (EMT) in columnar epithelial cells characterized by the presence of tight junctions (TJ) and adherens junctions (AJ) at their apical aspect and adherence to the glomerular basement membrane (GBM) at the basal aspect. Columnar epithelial cells lose cell-cell junctions and their cobblestone morphology and develop a spindle shape, permitting increased motility and promoting migration into and through a degenerating GBM. (Bottom panel) Phenotype change in podocytes. Arborized podocytes have interdigitating foot processes linked by slit diaphragms (SD), but retain more mesenchymal characteristics at baseline, with spindle-shaped processes and expression of vimentin. In diabetic nephropathy, podocytes become effaced and have increased expression of mesenchymal markers. They do not invade the GBM, but rather increased motility results in detachment and loss of podocytes. Abbreviations: α-SMA, α smooth muscle actin; FSP-1, fibroblast-specific protein 1; ILK, integrin-linked kinase; ZO-1, zonula occludens 1.
lular matrix invasion. For these reasons, podocyte EMT remains a controversial issue.

What is unique about the present study is that it also links EMT to podocyte loss in human diabetic kidney disease. The investigators identify FSP-1 expression in the majority of urinary podocytes from patients with diabetic nephropathy, and these podocytes did not have apoptotic nuclei, although no direct assay for apoptosis was performed. Notably, urinary FSP-1− podocytes correlated with the severity of diabetic lesions, and increased glomerular expression of FSP-1 and urinary loss of FSP-1+ podocytes was specific to diabetic lesions and not identified in patients with minimal change disease and proteinuria. This finding is important because it has long been unclear why patients with proteinuria and minimal change disease do not develop a decreased glomerular filtration rate, whereas in other kidney diseases, proteinuria correlates with progressive decrease in glomerular filtration rate.

The investigators also show increased expression of snail1 and downregulation of ZO-1 in human diabetic glomeruli. In addition, they identify upregulation of integrin-linked kinase in FSP-1+ podocytes of patients with diabetic nephropathy; integrin-linked kinase has been shown to mediate EMT in tubular cells and functions in adhesion of podocytes to the glomerular basement membrane. Taken together, the data indicate that in vitro under the influence of TGF-β, podocytes might take on mesenchymal characteristics, and this mesenchymal phenotype may contribute to podocyte dehiscence and loss.

It is unclear to date how expression of mesenchymal genes results in detachment of podocytes; however, it is clear that podocytes that detach undergo a change in their transcription profile. It is interesting to note that although both snail and integrin-linked kinase have been implicated in EMT, they also are regulators of apical-basal polarity and cell motility. Because apical-basal polarity is required to properly localize ZO-1 and adherens junction proteins at cell-cell interfaces and for adhesion to matrix components, these findings suggest the possibility of a defect in podocyte polarity in diabetic nephropathy. Podocyte polarity recently has been a topic of interest, with demonstration that the apical basal polarity factors Crumbs and Par3/Par6/β-aPKC are required to localize nephrin to the podocyte SD, with loss of function inducing proteinuria and glomerulosclerosis.

From a therapeutic standpoint, the important role of TGF-β in EMT provides yet another mechanism by which TGF-β inhibitors could be protective in progressive proteinuric kidney disease. It also suggests that ongoing trials using anti–TGF-β agents in the treatment of progressive proteinuric kidney diseases should examine urinary podocyte loss as an outcome measure.

In sum, phenotypic changes in podocytes associated with an increase in podocyte motility and expression of new markers is documented in human diabetic kidney disease and many different animal models and appears to be a common theme associated with proteinuria. The functional role of these changes and whether we can call these changes EMT remains to be determined. Nevertheless, Yamaguchi at al provide a new paradigm of podocyte detachment, with EMT contributing to podocyte loss in diabetic nephropathy.

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