Glomerular endothelial cell injury and cross talk in diabetic kidney disease

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The epidemic of diabetic kidney disease (DKD) has been rising significantly over the last several decades and will continue to be the leading cause of renal failure, being the primary diagnosis in ~50% of people starting renal replacement therapy for end-stage renal disease in the United States (105). However, the current treatment of DKD has been limited to hyperglycemic control, blood pressure control, and renin-angiotensin system (RAS) blockade. Several recent clinical trials for new therapies of DKD have been unsuccessful. Therefore, there is an urgent need to better understand the pathogenesis of DKD to develop early and more effective therapy for this disease.

Endothelial cell injury is a major event in diabetes, leading to multiple macro- and microvascular complications. Glomerular endothelial cells (GEC) have a unique feature of fenestrations and are able to handle a large amount of filtration. GEC injury is thought to contribute to the development of microalbuminuria, an early event of DKD. However, despite their well-recognized association, the mechanism by which GEC dysfunction results in albuminuria is poorly understood. In addition, recent studies suggest that GEC may contribute to DKD by paracrine communication with other glomerular cells such as podocytes and mesangial cells. Discussed in this review are the potential roles of GEC dysfunction in the pathogenesis of DKD, with a particular focus on the mechanism of GEC injury in the development of albuminuria and the role of the paracrine network between GEC and other glomerular cells in DKD.

GEC Injury and Albuminuria

The glomerular filtration barrier functions as a whole in albumin handling. The glomerulus is a highly specialized structure in which the capillary walls function as an efficient filtration barrier that restricts passage of larger molecules, predominantly proteins, but remains highly permeable to water and small molecules (46). These functions are achieved by the glomerular filtration barrier (GFB) comprised of an innermost fenestrated GEC layer, a glomerular basement membrane (GBM), and an outermost layer of podocytes with their interdigitating foot processes bridged by a slit diaphragm. Injury of any of these three components of the GFB will lead to albuminuria and glomerular disease.

Role of GEC in the development of albuminuria. An extensive body of evidence indicates that proteinuria is often a result of ultrastructural changes in podocyte foot processes.
ever, many examples demonstrate that proteinuria may also occur independently of foot process effacement. For instance, mice with podocyte-specific overexpression of angiopoietin-2 (Ang-2) had significant increases in both albuminuria and GEC apoptosis, while their podocytes remained structurally intact (25). Increased circulating sFlt1 is associated with preeclampsia and resulted in hypertension, proteinuria, and glomerular endotheliosis (a classic lesion of preeclampsia) through inhibition of VEGF in the absence of podocyte structure changes (85, 111). These results provide insight into a critical role of GEC in the development of proteinuria and highlight the importance of podocytes and GEC cross talk.

A study of Pima Indians with type 2 diabetes found that both podocyte damage and glomerular endothelial injury were commonly present in a cohort with macroalbuminuria (122). Interestingly, compared with podocyte injury, endothelial abnormalities were more closely associated with increasing urine albumin excretion, suggesting that endothelial cell injury may be more critical to glomerular alterations in DKD compared with the commonly viewed importance of podocyte injury (122). These findings in DKD, in addition to those in preeclampsia (109) and rare familial nephrotic syndrome (5), suggest that GEC play a critical role in the development of albuminuria.

Mechanism of GEC injury in the development of albuminuria. How GEC injury leads to albuminuria is not completely understood. Each GEC comprises the proximal component layer of the glomerular filtration unit, characterized by the presence of individual fenestrae on the order of 70–100 nm in diameter (99). While it was well appreciated that the endothelial fenestrations contribute to the high hydraulic permeability, it had been thought to contribute little to the protein barrier function of the glomerular capillary wall.

The glomerular endothelium is coated with a polysaccharide-rich layer composed primarily of glycocalyx, a meshwork of membrane-bound glycoproteins and proteoglycans, and a loosely attached endothelial cell coat of secreted proteoglycans, glycosaminoglycans, glycoproteins, and plasma proteins, referred to as the endothelial surface layer (ESL) (46, 71, 97). Recent findings showed that the ESL provides an important barrier to protein permeability.

Loss of the ESL has been reported in experimental models in association with albuminuria and increased glomerular albumin clearance without alteration of the GBM and podocyte foot processes (39, 85, 111, 133). Loss of the ESL or components of the ESL can be caused by enzymatic digestion induced by increased oxidative stress and high glucose concentration: Kuwabara and coworkers (49, 70) found that oxidative stress causes deterioration of the glomerular ESL by induction of heparanase, a degrading enzyme of the ESL, resulting in increased glomerular permeability. Mice that lack heparanase do not develop proteinuria or structural injury in diabetes induced with streptozotocin (STZ) (42). Notably, widespread ESL loss has been reported in patients with type 1 (26, 92) and type 2 diabetes (6, 72), and the development of microalbuminuria in diabetic patients results in further reductions of the systemic ESL, leading to systemic vascular dysfunction (92). Experimental injury to the glomerular endothelial glycocalyx also results in increased clearance of unmodified, negatively charged albumin, which is particularly recapitulated in the early albuminuric phase of DKD (23, 52, 70, 86, 97). Indeed, microalbuminuria may be a marker for systemic endothelial glycocalyx disruption, causing endothelial cell injuries, such as preeclampsia and thrombotic microangiopathy (71, 93).

Communication Between GEC, Podocytes, and Mesangial Cells

Gene and protein expression profiling shows that glomerular changes in diabetes involve many metabolic and signaling pathways that may occur in the individual glomerular cells or through cross talk between them (43, 50, 103, 112). Several studies have confirmed that interactions occur between glomerular cells. As seen in Fig. 1, GEC and podocytes both sit on the GBM, where cross talk may occur through a bidirectional diffusion of cytokines/growth factors following a gradient. In addition, direct communications between cells also occur through gap junctions, such as between interdigitating mesangial and endothelial cells in the paramesangial region of the capillary loop. Although some of these interactions have been shown, many others remain unknown. Discussed below are the known interactions among glomerular cells and their roles in DKD (Table 1).

Interaction between GEC and mesangial cells. PLATELET-DERIVED GROWTH FACTOR B (PDGF-B)/PDGFRB. Mesangial cells provide structural support for and regulate blood flow of the glomerular capillaries by their contractile activity. The generation and maintenance of mesangial cells in glomerular development are dependent on PDGF-B/PDGFRB signaling, which occurs through the interaction between GEC and mesangial cells. This paracrine signaling is mediated by PDGF-B produced by GEC and PDGFRB on the mesangial cells. Genetic ablation of either PDGF-B or PDGFRB leads to a similar embryonic lethal phenotype, displaying a failure of mesangial cell ingrowth into the developing glomeruli and subsequent capillary loop sprouting, resulting in a single vascular sac per glomerulus (35, 77). Endothelium-specific deletion of PDGF-B, although not lethal, also showed similar defects in the glomeruli, confirming that endothelium-derived PDGF-B is necessary for mesangial recruitment (4).

Fig. 1. Normal glomerular filtration barrier complex and its disruption in diabetic kidney disease. A: schematic illustration of the normal glomerular filtration barrier complex, consisting of podocyte foot processes with their slit diaphragm, glomerular basement membrane (GBM), and the glomerular surface layer (ESL). Glomerular endothelium and fenestrae are covered by the glycocalyx, a meshwork of membrane-bound glycoproteins and proteoglycans. Cell-to-cell communication occurs through a bidirectional diffusion of cytokines/growth factors produced by one or more of the glomerular cell types. B: schematic illustration of a diabetic kidney, which is characterized by GBM thickening, mesangial expansion, and podocyte foot process effacement and detachment. In addition, the fenestrated area of endothelial cells is reduced, the amount of glycocalyx is diminished, and the communication between glomerular endothelial cells (GEC) and neighboring glomerular cells is altered. The abnormal renal expression of VEGF, angiopoetins, and endothelin-1 in early diabetic kidney disease (DKD) induces neoangiogenesis and may contribute to the initial hyperfiltration. GEC also produce other mediators, such as HGF and IGF, and their dysregulation is associated with glomerular hypertrophy and microalbuminuria in early DKD. See text for additional definitions.

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**Podocyte-GEC:**
- VEGF-A
- ANG1, ANG2
- ET-1

**GEC-Podocyte:**
- HGF
- IGF
- KLF2

**GEC-Mesangial cell:**
- PDGFB

**Mesangial cell-GEC:**
- Integrin αβ8, TGF-β

**Other mediators:**
- COX-2–derived Prostanoids
- MicroRNA

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**Podocyte:**
1. Podocyte hypertrophy
2. Podocyte foot process effacement
3. Podocyte apoptosis
4. Podocyte detachment
5. Disruption of slit diaphragm

**GBM:**
- Thickening

**Endothelial cell:**
- Glycocalyx Disruption

**Mesangial cell:**
- Mesangial hypercellularity, matrix expansion

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**Glomerular injuries:**
- VEGF-A
- ANG1↓ / ANG2↑
- Tie1↓ / Tie2↑
- ETA

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**Early DKD**
- VEGF-A
- VEGF
- VEGF-A-VEGFR2
- Neangiogenesis
- Glomerular injuries
- Vascular rarefication
- Renal fibrosis

**Later DKD**
- Podocyte loss
- VEGFR2

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**Renal fibrosis**
- Klf2↓
- eNOS↓
- HGF? PDGFB↑

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**Angiogenesis**
- VEGF1
- VEGFR2↑
- eNOS Ser1177↑ phosphorylation

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**Renal fibrosis**
- ETA
- eNOS↓

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**Neangiogenesis**
- VEGF↓
Table 1. Summary of mediators for glomerular endothelial cell and podocyte cross talk

<table>
<thead>
<tr>
<th>Cross Talk</th>
<th>Ligand</th>
<th>Receptor</th>
<th>Physiological Role</th>
<th>Role in DKD</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Podocyte→endothelial cell</td>
<td>VEGF-A</td>
<td>VEGFR-1</td>
<td>Mediators of podocyte and glomerular endothelial cells; regulates development and function of the glomerular filtration barrier in the kidney.</td>
<td>Renal expression of both VEGF and VEGFR2 is increased in early DKD, and the reduction of VEGF signaling may occur due to loss of podocytes at later stages, which contributes to vascular rarefication and renal fibrosis.</td>
<td>33, 34, 53, 85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VEGFR-2</td>
<td>Mediators of podocyte and glomerular endothelial cell migration, proliferation, and differentiation.</td>
<td></td>
<td>120, 121</td>
</tr>
<tr>
<td>Ang-1→podocyte</td>
<td>Tie-2</td>
<td></td>
<td>Promotes angiogenesis, podocyte survival; reduces endothelial cell permeability, and modulates effects of VEGF.</td>
<td>Decreased Ang-1/Ang-2 ratio contributes to development of DKD. Ang-1 could potentially protect glomerular microvasculature from diabetes-induced injury.</td>
<td>41, 119</td>
</tr>
<tr>
<td>Ang-2→podocyte</td>
<td>Tie-2</td>
<td></td>
<td>Antagonistic ligand for Tie-2 in endothelial cells that inhibits the binding of Ang-1 to Tie-2 in an autocrine fashion.</td>
<td></td>
<td>82</td>
</tr>
<tr>
<td>ET-1→podocyte</td>
<td>ETα</td>
<td></td>
<td>ET-1 is a potent vasoconstrictory peptide with proinflammatory and profibrotic properties.</td>
<td>ET-1 is upregulated in DKD, and selective blockade of the ETα receptor provided renal protection by reduced chemokine and cytokine expression, as well as attenuation of various mediators of renal fibrosis.</td>
<td>59</td>
</tr>
<tr>
<td>ANGPTL4→podocyte</td>
<td></td>
<td></td>
<td>Inhibits endothelial cell adhesion, migration, and sprouting; alters actin cytoskeleton.</td>
<td>A significant contributor to proteinuria in experimental DKD. An upregulation of podocyte-secreted Angptl4 in experimental DKD has been described, and treatment with ManNAc resulted in a significant decline in proteinuria.</td>
<td>9, 16, 17</td>
</tr>
<tr>
<td>SDF-1→podocyte</td>
<td>CXCR4</td>
<td></td>
<td>Essential for development of renal vasculature and primarily through blood vessel formation.</td>
<td>SDF-1 promotes pancreatic β-cell survival through Akt activation, suggesting that SDF-1 agonists may prove beneficial for treatment of diabetes.</td>
<td>117, 131</td>
</tr>
<tr>
<td>IL6→podocyte</td>
<td></td>
<td></td>
<td>Podocytes secrete soluble IL-6 and influence the ability of GEC to recruit neutrophils during inflammation dependently via paracrine manners.</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>Endothelial cell→podocyte</td>
<td>HGF</td>
<td>c-MET</td>
<td>Antifibrotic and regenerative properties that prevent onset and progression of a wide variety of chronic kidney diseases mediated by a single HGF receptor, c-MET.</td>
<td>The role of HGF in DKD remains controversial.</td>
<td>32, 87, 130</td>
</tr>
<tr>
<td>IGF→podocyte</td>
<td>IGFBPs</td>
<td></td>
<td>Plays a critical role in maintenance of normal renal function and pathogenesis and progression of CKD.</td>
<td>Dysregulation of the growth hormone/IGF system is found in early DKD and is associated with both glomerular hypertrophy and microalbuminuria.</td>
<td>2</td>
</tr>
<tr>
<td>TNF-α→podocyte</td>
<td>TNFR1</td>
<td></td>
<td>Induces changes in glomerular endothelial fenestrae and ESL during severe experimental endotoxemia through TNFRI signaling.</td>
<td>Unknown.</td>
<td>47, 126, 127</td>
</tr>
<tr>
<td>Endothelial cell→mesangial cell</td>
<td>PDGFB</td>
<td>PDGFRβ</td>
<td>Generates and maintains mesangial cells in glomerular development.</td>
<td>PDGFRβ signaling is activated in glomeruli and tubules of diabetic mice. It may contribute to the progress of diabetic nephropathy, with an increase in oxidative stress and mesangial expansion.</td>
<td>35, 77</td>
</tr>
<tr>
<td>Mesangial cell→endothelial cell</td>
<td>Integrin-αβ8</td>
<td>Latent TGF-β</td>
<td>Protects kidney from glomerular dysfunction, endothelial apoptosis, and development of proteinuria.</td>
<td>Unknown.</td>
<td>60</td>
</tr>
</tbody>
</table>

Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; ANGPTL4, angiopoietin-like 4; CXCR4, C-X-C chemokine receptor type 4; DKD, diabetic kidney disease; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; ETα, endothelin receptor A; ESL, endothelial surface layer; GEM, glomerular basement membrane; GEC, glomerular endothelial cell; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; IL-6, interleukin 6; PDGFB, platelet-derived growth factor B; SDF-1, stromal cell-derived factor 1; TGF-β, transforming growth factor-β; Tie2, angiopoietin 1 receptor; TNF-α, tumor necrosis factor-α; TNFR1, tumor necrosis factor receptor 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
INTEGRIN αβ8. Another example of the mesangial-to-endothelial cell cross talk necessary for the maintenance of normal glomerular integrity is provided by integrin αβ8 and its ligand latent transforming growth factor-β (TGF-β) (60). Integrin αβ8 expressed in kidney mesangial cells binds and sequesters latent TGF-β, thereby decreasing TGF-β-mediated signaling in neighboring endothelial cells. In the absence of integrin αβ8, increased levels of TGF-β lead to glomerular damage as demonstrated by glomerular dysfunction, endothelial apoptosis, and development of proteinuria in mice with homozygous Itgb8 deletion, providing the evidence that mesangial cells can maintain glomerular endothelial cell stability through regulating the bioavailability of TGF-β.

Interaction between GEC and podocytes. VEGF-A/VEGF RECEPTOR 2 (VEGFR-2). One of the most intensively studied signaling mediators of endothelial and podocyte function within the glomerulus is the vascular endothelial growth factor (VEGF) and their receptors VEGFR-1 (or Flt-1) and VEGFR-2 (or Flk-1/KDR). The VEGF-A/VEGFR system is essential for normal glomerular development and adult renal homeostasis, and its alteration plays a major role in DKD. Nevertheless, the precise source of the ligand and localization of receptors remain controversial. Reports of VEGFR1 and/or VEGFR2 expression in podocytes, direct effects of VEGF on cultured podocytes (3, 12, 44, 67, 121) and podocyte-specific overexpression of VEGF in vivo resulting in pathological features, including foot process effacement (120, 121), suggest that podocytes may respond to VEGF in an autocrine regulatory system.

However, other studies also provide compelling evidence for a paracrine VEGF/VEGFR2 regulatory loop between GEC and podocytes. A global deletion of Vegfr2 resulted in marked abnormalities in the kidney and defects in the glomerular microvasculature, while podocyte-specific Vegfr2 deletion did not affect glomerular development or function nor ameliorate the glomerular injury induced by podocyte-specific VEGF overexpression (106). These findings suggest that VEGF produced by the podocyte regulates the structure and function of the adjacent endothelial cells.

Experimental models have shown that the renal expression of both VEGF and VEGFR2 is increased in early DKD (10, 18) and that the inhibition of VEGF-A or VEGF receptors in diabetic animals results in prevention of proteinuria and amelioration of glomerular injuries (29, 36, 67, 113). Furthermore, podocyte-specific overexpression of Vegfa is sufficient to induce GBM structural and functional abnormalities, similar to those observed in rodent models of DKD (121). However, a contradictory result was shown in patients with more advanced stage of DKD of a significant inverse correlation between Vegf-A expression and proteinuria (78). The authors suggest that the apparent discrepancy between rodent and human may be due to a limited nature of DKD progression in rodent models. It is plausible that while the activation of VEGF/VEGFR in early DKD causes neoangiogenesis and other glomerular injury, at later stages the reduction of VEGF signaling may occur due to the loss of podocytes, contributing to vascular rarefaction and renal fibrosis. Therefore, approaches to intervene in VEGF signaling in DKD have to be taken with caution and possibly with stage-specific considerations.

ANGIOPoitInS ANG-1 AND ANG-2. Although VEGF-A/VEGFR2 has been most extensively investigated, recent studies indicate that this exciting story may represent only the tip of the iceberg of glomerular cell cross talk and interaction. Podocytes can communicate with adjacent endothelial cells through multiple secreted molecules.

Angiopoietins (Ang) are other important growth factors that promote angiogenesis together with VEGF and are involved in the pathophysiology of DKD. As demonstrated by mouse knockout studies, among the four angiopoietins (Ang-1–4), Ang-1 and Ang-2 are required for the formation of mature blood vessels (40, 114). Ang-1 and Ang-2 act by binding to the endothelium-specific receptor tyrosine kinase 2 (Tie-2), but they exert opposing effects (124). Ang-1 is expressed mostly in the podocyte and is able to promote cell survival, to reduce endothelial cell permeability (119), and to modulate the effects of VEGF (41). Ang-2 is an antagonistic ligand for Tie-2 in endothelial cells that inhibits the binding of Ang-1 to Tie-2 in an autocrine fashion (82).

A decreased Ang-1/Ang-2 ratio contributes to the development of DKD. Rizkalla et al. (96) reported that after 8 wk administration of STZ in adult rats, Ang-1 levels were lower than in nondiabetic controls, whereas Ang-2 remained elevated. The same observation is found in STZ-induced DKD mice (129) and humans with type 2 diabetes (76). To further investigate the role of Angpt1 in diabetes, Jeansson et al. compared diabetic controls and Angpt1-deleted mice induced with STZ. The Angpt1 knockout kidney showed accelerated diabetes-mediated glomerular damage, suggesting that Ang-1 could potentially protect the glomerular microvasculature from diabetes-induced injury (51). Recently, the Gnudi group (28) reported that mice with podocyte-specific inducible Ang-1 overexpression in early stage of DKD led to a 70% reduction of albuminuria and prevented diabetes-induced GEC proliferation via increased Tie-2 phosphorylation. In addition, they reported elevated soluble VEGFR1, decreased VEGFR2 phosphorylation, and increased Ser1177 phosphorylation of endothelial nitric oxide synthase (eNOS) in these mice, suggesting a critical role of Ang-1/Ang-2 in DKD.

ENDOTHElIN-1. Endothelin-1 (ET-1) has been strongly implicated in renal injury and in the progression of DKD. Global overexpression of ET-1 induces glomerulosclerosis and interstitial fibrosis without concurrent hypertension, suggesting that elevation in ET-1 could directly contribute to renal fibrosis. ET-1 is a potent vasoconstrictory peptide with proinflammatory and profibrotic properties that exerts its biological effects through two ET receptor isoforms, ETα and ETβ. In normal physiology, ETα receptors promote vasoconstriction, cell proliferation, and matrix accumulation, while ETβ activation is vasodilatory, antiproliferative, and antifibrotic (59).

Many preclinical studies with animal models have suggested that selective blockade of the ETα receptor is associated with renal protection when used together with the standard therapy, such as RAS blockade. In STZ-induced diabetic rats, selective ETα antagonists provided renal protection in association with reduced chemokine and cytokine expression, as well as attenuation of various mediators of renal fibrosis (4, 90). In clinical trials, the similar beneficial effects of ETα antagonists have been obtained including systemic and renal vasodilation and albuminuria-lowering effects (84). However, endothelin antagonists have not yet emerged in clinical practice, due to significant side effects, such as fluid overload and liver toxicity (102). More recently, de Zeeuw et al. (31) demonstrated that
Atrasentan, a selective ET$_\text{A}$ receptor antagonist, reduces albuminuria and improves BP and lipid spectrum with manageable fluid overload-related adverse events in patients with type 2 DKD receiving RAS inhibitors.

A recent report by Daehn et al. (106) showed that podocyte-specific activation of TGF-$\beta$ signaling is associated with ET-1 release by podocytes, which mediates mitochondrial oxidative stress and dysfunction in adjacent GEC via paracrine ET$_\text{A}$ activation (20). GEC dysfunction promoted podocyte apoptosis, and inhibition of ET$_\text{A}$ or scavenging of mitochondrial-targeted ROS prevented podocyte loss, albuminuria, glomerulosclerosis, and renal failure. These studies suggest a reciprocal cross talk between podocytes and GEC through the ET-1/ET$_\text{A}$ pathway, and targeting the reciprocal interaction between podocytes and GEC may provide opportunities for therapeutic intervention in FSGS.

eNOS. Another evidence of glomerular endothelial-podocyte cross talk in the development of DKD comes from the study showing that diabetic mice with endothelial dysfunction induced by genetic deficiency of eNOS develop a podocyte-specific injury with heavy albuminuria (134). These findings suggest that podocytes may receive signals from the endothelium, highlighting the importance of communication between endothelial cells and podocytes in diabetes. Interestingly, maintenance of endothelial levels of the essential eNOS cofactor tetrahydrobiopterin ameliorates diabetic nephropathy (61). In addition, polymorphisms in the NOS3 gene that code for eNOS are associated with more advanced diabetic nephropathy (135). However, there are some caveats. The heavy albuminuria in diabetic eNOS-deficient mice is unlikely a result of reduction in NO production per se, since NO itself has been shown to increase glomerular albumin permeability (73). Also, since the animal model used by Yuen et al. (134) is a global knockout of eNOS, additional studies using an endothelial-specific eNOS ablation is necessary to further determine the importance of eNOS in GEC/podocyte cross talk.

KRPPEL-LIKE FACTOR 2. A recent report demonstrated that the expression of Krüppel-like factor 2 (KLF2), a shear stress-inducible transcription factor, is reduced in diabetic kidneys and its lack aggravates endothelial injury in diabetic nephropathy (138). Endothelial-specific reduction of KLF2 exacerbated diabetes-induced glomerular injury and albuminuria by differentially modulating the expression of key angiogenic markers, accompanied by a reduced endothelial glyocalyx. Interestingly, endothelial-specific KLF2 reduction also led to prominent podocyte injury in diabetic kidneys, suggesting that KLF2 is involved in the GEC/podocyte cross talk.

OTHER MECHANISMS OF CROSS TALK BETWEEN GEC AND PODOCYTES. Utilizing a cocultured system in which the podocytes were treated with conditioned medium from GEC under chronic laminar shear stress, Slater et al. (107) found that GEC-podocyte communication is regulated by shear stress. Shear sensing is dependent on the endothelial glyocalyx (118), indicating an additional important role for the glomerular endothelial glyocalyx in glomerular physiology. Recent studies also observed that the cocultured system led to an altered organization of extracellular matrix (ECM). Electron microscopy revealed basement membrane-like ECM deposition between cocultured cells, providing evidence that the assembly of ECM requires cross talk between glomerular cells and further suggests that the soluble factors (e.g., growth factors or ECM components) released by podocytes mediate this effect (8, 138).

Another important pathway through which GEC might modulate the phenotype of other glomerular cells is via increased permeability of the glomerular endothelium, by alteration of the ESL (98). The increased permeability of the endothelium is thought to be the leading cause of increased delivery of macromolecules into podocytes and results in their dysfunction (100, 123). When ESL dysfunction is caused by either increased shear stress or by enzymatic degradation, the albumin is exposed to the podocyte surface, inducing injury. In vivo evidence has confirmed that protein overload results in podocyte damage (1, 88).

Other important mediators for glomerular cell cross talk. TGF-$\beta$. TGF-$\beta$ is a superfamily of cytokines that exerts pleotropic cellular effects, including regulation of cell growth, differentiation, inflammation, and apoptosis and is a well-recognized central mediator of glomerulosclerosis and DN pathogenesis. In response to the ligand binding of TGF-$\beta$, activated TGF-$\beta$ receptors in the cell membrane induce transcription of target genes through the canonical Smad-dependent and noncanonical Smad-independent signaling pathways (27).

In DKD, TGF-$\beta$ signaling is initiated by diacylglycerol (DAG) production and subsequent activation of PKC, increased intracellular glucosamine production, enhanced renal production of vasoactive agents, and various mediators produced under hyperglycemic conditions, such as advanced glycation end products (AGE) and reactive oxygen species (ROS) (55).

Increased TGF-$\beta$1, the prototypical TGF-$\beta$, is associated with podocyte loss, mesangial expansion, and interstitial fibrosis in CKD (65, 101, 104). As podocyte loss is an early feature in DN (94), albeit the existence of some debate as to whether podocyte apoptosis or detachment is quantitatively more important for the loss, in vitro and in vivo findings suggest that TGF-$\beta$1-mediated podocyte apoptosis and dedifferentiation contribute to podocyte loss (101, 115, 125). Increased TGF-$\beta$1 also induces mesangial proliferation and hypertrophy, as well as mesangial matrix deposition in kidney disease, resulting in mesangial expansion (24, 116, 132). Furthermore, TGF-$\beta$ has been shown to induce endothelial-mesenchymal transition in endothelial cells (63, 137), contributing to endothelial-derived myofibroblast generation in early diabetic renal fibrosis (74, 75, 136).

Given these roles of TGF-$\beta$1 in the glomerular cells of the diabetic kidney, it is likely that it also mediates the cross talk among these cells. Further studies are required to demonstrate whether TGF-$\beta$1 is synthesized mostly in a particular type or all glomerular cells and whether TGF-$\beta$1 exerts autocrine or paracrine effects in diabetic conditions.

HEPATOCYTE GROWTH FACTOR. Hepatocyte growth factor (HGF) has antifibrotic and regenerative properties that prevent the onset and progression of a wide variety of chronic kidney diseases, including DKD (32, 87, 130). The biological activities of HGF are mediated by a single HGF receptor, c-MET (95). The expression of c-MET receptors is limited to epithelial cells of various organs, whereas its ligand HGF is primarily derived from the mesenchyme (108), suggesting a possible paracrine role of HGF. Renal HGF is mainly produced by mesangial cells, endothelial cells, and interstitial fibroblasts.
Interestingly, podocyte are shown to express c-MET, suggesting the capacity of responding to HGF stimulation. The possible linkage of HGF signaling to podocyte function under physiological and pathological conditions is demonstrated from the in vivo studies, in which administration of HGF protein has been shown to ameliorate proteinuric kidney diseases (7, 19, 22).

In DKD, however, the role of HGF remains somewhat controversial. Liu et al. (79) showed both in vitro and in vivo that hyperglycemia increases renal expression of HGF and c-Met and may contribute to the renal hypertrophy of diabetes. In contrast, Nakamura et al. (91) found a decrease in circulating and renal HGF levels in a mouse model of type 2 diabetes (91). These discrepancies may be a reflection of changing HGF expression throughout the progression of DKD. HGF in general seems to play a role in preserving podocyte structure and function in DKD, thereby preventing the development of proteinuria. The protective role of HGF in podocytes seems to be associated with antiproliferative effects (19, 22), preservation of the actin cytoskeleton (7), and maintenance of nephrin and synaptopodin expression (21, 38).

INSULIN-LIKE GROWTH FACTOR. Dysregulation of the growth hormone/insulin-like growth factor (IGF) system is found in early DKD and is associated with both glomerular hypertrophy and microalbuminuria. IGF-1 and -2 share structural similarities to insulin and are able to signal through a family of insulin/IGF receptors. Recent studies suggest that podocytes are a specific target for insulin or IGF (14, 15) and that podocyte-specific insulin signaling is biologically important for normal glomerular function and DKD (45, 54, 68).

IGF binding proteins (IGFBPs) can either enhance or inhibit the IGF biological effects by modulating IGF access to their specific receptors (15). GEC produce IGFBP-4, IGFBP-2, and IGFBP-3 and express mRNA for IGFBP-2 to IGFBP-5 (62), which could regulate IGF signaling in podocytes. However, further studies are required to further confirm the role of these mediators in GEC-podocyte cross talk.

CYCLOOXYGENASE-2-DERIVED PROSTANOIDS. Some lipid mediators, such as the arachidonic acid-derived prostanoids, have been identified as important autocrine and paracrine mediators. Cyclooxygenase (COX)-derived prostanoids play a complex role in renal pathophysiology. Effects of prostanooids are mediated via specific receptors, and several prostanoid receptors have been identified on the surface of glomerular podocytes. Cheng et al. (13) demonstrated that transgenic mice that overexpress COX-2 selectively in podocytes are more susceptible to glomerular injury induced by adriamycin or puromycin. Upon podocyte-specific overexpression of COX-2, selective deletion of podocyte PGE2 receptor subtype 4 (EP4) had no effects on adriamycin-induced kidney injury, while deletion of the thromboxane receptor (TP) in these mice showed attenuated albuminuria and foot process effacement and protection against adriamycin-induced injury (37). However, in a remnant kidney model of injury, mice with podocyte-specific overexpression of the EP4 receptor were more susceptible to the development of albuminuria, whereas mice with podocyte-specific deletion of the EP4 receptor were partially protected from GFB damage, suggesting that PGE2-mediated EP4 receptor signaling contributes to podocyte injury (110). Taken together, these data suggest that increased expression of podocyte COX-2 in the injured glomerulus triggers increases susceptibility to podocyte injury, likely mediated by the activation of the TP and EP4 receptors.

Prostanoids PGE2, PGI2, and TxB2 are also reported to be increased in the diabetic kidney (14, 30, 64). Selective COX-2 inhibition significantly reduces glomerular hyperfiltration in STZ-induced diabetic rats, consistent with the role of COX-2-derived prostanoids in the regulation of renal blood flow. Treatment with EP1 receptor and TP antagonists ameliorates renal and glomerular hypertrophy and decreases mesangial expansion (80, 83, 128). The role of COX-derived prostanoids in the cross talk of glomerular cells and the pathogenesis of DKD remains to be further explored.

MicroRNAs as the new mediators of cell-cell cross talk.

MicroRNAs (miRNAs) are short noncoding RNAs that repress target gene expression via posttranscriptional mechanisms. Emerging evidence shows that miRNAs have diverse cellular and biological functions and play a key role in the pathogenesis of DKD (56, 57, 80, 89), and have been recently reviewed in depth (11, 66, 81). Recently, a paracrine role of miRNA in the communication between endothelial cells and their neighboring cells has been reported by Hergenreider et al. (48), where a novel mechanism of miR-143- and miR-145-mediated communication between endothelial cells and vascular smooth muscle cells (VSMC) maintains normal VSMC function. This landmark study suggests that miRNA may also be potential mediators for glomerular cell cross talk in DKD. However, this needs to be yet proven in the future studies.

Conclusion

A large amount of evidence suggests that GEC injury occurs at early stages of DKD and contributes to microalbuminuria (Fig. 1). The alteration of glomerular ESL or glycocalyx is a major cause leading to albuminuria in DKD. Abnormal glomerular cell cross talk plays a critical role in the development of DKD, where VEGF/VEGFR2, angiopoietins/Tie2, ET-1/ETα, and eNOS are the major mediators involved in the pathogenesis of DKD. GEC dysfunction may release soluble mediators to cause podocyte damage, which in turn exacerbates GEC injury, thus forming a vicious cycle. Therefore, inhibition of these major mediators may be helpful in blocking this vicious cycle of injury and the progression of DKD. A deeper understanding of the mechanism of GEC injury and glomerular cell cross talk will be valuable in development of new targeted therapies for DKD.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

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