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Renal Endothelial Dysfunction in Diabetic Nephropathy

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Abstract

Endothelial dysfunction has been posited to play an important role in the pathogenesis of diabetic nephropathy (DN). Due to the heterogeneity of endothelial cells (ECs), it is difficult to generalize about endothelial responses to diabetic stimuli. At present, there are limited techniques fordirectly measuring EC function *in vivo*, so diagnosis of endothelial disorders still largely depends on indirect assessment of mediators arising from EC injury. In the kidney microcirculation, both afferent and efferent arteries, arterioles and glomerular endothelial cells (GEnC) have all been implicated as targets of diabetic injury. Both hyperglycemia *per se*, as well as the metabolic consequences of glucose dysregulation, are thought to lead to endothelial cell dysfunction. In this regard, endothelial nitric oxide synthase (eNOS) plays a central role in EC dysfunction. Impaired eNOS activity can occur at numerous levels, including enzyme uncoupling, post-translational modifications, internalization and decreased expression. Reduced nitric oxide (NO) bioavailability exacerbates oxidative stress, further promoting endothelial dysfunction and injury. The injured ECs may then function as active signal transducers of metabolic, hemodynamic and inflammatory factors that modify the function and morphology of the vessel wall and interact with adjacent cells, which may activate a cascade of inflammatory and proliferative and profibrotic responses in progressive DN. Both pharmacological approaches and potential regenerative therapies hold promise for restoration of impaired endothelial cells in diabetic nephropathy.

Keywords

diabetic nephropathy; endothelial cell; eNOS; heterogeneity; kidney; microvascular complication; nitric oxide; VEGF

INTRODUCTION

Diabetic Nephropathy (DN) is the leading cause of chronic kidney diseasein the developed world and affects about 15–25% of type 1 and 30–40% of type 2 diabetic patients [1]. Despite extensive research, underlying pathogenic mechanisms have yet to be completely elucidated. Hyperglycemia *per se*, as well a metabolic consequences of hyperglycemia, such

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

as Advanced Glycation End-products (AGEs), have been implicated in therenal pathological changes [2], various cells, including glomerular podocytes, mesangial and glomerular endothelial cells (GEnCs), as well as tubular epithelial cells, interstitial fibroblasts and vascular ECs have all been implicated in the development of DN [3]. The ECs play critical roles in many physiological functions: vascular tone adjustment, blood cell trafficking, hemostatic balance, permeability status, cell proliferation and survival, as well as being involved in mediation of innate and adaptive immunity. EC dysfunction promotes progression of DN [4]. A correlation between diabetes and vascular endothelial dysfunction has been confirmed in various studies [5-9]. There is suggestive evidence that endothelial dysfunction may even exist in subjects with normal glucose tolerance and with a family history of diabetes [10]. In addition to hyperglycemia, endothelial dysfunction might also be exacerbated by hypertension, dyslipidemia, obesity, micro albuminuria, inflammation and insulin resistance [11]. This review discusses the underlying mechanisms and consequences of endothelial dysfunction in diabetic nephropathy.

CHARACTER AND HETEROGENEITY OF RENAL ENDOTHELIAL CELLS

The endothelium is the thin layer lining the interior surface of blood vessels and lymphatic vessels [12]. ECs form the endothelium as an interface between circulating blood in the lumen vessel wall. In kidney, blood flows successively through the renal arteries, interlobular arteries, and afferent arterioles to enter the glomerular capillary tufts and then exits glomeruli *via* efferent arterioles, which give rise to the plexus of peritubular capillaries, the vasa recta. ECs from renal arteries, arterioles, capillaries, venules, veins and glomerular capillaries each have distinctive phenotypic features. In spite of accumulating research on endothelial dysfunction, relatively little attention has been paid to varying phenotypes regulated by location and time, so called “endothelial heterogeneity”[13, 14]. The heterogeneity is also species and tissue dependent. Moreover, EC may possibly transdifferentiate into other cell types or vice versa in pathologic conditions [15].

Structural Heterogeneity of Endothelial Cells

Endothelial shape and thickness may vary among vascular beds. Electron microscopy demonstrates elongated, spindle-shaped ECs in rat arterioles, irregularly shaped ECs in capillaries and comparatively large, elliptical or irregularly shaped ECs in post-capillary venules [16]. The intercellular junctions of ECs are either continuous or discontinuous. Brain, skin, cardiac, and pulmonary endothelium in arteries, veins, and capillaries is non-fenestrated and continuous. The density of fenestrae also depends on vascular phenotype. Glomerular capillary endothelium is fenestrated and continuous [17].

ECs also have a diversity of other cellular components. Connexins, a family of structurally related transmembrane proteins that assemble to form gap junctions, have a different pattern in ECs from glomerular afferent and efferent arterioles, which could contribute to the altered renal autoregulation in DN [18]. Plasma lemmal vesicle (PV)-1 protein is an endothelium-specific integral membrane glycoprotein, enriched on the endothelium of renal peritubular capillaries during development, which is fenestrated with diaphragms; it is absent from adult GEnCs, which are also fenestrated but not apertured by diaphragms [19]. Glycocalyx is the glycoprotein oating the luminal surface of the glomerular capillaries; its heterogeneous

distribution along the vascular tree can also be seen by electron microscopy [20]. The EC common marker, von Wille brand factor (vWF) has an uneven distribution indifferent types of vessels [21, 22], although the physiologic significance remains incompletely understood. eNOS expression in ECs of the renal medulla (vasa recta) appears to be stronger than in cortex (glomeruli and peritubular capillaries) [23].

Functional Heterogeneity of Endothelial Cells

Endothelium not only forms a passive barrier, dynamically regulating permeability of the microvasculature [24] but also acts as an active signal transducer for leukocyte trafficking [25], modulating hemostasis [26] and playing a pivotal role in angiogenesis and vasculogenesis, such that it is recognized as a multifunctional paracrine and endocrine "organ" that responds to metabolic, hemodynamic and inflammatory stimuli [27]. Phenotypic variation among endothelial cells may be related to their location in the vascular tree. In general, ECs in post capillary venules, where shear stress is lowest, are mainly responsible for mediating adhesion and recruiting leukocytes [28], while those in arterioles are primarily for vasomotor tone. There are two major functions for the renal endothelium-oxygen/nutrient delivery and filtration [29]. In contrast to other vascular beds, glomerular capillaries serve predominantly as a sieve of fluids and solutes. GEnCs covering 20% of the endothelial surface serve as a barrier for efficient absorption, secretion, and filtering [17]. After approximately 30% of the blood volume is filtered by the glomerulus, blood enters the efferent arterioles with increased viscosity. At each step from the hyperosmolar, hypoxic depths of the inner medulla to cortex, ECs in individual compartments perform different functions to maintain kidney homeostasis. Therefore, it is not surprising that ECs derived from each intrarenal compartment demonstrate individual chemokine expression patterns, mediating compartment-specific T cell and monocyte recruitment in inflammatory injury [30]. This variability may also lead to differential susceptibility to apoptosis and differential responses to microenvironment changes or stimuli [28].

In summary, the vasculature in the kidney is not only distinct from that of other organs, but also displays striking intra-renal heterogeneity in term of surface phenotype and protein expression in different vascular compartments. Understanding this heterogeneity may help to further direct research in EC dysfunction in progressive kidney injury and properly translate the results from bench to bedside.

RENAL ENDOTHELIAL DYSFUNCTION IN DN

Micro- and macrovascular impairment are major complications in diabetic mellitus. The former involves small vessels, such as capillaries, the latter predominately large vessels, such as arteries and veins. Nephropathy has been recognized as a common microvascular complication of diabetes. The so-called "silent phase" of DN usually leads to an underestimation of the underlying EC disorder [31].

How to Measure Renal EC Dysfunction?

As discussed above, renal EC functions vary according to their phenotype and compartmentalization in kidney. *In vitro* experiments are able to provide direct measurement

of vasodilation/vasoconstriction from isolated vessels and characteristics of both structural and functional characteristics of cultured ECs– from their morphology to proliferation, migration, adhesion, permeability, matrix secretion and angiogenesis properties. However, caution must be taken when these data are translated to clinical situations, because commonly used culture conditions may activate or transform endothelial phenotypes [32]. *In vivo*, new techniques to measure peripheral circulation non-invasively, such as peripheral arterial tonometry and brachial artery flow-mediated dilatation (FMD), have been developed in order to assess vascular health and endothelial function [33, 34], but their value to predict the risk factor in DN is still debated [35]. No gold standards have been developed yet to evaluate EC function *in vivo*. In general, EC function is largely evaluated experimentally by: 1) assessment of the functional consequences of EC activity; 2) measurement of the concentration of chemical mediators for EC function [11]; and 3) testing competence of endothelial progenitor cells (EPCs), which may mediate regeneration, since EC has limited intrinsic capacity of self-repair with a low proliferative potential [36].

Endothelial Dysfunction-Related Pathophysiologic Abnormalities in DN

DN is a serious and progressive “microvascular” complication from both type 1 and type 2 diabetes mellitus. Its pathophysiologic alterations manifest as microalbuminuria and hyperfiltration at early stages followed by deterioration to end-stage renal disease. Microalbuminuria is usually the first signal of renal complications and may progress to overt albuminuria [37, 38]. Approximately one fourth of people with type 2 diabetes have albuminuria, and the rate is still rising by 2% to 3% per year [39, 40]. Albuminuria can result from higher intra-glomerular pressure and glomerular basement membrane (GBM) permeability, and may be indirectly influenced by interactions of ECs with mesangial cell and podocytes in a paracrine fashion [41]. In type 2 diabetes, markers of endothelial dysfunction occur in patients with normal urine albumin excretion [42], which supports the hypothesis that endothelial dysfunction may not be a simple consequence, but may also play a key etiologic role in the vasculopathy [11].

Animal experiments have suggested that dilatation of the afferent glomerular arteriole is mainly responsible for the hyperfiltration response, *via* increasing intraglomerular pressure and renal blood flow [43, 44]. Renal hyperfiltration has been proposed to be a common factor in early diabetes associated with vascular dysfunction, which may eventually lead to a decline of renal function and the development of glomerulosclerosis and tubulointerstitial fibrosis [45-47]. Endothelial dysfunction also diminishes the an tiatherogenic ability of ECs, which may also contribute to the abnormal renal function.

Vascular Lesion-Associated Pathologic Changes in DN

In addition to mesangial expansion, GBM thickening as a consequence of extracellular matrix accumulation, and Kimmelstiel–Wilson lesionsor global glomerulosclerosis are common pathologic features in DN [48, 49]. Dissociation of endothelial cells may disrupt the connections between the mesangial area and the GBM. Nodular sclerosis (Kimmelstiel–Wilson lesions) or global glomerulosclerosisis seen in late stages of DN. Atherosclerosis links endothelial injury, dysfunction and activation [27], and arteriolosclerosis in both afferent and efferent is recognized as characteristic of vascular lesions in DN [48]. However,

hyalinosis of the efferent arteriole is relatively specific for DN, since afferent medial thickness may also be associated with concurrent hypertension [49] and is also seen in other settings [48, 50]. Intraglomerular capillary pressure secondary to an increased glomerular filtration rate is influenced by the constriction or relaxation of both glomerular afferent and efferent arteriole, but the latter may be more sensitive to angiotensin II, resulting in relatively decreased afferent arteriolar resistance and higher glomerular capillary pressure [51].

Endothelial-Myofibroblast Transition (EndoMT)

During EndoMT, endothelial cells lose endothelial markers (such as CD31 and vascular endothelial cadherin), while exhibiting mesenchymal markers, including α-smooth muscle actin (α-SMA). EndoMT has been suggested to play an important role in organ fibrosis and cancer progression [52]. Kidney fibrosis in DN, resulting from excess fibrous connective tissue, is a major sign of an advanced stage of disease and may develop in both the tubulointerstitial space and glomerulus. Zeisberg *et al*., presented the first evidence of possible EndMT in diabetic kidney fibrosis [53]: in a 6-month STZ-induced diabetic mouse model, they found that about 40% of all fibroblast-specific protein-1-positive and 50% of the α-SMA-positive cells co-labeled with CD31, implying that EndoMT may exist in the development and progression of DN. Based on these studies, EndoMT was considered as a potential new player in diabetic renal fibrosis. However, confirmation in human disease and elucidation of the underlying molecular pathways of EndoMT leading to renal fibrosis remain to be elucidated. Debate on the source of fibrosis-generating myofibroblasts (either from endothelial cells or vascular pericytes) *in vivo* is still raging [54].

Markers of EC Dysfunction

In addition to renal pathological abnormalities, chemical mediators can be utilized to estimate EC function. Renal endothelium secretes numerous vasoactive substances, such as the vasodilators, prostacyclin (PGI_2) and nitric oxide (NO), and the vasoconstrictor, endothelin (ET)-1. Increased urinary and plasma endothelin are linked to renal damage progression in diabetic animal models [55] and patients with type 2 DN [56], while decreased urine PGI_2 excretion has been reported in diabetic patients [57] and animal models [58]. NO is a particularly important molecular marker and endothelium-derived mediator in DN, due to its vasodilator, anti-platelet, anti-proliferative, anti-adhesive, permeability-decreasing and anti-inflammatory properties [59]. NO derived from L-arginine, is a free radical gaseous molecule and is synthesized by the action of nitric oxide synthases (NOS) [60]. Although both eNOS (endothelial NOS) and iNOS (inducible NOS) are expressed in EC, studies in mice with specific eNOS or iNOS deletion suggested thateNOS plays a predominant role in VEGF-induced angiogenesis and vascular permeability [61]. Alterations in eNOS-driven NO production and/or bioactivity are a well-accepted component of diabetic endothelial dysfunction [62]. In spite of the conflicting reports regarding eNOS gene regulation in animal model of diabetes mellitus, with reports of unchanged [63], diminished [64, 65] or increased [66, 67] expression, we and others have found that eNOS insufficiency accelerates nephropathy in mouse models of both type 1 and type 2 diabetes [68]. ADMA (asymmetric dimethylarginine), a product of arginine methylation, represents an endogenous inhibitor of endothelial NO synthase [69], and

elevated plasma levels have been found in patients with type 1 [70] or type 2 [71] DN, although it is remains uncertain whether alterations in ADMA levels are causal or are simply increased as a consequence of impaired renal function [72].

Given the broad function of renal EC and the complicated endothelial pathophysiology observed in DN, hemostatic (plasminogen activator and its endogenous inhibitor, PAI-1), inflammation (IL-1β, IL-6 and TNF-α) and oxidative stress biomarkers are also of significance in EC research. It is likely that new biomarkers will emerge to assist risk prediction, prognosis and pharmaceutical responses in EC injury.

MECHANISMS OF RENAL ENDOTHELIAL INJURY DURING DN

Glucose acutely autoregulates its uptake into muscle cells [73] but may not have similar autoregulation in endothelial cells [74]; thus, hyperglycemia may increase the intracellular accumulation of glucose and its metabolites in ECs. Extended exposure to high glucose may result in increased susceptibility to vascular endothelial cell injury in diabetes. The pathogenesis of diabetic EC injury is a complicated process, with multiple signal pathways being activated and numerous mediators being involved (Fig. **1**).

Etiology of Diabetic Renal Endothelial Impairment

Both genetic and environmental factors are involved in the development of endothelial dysfunction during DN. A large multiethnic populations genome-wide association study (The Family Investigation of Nephropathy and Diabetes study) mapped genes underlying susceptibility to DNA and found the strongest evidence for linkage to chromosomes 7q21.3, 10p15.3, 14q23.1 and 18q22.3 [75]. Association of chromosome 18q22.3 with DN in type 2 diabetes was also confirmed in Turkish patients [76]. Environmental factors, such as smoking [77] and high fat diet [78] may also be important factors that superimpose to produce microvascular complications. Nevertheless, there is no doubt that chronic hyperglycemia and the subsequent metabolic derangements play a major role in diabetic EC injury and can lead to the over production of advanced glycation end products (AGE), activated protein kinase C (PKC) signaling cascades and accumulated reactive oxygen species (ROS). Hemodynamic alterations along with renin-angiotensin system (RAS) regulation seem to be another pivotal contributor to dysfunction of renal endothelium in both glomerular afferent and efferent arteries.

Hyperglycemia Activated Signal Pathways

Various signal pathways are activated during DN; here we concentrate on the three signaling pathways likely to be involved in endothelial dysfunction:

The DAG (Diacylglycerol)/PKC (Protein Kinase C) Pathway—PKC comprises a superfamily of isoenzymes, activated by cofactors such as DAG and phosphatidyl serine. Hyperglycemia, along with other metabolic and hemodynamic factors, induces an elevation in DAG, which activates the PKC pathway. PKC plays an important role inthe regulation of endothelial permeability, vasoconstriction, cell growth, angiogenesis, and leukocyte adhesion [79]. Indirectly, high glucose-induced reactive oxygen species (ROS) contribute to vascular dysfunction *via* a PKC-dependent activation of nicotinamide adenine dinucleotide

phosphate (NADPH) oxidase [80]. Multiple PKC isoforms have been documented to be involved in mediation of endothelial dysfunction in diabetic nephropathy. Based on experiments with isoform-specific knock-out mice and a specific PKC-β inhibitor (ruboxistaurin, a bisindolylmaleimide) treatment, PKC-α activation appears crucial for the development of albuminuria, whereas PKC-β activation appears to be mainly involved in mesangial expansion, basement membrane thickening and renal hypertrophy in the development of DN [81]. Activation of other PKC isoforms: PKC-δ and -ε by hyperglycemia-induced oxidative stress has also been reported in diabetic rat kidney [82], but the linkage of other PKC isoforms with diabetic vascular dysfunctions remains under investigation [83-85].

The Polyol Pathway—Hyperglycemia increases glucose metabolism *via* the polyol pathway, causing the accumulation of intracellular sorbitol. It has been suggested that elevated levels of sorbitol increase superoxide production, interfere with NO bioavailability and promote PGH_2 (prostaglandin H_2) /TXA_2 (thromboxane A_2) release [86], which disturbs regulation of arterial vasomotor responses. Hence activation of the polyol pathway may contribute to the development of microvascular dysfunction in diabetes mellitus. Inhibition of aldose reductase, a key enzyme in the polyol pathway, attenuated proteinuria, decreased GBM thickening in diabetic rats [87] and reduced glomerular hyperfiltration in humans [88].

TGF-β (Transforming Growth Factor-β) Signaling—Hemodynamic alterations and hyperglycemia and its associated metabolic alterations stimulate secretion of inflammatory molecules, including TGF-β1, in diabetic animal models [89] and in patients [90]. Increased TGF-β1 promotes extra cellular matrixprotein accumulation in the vasculature [91], activates Smad (Mothers against decapentaplegic homolog) -2 and Smad-3 and is involved in angiogenesis by mediating the balance of the proangiogenic factor, VEGF, and antiangiogenic growth factor, thrombospondin-1 (TSP-1) [92]. TGF-β signaling may also interact with other signal pathways [93]. Various anti-TGF-β1 approaches are under investigation in clinical trials [94]. Hopefully they will lead to potential therapeutic innovations. However, activation of the TGF-β1-Smad signaling pathway also induces upregulation of eNOS in endothelial cells [95], which may be beneficial to ECs. Inhibition of TGF-β could be a double-edged sword in DN, due to its anti-proliferative and anti-inflammatory properties [96].

Impairment of eNOS/NO Bioactivity

There are multiple mechanisms by which hyperglycemia may impair NO production in renal ECs. Hyperglycemia-induced eNOS impairment leads to increased oxidative stress and scavenging of NO, which represents initiation event(s) for development of endothelial dysfunction [97]. There is increasing evidence of eNOS /NO dysfunction during DN [98, 99]. Patients with either type 1 or 2 diabetes exhibit abnormal endothelium dependent vasodilation [100]. Studies from our own group and others have demonstrated accelerated glomerular injury in diabetic mice with eNOS deficiency, which strongly supports a key role for eNOS dysfunction in the pathogenesis of DN [68a, 101]. Investigation in patients with type 2 DN suggested that certain eNOS gene polymorphisms are linked to eNOS function

and associated with advanced DN [102]. Nevertheless, there are conflicting reports for eNOS gene expression in animal diabetic models: with eNOS expression described to be either unchanged [63], diminished [64, 65] or increased [66, 67]. Regulation of eNOS bioactivity is multifactorial. In our study from glomeruli of *db/db* mice at 34 weeks, there was no significant change in eNOS monomer expression, but a significant decrease in the dimerized form [99]. Under physiological conditions, eNOS functions as a "homodimer". Coupled eNOS transports electron from a flavin-containing reductase domain to a heme-containing oxygenase domain. Homodimer uncoupling leads to superoxide anion (O_2^{-}) formation instead of NO production [103]. Optimal concentrations of the eNOS substrate, L-arginine and the co-factor tetrahydrobiopterin (BH_4) are essential to maintain eNOS dimerization. Active eNOS requires formation of a homodimer through a linkage between the N-terminal oxygenase domains; BH_4 stabilizes formation of the eNOS dimer, increases NOS affinity for L-arginine, and undergoes distinct redox transitions with the heme group [104]. When either the essential substrate, L-arginine, or the essential cofactor, BH_4, is limited, electron transfer from eNOS flavins becomes uncoupled from L-arginineoxidation, and superoxide is produced from the oxygenase domain [60, 103, 105], which may further reduce NO bioactivity and increase oxidative stress within endothelial cells by scavenging NO and forming peroxynitrite [106]. Insufficient L-arginine may link to onset of microalbuminuriain type 2 diabetic patients [107]; and long term L-arginine supplementation therapy has been shown to improve vascular function and glucose homeostasis instreptozotocin-induced diabetic rats [108] and diabetic patients [109]. Reduced BH_4 has been observed in diabetic rats [109a, 110], with more profound depletion in endothelial cells than plasma [109a]. Acute intraarterial infusion of BH_4 induced acute increases in forearm blood flow in response to endothelium-dependent vasodilators in patients with type 2 diabetes [111]. Sepiapterin is the immediate precursor of BH_4 *via* the biopterin salvage pathway, but is less sensitive to oxidative stress than BH_4 [112]. Our recent studies provided further evidence for a direct beneficial role of both arginine and BH_4 supplement, sepiapterin on GEnCs in DN, independent of vasodilation [99].

Post-translational regulation also plays a vital role in control of eNOS bioactivity due to its a long half-life at baseline (10–35 h) [113]. eNOS is subject to a variety of posttranslational regulatory mechanisms, including reversible enzyme acylation, regulation of subcellular localization, protein–protein interactions, S-nitrosylation and phosphorylation [114]. Our studies in both diabetic mouse glomeruli and high glucose-stimulated GEnCs demonstrated a decrease in eNOS phosphorylation at Ser1179 without a significant alteration at Thr497. Akt-dependent phosphorylation of eNOS at Ser1179 is critical for endothelium-dependent relaxation [115], while phosphorylation at Thr497 is considered inhibitory [116]. Reduced phosphorylation of eNOS at Ser1179 has previously been reported in moderately hyperglycemic diabetic rats [117]. These post-translation modifications may contribute to eNOS dysfunction.

eNOS activity is also regulated by its location within the cell. Under baseline conditions, eNOS predominately localizes to the plasma membrane, but it may traffic into the cytoplasm in response to certain stimuli. Microenvironments in cytoplasmic regions of the Golgi, the mitochondria and the nucleus may be less optimal for NO production, primarily due to

insufficient access to calcium-calmodulin [118]. Caveolin-1 (Cav-1) has been suggested to be an inhibitor of eNOS in some phenotypes of ECs; conjugated Cav-1-eNOS in caveolae maydecrease eNOS-dependent NO release [119]. eNOS activity may also be inhibited by its endogenous inhibitor, ADMA (Asymmetric Dimethylarginine). Elevated plasma ADMA has been found in patients with both types DN [71, 72, 120].

Oxidative Stress

The imbalance between NO and reactive oxygen species (ROS) generation is a central pathophysiologic denominator in diabetic endothelial dysfunction. High glucose increases ROS production in ECs [2], and reduces endogenous antioxidant systems [121], resulting in oxidative stress. The superoxide anion may interact with NO, generating peroxynitrite [122]. Peroxynitrite is increased in patients with type 2 diabetes [123] and diabetic mice [99] and peroxynitrite-mediated endothelial dysfunction has been reported in DN [124]. It is well known that hyperglycemia leads to increased AGEs and upregulates its receptor, RAGE. ROS stimulates the formation of AGEs [125]. Furthermore, ROS oxidizes BH_4 to an inactive metabolite, promoting eNOS uncoupling [126], in addition to inactivating prostacyclin synthetase [127].

Interaction of Renal ECs with Other Cells in DN

In kidney, ECs is in close contact with other renal resident and blood cells. Together, they not only provide a permeability barrier, but also act as multifunctional paracrine and endocrine regulators, coordinating immune responses, hemostasis, angiogenesis, extracellular matrix accumulation and modulation of blood flow and vascular tone (Fig. **2**). Growth factors, cytokines and diabetic vasoactive agents mediate the cross talk [128], including angiogenesis factors, such as vascular endothelial growth factor (VEGF) and pro- or anti-inflammatory cytokines, such as tumor necrosis factor (TNF)-α.

Cross-Talk of GEnCs with Podocytes and Mesangial Cells—The glomerular filtration barrier is a multicomponent apparatus [129]; renal glomerular capillaries consist of three layers: a fenestrated endothelium, the intervening glomerular basement membrane, and podocytes. Intraglomerular mesangial cells are located in the interstitium between GEnCs (Fig. **2A**). Podocytes generate several angiogenic growth factors, such as vascular endothelial growth factor (VEGF-A), and Angiopoetin-1 (Ang-1) [130], while GEnCs express corresponding receptors. There are several members (at least A, B, C, D) of the VEGF family; VEGF –A appears to play the major role in podocyte-EC interactions. Podocyte derived VEGF–A regulates GEnCs function mainly *via* paracrine action [131], although the contribution *via* autocrine VEGF–A signaling to normal barrier function cannot be completely excluded. Elevated VEGF–A has been reported in the initial phases of diabetes [132], while a subsequent decrease has been documented in numerous human or animal studies [133-135].

Elevated VEGF has been shown in a diabetic mouse model with NOS deficiency [136], while NO attenuated VEGF–A induced endothelial proliferation [137]. This condition of low NO bioavailability associated with high VEGF-A expression was termed as “uncoupling of VEGF-A with NO” [135]. Both activation and inhibition of the VEGF-A dependent

signal transduction cause functional defects of the renal glomeruli [138, 139]. Initial upregulation of glomerular VEGF-A expression may increase glomerular permeability, while the subsequent decreases in receptor-bound VEGF on the endothelium may underlie inability of effective capillary repair with more advanced disease [140]. Despite a few studies showing marked amelioration of albuminuria in somediabetic animal models from inhibition of VEGF activity by neutralizing antibodies or small molecule inhibitors of VEGF receptor kinase signaling [141, 142], VEGF displayed protective effects for glomerular microvasculature in diabetes overall; deletion of VEGF-A in type 1 diabetic mice promotes endothelial injury, accelerating the progression of glomerular lesion [143].

Another podocyte-derived angiogenesis growth factor, Ang-1, also contributes to the maintenance of the integrity of the glomerular filtration barrier [144]. Ang-2, from the same angiopoietin family, is also expressed in the kidney during development, but is significantly downregulated in adult [145]. Ang-2 is a competitive antagonist of Ang-1, since both of them share the same receptor in ECs [144]. Upregulated Ang-2 that stimulates vascular permeability has been reported in DN [146]. Imbalance of Ang-1/2 may underlie dysfunctional crosstalk between podocytes and GEnCs during DN.

Mesangial cells provide structural support for glomerular capillary loops and respond to capillary stretch, possibly playing an important role inregulation of glomerular flow and pressure. Hyperglycemia-activated mesangial cells are responsive to the increased EC-derived PDGF (platelet-derived growth factor), the major mediator between mesangial cells and GEnC [147]. It has been suggested that GEnC promote mesangial cell growth *via* a PDGF-Like substance [148].

Cross-Talk between Renal Proximal Tubular Cells (PTCs) and Endothelial Cells—Diabetic injury initially is detected in glomeruli, but the decline of renal function correlates with the degree of renal tubule interstitial fibrosis [149]. There is direct evidence of high glucose-induced collagen secretion by PTC [150]. Peritubular capillaries are essential for renal transport, reabsorption and oxygen supply to the tubules [151]. Recently, Tasnim *et al.*, investigated the interactions between renal tubular epithelial cells and adjacent endothelial cells in a co-culture system and found that primary cultured renal proximal tubular cells stimulated endothelial cells to express a functionally balanced combination of various factors, including VEGF, TGF-β along with its antagonist α2-macroglobulin and HGF (Hepatocyte growth factor). In turn, endothelial cells appeared to promote survival, proliferation and differentiation of the proximal tubule cells [152].

Interaction of ECs with Inflammatory Cells—Diabetes has been recognized as an inflammatory process [153]. Vascular cell adhesion molecule-1 (VCAM-1) was found to be upregulated in patients with DN [154], which may promote the adhesion of inflammatory cells to the endothelium and recruit circulating immune cells into the diabetic kidney. Renal tissue macrophages, T cells, and neutrophils, along with reactive oxygen species, pro-inflammatory cytokines, metalloproteinases, and growth factors, modulate the local response and promote inflammation and fibrosis within the diabetic kidney [153].

EC-Pericyte Communication—Pericytes are vascular mural cells embedded within the vascular basement membrane of blood microvessels (Fig. **2B**). In the kidney, they are localized in the tubulointerstitial space on peritubular capillaries. Mesangial cells are categorized as specialized pericytes within the glomerulus [155]. These cells closely contact with the endothelium, regulating vascular development, stabilization, maturation, and remodeling. Endothelial–pericyte communication largely depends on growth factors, including TGF β, angiopoietins, PDGF, spingosine-1-phosphate, and Notch ligands along with their respective receptors [156]. Among them, PDGF receptor-β (PDGFRβ) is critically involved in pericyte recruitment and proliferation [157]. It has been suggested that pericytes play a central role in diabetic complications. Loss of pericytes is one of the first observable changes in diabetic retinopathy, ultimately followed by increased vascular permeability [158]. Knockout of PDGF-B or PDGFR-β results in deformity of pericyte-like mesangial cells, leading to defective glomerulogenesis and glomerulosclerosis [159]. Duffield *et al.*, recently described renal pericytes as a major source of myofibroblast precursors in the kidney [54, 155].

In summary, high glucose along with its metabolites and other stimuli activate various pathways *via* similar mechanisms in different cell types of the kidney; induce numerous growth factors, cytokines, ROS generation and eNOS impairment, leading to renal EC dysfunction. The injured endothelium acts as an active signal transducer for metabolic, hemodynamic and inflammatory factors that modify the function and morphology of the vessel wall and interacts with adjacent cells. The self-protective mechanisms in response to oxidant, chemical, and shear stress may in turn produce a cascade of factors that promote inflammatory, proliferative and profibrotic responses in progressive diseases. Hence EC dysfunction is considered as a potential contributor in the progression of DN [160] (Fig. **1**).

POTENTIAL EC REGENERATIVE THERAPIES IN DN

With the development of regenerative medicine, studies have suggested potential strategies for EC regenerative therapy in DN [161]. In addition to the mechanism-based pharmacological and growth factors (or their inhibitors) therapeutic innovation mentioned previously, stem/progenitor cellular strategies may prove to be effective approaches to regeneration of ECs. Rat experiments have suggested that bone marrow-derived endothelial progenitor cells may participate in glomerular endothelial cell turnover [162]. However, the effect of endothelial progenitor cells in long-standing asymptomatic type 1 diabetic patients remains inconclusive [163]; it provides hope, but its long term safety and beneficial impact need to be cautiously evaluated.

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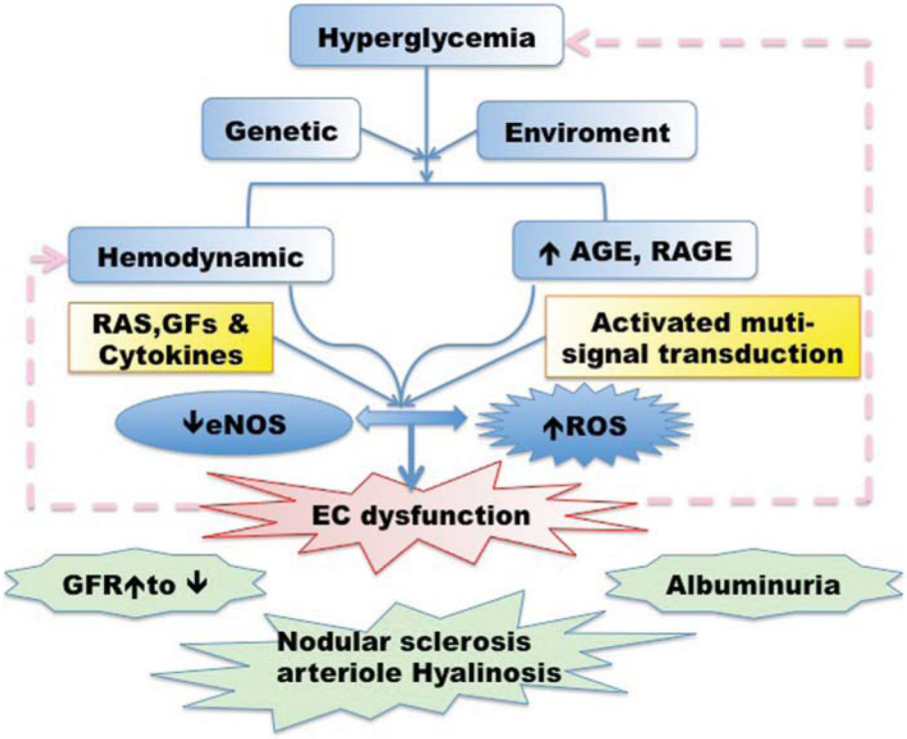

Fig. (1). Pathogenesis of Renal EC dysfunction during DN

Hyperglycemia, along with its metabolites and other stimuli, activates various signaling pathways and induces numerous growth factors, cytokines, ROS generation and eNOS impairment, leading to renal EC dysfunction. Injured ECs may further contribute to the progression of DN in turn. RAS: renin-angiotensin System; GFs: growth factors; eNOS: endothelial Nitric Oxide; AGE: advanced glycation end products; RAGE: the receptor of AGE; ROS: reactive oxygen species; GFR: glomerular filtration rate.

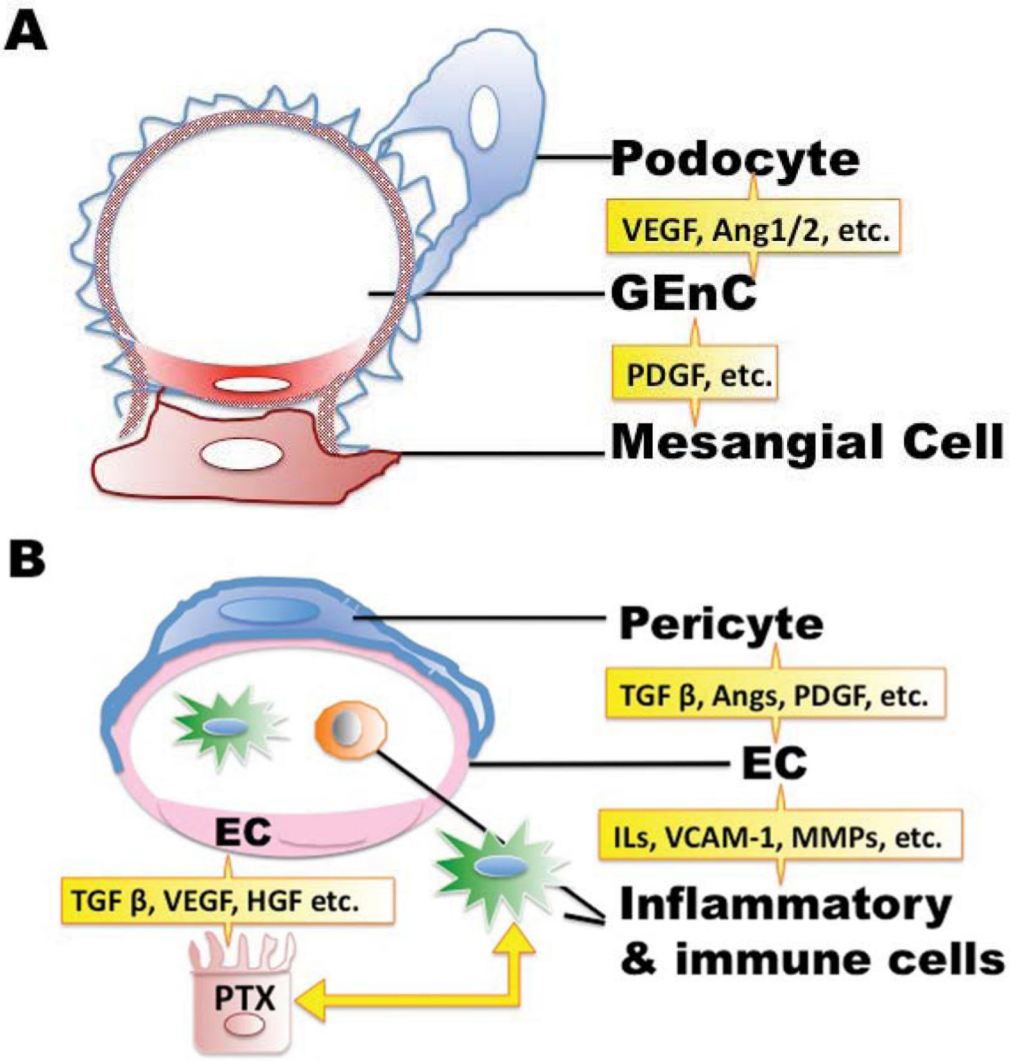

Fig. (2). Interaction of renal ECs with adjacent cells

A. In glomeruli, GEnCs are surrounded by the interdigitated foot process from podocytes. Glomerular mesangial cells (glomerular pericytes) are located in the area between GEnCs. The injured endothelium serves as an active signal transducer for metabolic, hemodynamic and inflammatory factors that modify the function and morphology of the vessel wall and interacts with adjacent cells. Growth factors involved in this crosstalk include VEGF:Vascular endothelial growth factor; Ang: Angiopoietins and PDGF: Platelet-derived growth factor. **B**. Pericytes are embedded within the vascular basement membrane of blood microvessels. Circulating and renal resident inflammatory/immune cells, pericytes and renal ECs interact each other, influence to effect tissue repair/fibrosis processes. Pro-inflammatory, pro-fibrotic and adhesive cytokines, metalloproteinases, and growth factors mediate this communication.VCAM-1: Vascular cell adhesion molecule-1, ILs: interleukins, MMPs: Matrix metalloproteinases. PTX: proximal tubular cell, HGF: hepatic growth factor.