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Endothelin and the Glomerulus in Chronic Kidney Disease

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Summary

Endothelin-1 (ET-1) is a 21-amino acid peptide with mitogenic and powerful vasoconstricting properties. Under healthy conditions, ET-1 is constitutively expressed in all cells of the glomerulus and participates in homeostasis of glomerular structure and filtration function. Under disease conditions, increases in ET-1 are critically involved in initiating and maintaining glomerular inflammation, glomerular basement membrane hypertrophy, and injury of podocytes (visceral epithelial cells), thereby promoting proteinuria and glomerulosclerosis. Here, we review the role of ET-1 in the function of glomerular endothelial cells, visceral (podocytes) and parietal epithelial cells, mesangial cells, the glomerular basement membrane, stromal cells, inflammatory cells, and mesenchymal stem cells. We also discuss molecular mechanisms by which ET-1 – predominantly through activation of the ET_A receptor - contributes to injury to glomerular cells and review preclinical and clinical evidence supporting its pathogenic role in glomerular injury in chronic renal disease. Finally, the therapeutic rationale for endothelin antagonists as a new class of antiproteinuric drugs is discussed.

Keywords

chronic kidney disease; endothelin; endothelin receptor antagonists; ERA; albuminuria; proteinuria; podocyte; glomerulus; GFR; blood pressure; FSGS; mesangial cells; epithelial cell

Glomerular injury is the underlying cause of the majority of forms of chronic kidney disease, resulting in progressive loss of glomerular filtration function.^{1, 2} Two of the most prevalent diseases in the general population, diabetes and arterial hypertension, represent the main causes underlying chronic kidney disease (CKD) development.³ Pathological mechanisms of CKD involve cellular injury, inflammation, podocyte effacement, hypertrophy of the glomerular capillary, proteinuria, and sclerosis of the glomerulus.²⁴ Untreated, CKD leads to progressive loss of functional nephrons and finally to end-stage

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renal disease (ESRD).⁵ In the United States and in other parts of the world, the incidence of ESRD continues to rise.^{6,7} Moreover, CKD worsens overall health status as the loss of glomerular and renal function aggravates preexisting cardiovascular risk factors;² these include arterial hypertension and compensatory dyslipidemia due to glomerular loss of lipoproteins.^{6,8} Accordingly, patients with ESRD carry a high risk of coronary artery disease and myocardial infarction and most of them die from cardiovascular complications.⁹⁻¹¹

Cellular Targets in Chronic Kidney Disease

Glomerular endothelial cells of the renal capillary (Figure 1) represent the first barrier of cells involved in filtration function of glomerulus.^{12, 13} The glomerular basement membrane (GBM) is the second barrier of filtration in glomerulus. It is a product of fusion of materials secreted by endothelial and epithelial glomerular cells and has a complex structure. Similar to endothelial cells, the GBM is negatively charged, thereby enabling charge selectivity in the filtration process. Podocytes (glomerular epithelial cells) are the third and the last barrier to filtration in glomerulus (Figure 2) and essential for maintaining glomerular function.^{14, 15} Podocytes extend numerous foot-shaped processes away from the main cell body to surround individual capillary loops that are attached to the GBM (Figure 1). Glomerular mesangial cells provide structural support for the glomerular capillary loops and are surrounded by a matrix consisting of fibronectin, laminin and glycosaminoglycans.¹⁶ Glomerular mesangial cells possess contractile properties and when exposed to vasoactive agents modulate glomerular filtration. Proliferation of glomerular mesangial cells, which also show immunomodulatory properties,¹⁷ is a hallmark of glomerular injury progression.^{18, 19} Glomeruli also contain a population of resident mesenchymal stem cells (multipotent renal progenitor cells) which are the major progenitors of scar-forming cells termed myofibroblasts and involved in cell turnover during renal injury.^{20, 21} Parietal epithelial cells within the glomerulus, which form the outer wall of Bowman's capsule (Figure 1),²² are now thought to function as a second barrier to protein by interacting with the GBM.²³ Proliferation and matrix deposition of parietal epithelial cells, of which different subpopulations have been identified,²⁴ have also been implicated in glomerulosclerosis.

The Endothelin System

Endothelin-1 (ET-) is the most potent and long-lasting vasoconstrictor known, being 100-times more potent than noradrenaline.²⁵ ET-1 was detected as a peptidergic vasoconstrictor activity in the supernatant of endothelial cells by two laboratories in the United States 30 years ago.^{26, 27} The sequence encoding the gene and a 21-amino acid peptide designated endothelin (ET) because of its endothelial origin were published in 1988 by Yanagisawa et al.,²⁸ and its two receptors (ET_A and ET_B) were identified shortly thereafter (reviewed in ²⁹). ET-1 is the predominant and biologically most relevant isoform of the ET peptide family (comprising ET-1, ET-2, and ET-3).²⁹

In the vasculature, ET_A receptors predominantly mediate vasoconstriction and mitogenesis, whereas ET_B receptors mediate mainly vasodilation and inhibition of growth and inflammation (reviewed in ²⁹). ET_B receptors can therefore be considered as endogenous

“antagonists” of the effects mediated by ET_A receptors.³⁰ Endothelins can be viewed as ubiquitously expressed stress-responsive regulators working in a paracrine and autocrine fashion.²⁹ Endothelins exert a number of physiological functions, including natriuresis, glucose homeostasis and regulation of immune cell function.^{29, 31} In the healthy vasculature and kidney, via activation of ET_A receptors, endogenous ET-1 elicits basal (“tonic”) vasoconstriction and contributes to normal vascular smooth muscle cell growth;³¹ thus, excessive production of ET-1 facilitates the development of arterial hypertension.³¹ Specific activities of ET receptors are discussed by Davenport and Maguire³², while components and functions of the ET system³³ are discussed in more detail by Kohan³³ and Benigni³ this issue. In the following section, we will review the expression, regulation, and function of ET-1 and its receptors in glomerular cells and address their function in glomerular diseases.

Glomerular Activities of Endothelin in Health and Disease

Glomerular Endothelial Cells

Endothelin was originally isolated from aortic endothelium, and endothelial cells of conduit arteries were initially considered as one of the primary sources of ETs.²⁸ In glomeruli, ET-1 was found in the endothelium of capillary loops (Fig. 1) and even though other glomerular cells were also shown to produce ET-1, endothelial cells emerged as the principal site of ET-1 secretion in normal human glomeruli.³⁴ Initial studies using in vivo binding of radioactively labeled ET-1 revealed that, at the electron microscopic level, ET-1 binding was specifically localized to the fenestrated endothelial cells of glomerular capillaries.³⁵ Early studies have suggested that ET receptor binding to fenestrated endothelial cells involves mainly ET_B receptors,³⁶ but since the design of autoradiographic studies does not allow reliable discrimination between receptor subtypes, additional evidence was needed. Initial findings were confirmed by immunofluorescence microscopy which revealed strong ET_B receptor immunoreactivity on glomerular endothelial cells.³⁷ It is now clear that endothelial cells express exclusively ET_B and serve as a major source of ET-1 in glomeruli. ET_B receptor signaling is strongly associated with production of NO and vasorelaxation.³¹ We previously proposed that ET-1 participates in the crosstalk between glomerular endothelial cells and neighboring cell types within the glomerulus¹⁴ (Figure 2). A similar concept was recently introduced for the pathogenesis of diabetic CKD.³⁸

Glomerular Basement Membrane

Only few studies have addressed the role ET as modulator of structure of function of the glomerular basement membrane (Fig. 1), both under physiological as well as under disease conditions. In the aging glomerulus, which is characterized pathologically by focal-segmental glomerulosclerosis (FSGS), hypertrophy of the GBM (Figure 3) and an increase in ET-1 expression has been found in laser-dissected glomeruli.³⁹ GBM structure and function depends on neighboring cells; podocyte injury, which will be discussed below, results in abnormalities in glomerular basement membrane matrix accumulation.⁴⁰ Accordingly, in cases in which regression of proteinuria and recovery of podocytes occurs, a regression of GBM thickening can be observed.^{39, 41, 42} Pharmacological approaches to induce regression of GBM thickening include ET receptor antagonists (ERAs) (Figure 3).³⁹

Parietal Epithelial Cells

In FSGS, parietal epithelial cells (PEC) (Figure 1) migrate to the glomerular tuft, produce Bowman's basement membrane matrix proteins and represent the predominant cell type in glomerular crescents.²⁴ The involvement of the ET system in this process has not been well studied. ET increases intracellular calcium concentration ($[Ca^{2+}]_i$) and contraction in freshly isolated parietal sheet of Bowman's capsule,⁴³ but this effect was mediated by myoepithelial cells, which are specialized smooth muscle cells. Analysis of the transcriptional landscape of glomerular PEC did not identify genes of components of the ET system among differentially expressed genes when capsulated (PEC-enriched) and decapsulated (PEC-derived) preparations were compared.⁴⁴ Whether different subpopulations of PEC synthesize and secrete ETs, what types of ET receptors they express, and whether ET-1 is involved in PEC migration to the glomerular tuft, and their contribution to glomerular diseases remains to be determined. Taking into consideration that PECs are emerging as important contributors to the pathogenesis of focal segmental glomerulosclerosis,⁴⁵ the analysis of activity of ET system in PECs may be an important avenue of investigation.

Visceral Epithelial Cells (Podocytes)

Visceral epithelial cells (podocytes) form the structural basis for the glomerular protein filter and embrace the glomerular capillary with a network of foot processes (Figure 1).⁴⁶ Podocyte functions include stabilisation of the capillary tuft and barrier filter function for proteins; other functions include cell-to-cell communication, turnover of the basement membrane of the glomerular capillary, and immunological functions.^{40, 47, 48, 49} Podocytes are not static cells, but are able to change their shape within minutes, causing swelling or retraction of their foot processes, or forming intercellular junctions.^{50, 51} Although thought to represent terminally differentiated, quiescent epithelial cells, podocytes can proliferate under certain conditions and migrate following injury.^{14, 40, 52} Podocyte injury is evident in numerous renal pathologies, including diabetic nephropathy, hypertensive nephropathy, autosomal dominant FSGS, and FSGS-human immunodeficiency virus nephropathy (HIV nephropathy).^{40, 47, 48}

Proteinuria may occur in the presence and absence of podocyte effacement.⁵³ The podocyte actin cytoskeleton is essential to provide structural support of cells but also contributes to podocyte signaling (Figure 2).^{54, 55, 56} Re-organization of the actin cytoskeleton in podocytes represents a stress-sensitive response pattern,⁵⁷ and podocyte loss or injury results in uncoupling of podocyte-specific proteins from the actin cytoskeleton.^{58, 59} Podocytes bind ET-1,⁶⁰ express ET receptors and prepro-ET-1, and respond with apoptosis and changes in their actin cytoskeleton following exposure to ET-1.^{39, 61, 62} Angiotensin II, which stimulates ET-1 formation in the renal cortex,⁶³ promotes podocyte actin cytoskeleton disruption and albumin permeability^{59, 64, 65} and causes podocyte apoptosis *in vitro*;⁶⁶ virtually identical effects on podocyte actin cytoskeleton disruption have been observed in response to ET-1.^{39, 67, 68} Exposure of cultured podocytes to protein stimulates ET-1 formation which, in turn, increases glomerular permselectivity for proteins, an effect that is regulated in an ET_A-receptor dependent manner.^{67, 68}

ET-1, as well as elevated glucose concentrations (and important causal factor in diabetic nephropathy), have been identified as factors underlying podocyte actin cytoskeleton disassembly, podocyte depletion and apoptosis,^{39, 67, 68, 69, 70} mechanisms that have all been implicated in diabetic nephropathy development. While in the healthy kidney senescent podocytes are shed in the urine, in diabetic nephropathy viable podocytes are also shed.^{62, 71, 72, 73} A causal role for ET in this process is likely, as recombinant ET-1 triggers nephrin shedding from podocytes which can be blocked by ET_A receptor antagonists.⁷⁴ Thus, in diabetics, glomerular podocyte loss likely occurs at least partly via ET-1. Loss of podocytes has also been observed in obese diabetic patients diagnosed with obesity-associated FSGS glomerulopathy.⁷⁵ That ET-1 – via ET_A receptors – plays a causal role in injury and depletion of podocytes in the pathogenesis of FSGS (and thus represents a target of prevention and therapy in chronic kidney diseases) is reinforced by a recent elegant study from Bottinger and associates.⁷⁷ These investigators found that podocyte-endothelial cell cross-talk – which we proposed several years ago as a possible mechanisms underlying ET-1 dependent glomerulosclerosis⁷⁶ – is present in FSGS, and that podocyte depletion is mediated via an ET-1/ET_A-dependent mechanism that involves mitochondrial oxidative stress in glomerular endothelial cells.⁷⁷ Recent research from Benigni's group further supports a role for ET_A receptor-dependent podocyte loss in adriamycin FSGS that can be rescued by ERA treatment^{78, 79} in line with our previous observations in aging-dependent FSGS.^{30, 39}

Glomerular Mesangial Cells

The ET system has been studied in detail in glomerular mesangial cells (GMC) (Figure 1). GMC express both types of ET receptors, but the pathological effects of ETs are mediated in large part through activation of ET_A receptors. Endothelin action in renal mesangium contributes to the development of multiple renal pathologies. Effects of ET-1 in GMC include stimulation of proliferation, triggering contraction and promoting synthesis of ECM proteins (Figure 4). Regulation of ET-1 gene expression in GMC and synthesis of ET-1 are well studied. Thrombin and cytokines (TNF and IL-1) synergistically increase ET-1 expression in GMC, and this process requires activation of p38 MAPK and PKC, but not ERK, JNK/SAPK, or intracellular Ca²⁺ release.⁸⁰ The ET-1 promoter in GMC is activated by phorbol myristate acetic acid or ectopic expression of PKC-β1.³⁴ GMC proliferation is essential for the development of focal or diffuse glomerulosclerosis as shown in rat models of CKD and acute mesangioproliferative glomerulonephritis.¹⁸ ET-1 is a potent mitogen for mesangial Cells both *in vitro* and *in vivo* and also stimulates GMC secretion of PDGF, one of the major regulators of mesangial cell proliferation.⁸¹⁻⁸⁴ Furthermore, ET-1-triggered transactivation of the EGF receptor (EGFR) contributes to ET-1 mitogenic activity.⁸⁵⁻⁸⁷

ET-1/ET receptor-dependent signaling pathways in GMCs are summarized in Figure 4. Members of the mitogen-activated protein kinase (MAPK) family are among the most thoroughly studied signaling molecules controlling multiple and diverse cellular functions. In GMC, ET-1 stimulates three major MAPK: ERK,⁸⁸ JNK/SAPK,⁸⁹ and p38 MAP kinase⁹⁰ as well as the less studied ERK5.⁹¹ As is the case with other ligands of G-protein coupled receptors, ET-1 signaling in GMC is mediated by a number of adaptor proteins which contain several classes of protein-binding domains. Among the best-characterized

adaptor proteins involved in ET-1 mitogenic signaling are *Shc* proteins which contain a number of protein-protein interaction domains. In GMC, three *Shc* isoforms are expressed: p46*Shc*, p52*Shc* and p66*Shc*. The p52*Shc* isoform is important for ET-1-mediated regulation of Ras and ERK activation.⁹² In ET-1-treated GMC, the continued tyrosine phosphorylation of p52*Shc* causes the lengthy association of p52*Shc* with the adaptor protein Grb2, usually responsible for coupling receptor tyrosine kinases with activation of Ras. The sustained ET-1-induced interaction between tyrosine-phosphorylated p52*Shc* and Grb2 SH2 domain results in biphasic Ras activation by the GEF Sos in GMC. It appears that the biphasic activation of Ras by ET-1 sequentially activates the ERK cascade and phosphatidylinositol 3-kinase in GMC.⁹²

The importance of ET-1-mediated regulation of cell cycle regulatory proteins for the proliferative effect of ET-1 was confirmed by inhibition of its mitogenic effect using antisense oligonucleotides directed against cyclin D1 and by overexpression of a nonphosphorylatable form of pRb.⁹³ *Src* family cytoplasmic tyrosine kinases contribute significantly to ET-1 mitogenic signaling in GMC and ET-1 induced expression of cell cycle signaling molecule cyclin D1 by signaling via *Src*.⁹⁴ Downregulation of p27kip1, expression of which is under control of transcription factor FOXO3a, results in cell cycle progression into S phase. ET-1 inactivates FOXO3a, resulting in downregulation of p27kip1 and progression of the cell through the cell cycle, which is dissimilar from Akt-mediated FOXO3a phosphorylation (Figure 4). This pathway utilizes the scaffolding activity of a guanine exchange factor GEF βPix. βPix, in addition to mediation of ET-1-induced GTP-loading of Cdc42⁹⁵, also participates in regulation of ET-1 signaling due to its scaffolding activity.⁹⁶ Short exposure of GMC to ET-1 induces serine phosphorylation of p66*Shc* isoform through Gai3^{85, 97} and increases β1Pix GEF activity.⁹⁵ Prolonged exposure to ET-1 increased p66*Shc* and βPix expression, followed by formation of multiunit signaling complex between p66*Shc*, β1Pix and FOXO3a.⁹⁸ Notably, shRNAs against either βPix or p66*Shc* are effective inhibitors of ET-1-induced GMC proliferation. We have also found that expression of β1Pix induced FOXO3a phosphorylation occurred through activation of Rac1, ERK1/2 and p66*Shc*. β1Pix-mediated FOXO3a phosphorylation and p27kip1 downregulation required p66*Shc* but was independent of Akt in GMC. Correspondingly, depletion of βPix prevented p27kip1 downregulation induced by ET-1.⁹⁸

Contraction of GMC, due to their location between glomerular capillaries and their supportive function (Figure 1), affects the efficiency of glomerular filtration. ET-1 lowers glomerular ultrafiltration coefficient (Kf) and contracts mesangial cells, at least partially through stimulation of inositol triphosphate (IP3) generation and elevation of [Ca²⁺]_i.⁹⁹ The early transient peak in [Ca²⁺]_i is due to IP3-released Ca²⁺ from intracellular sources, which accompanies cell alkalization through augmented Na/H exchange.^{100, 101} The continued plateau of [Ca²⁺]_i reflects the increased influx of Ca²⁺ across the plasma membrane, is independent of dihydropyridine-sensitive Ca²⁺ channels and is likely mediated through ET_A receptor activation.^{99, 102, 103} Cultured GMC possess L-type Ca²⁺ channel activity¹⁰⁴ and also express Ca²⁺-permeable cation channels from the family of canonical transient receptor potential (TRPC), including TRPC1.¹⁰⁵ Low concentrations of ET-1 (0.1-10 pM) cause a slow sustained increase in [Ca²⁺]_i mediated by Ca²⁺ influx through a voltage-channel-

independent mechanism, while higher ET-1 concentrations (100 pM and higher) cause a rapid and transient increase of $[Ca^{2+}]_i$ which is dependent on Ca^{2+} release from intracellular stores and requires activation of PLC and PKC.¹⁰¹ Activation of several protein kinase C isoforms, either PKC- α , - δ , or - ϵ , but not - ζ , are required for ET-1-stimulated contraction of GMC.¹⁰⁶ The ET-1-induced decrease in glomerular filtration rate *in vivo* was abolished by infusion of a TRPC1 antibody that targets an extracellular domain in the pore region of TRPC1 channel,¹⁰⁵ suggesting that the TRPC1 channel is important for ET-1-mediated GMC contraction.

There is evidence to suggest that tyrosine phosphorylation is also important role for GMC contraction; the contractile responsiveness of GMC could also depend upon activation of the p38 MAP kinases.⁸⁴ ET-1 activated Src kinases¹⁰² and ET-1-induced mobilization of $[Ca^{2+}]_i$ activates calcium regulated cytoplasmic proline-rich tyrosine kinase Pyk2 in GMC,⁹⁰ which appears to be important for activation of p38 MAPK. The role of p38 MAP kinase in cell contraction is likely due to its ability *in vivo* to activate MAP kinase activated protein kinase 2/3 which could in turn phosphorylates small heat shock protein 25 (HSP 25). Since small HSPs are involved in modulation of polymerization/depolymerization of F-actin, p38 MAP kinase could be considered as a player in regulation of GMC contractility.⁸⁴ As a rule, activation of major MAP kinase cascades requires GTP-loading of distinct small G proteins achieved by activity of particular guanine nucleotide exchange factors (GEF). In human GMC, ET-1, acting via ET_A receptors, induces Pyk2-mediated GTP-loading of the small GTPase Rap1 by promoting interactions between the scaffolding protein p130Cas and the GEF BCAR3.¹⁰⁷

The Pyk2/p130Cas/BCAR3/Rap1 signaling cascade activated via ET_A is involved in mesangial cell adhesion and spreading regulation.¹⁰⁷ Negative regulation of ET-1-triggered GMC contraction is mediated by prostaglandins (products of cyclooxygenase) and NO formation.³¹ ET-1 strongly inhibits cytokine induction of iNOS and formation of NO in cultured GMC, and this effect could be mediated via ET_A receptors by suppressing the expression of iNOS mRNA.^{108, 109}

Overexpression of another GEF - C3G was observed in renal mesangium after induction of the anti-Thy1 model of glomerulonephritis.¹¹⁰ Expression of C3G caused enhanced and prolonged Rap1 activation in response to ET-1 in rat GMC¹¹⁰ but not in immortalized human GMC.¹⁰⁷ In primary GMC of rat origin, C3G overexpression led to significant changes in cell spreading and migration patterns in response to ET-1 stimulation and also increased stress fiber formation, phenomena which were mimicked by Rap1A overexpression.¹⁰⁷ Thus, an increase in the C3G protein level contributes to the resolution stage of mesangioproliferative glomerulonephritis by modulating cellular motility and actin dynamics.¹¹⁰

ET-1, acting through ET_A receptors, promotes synthesis and accumulation of extracellular matrix proteins (ECM) in renal mesangium, one of the key features of progressive glomerular disease.^{82, 111-113} ET-1 increases fibronectin, type IV collagen and type I collagen synthesis by mesangial cells.^{82, 114} Analysis of the changes in mRNA transcripts in human GMC identified 122 genes regulated by ET-1.⁹⁴ Among the genes induced by

ET-1, as detected using Affymetrix U95 microarray platform, are genes encoding macrophage chemoattractant protein-1 (MCP-1) and IL-6.⁹⁴ It was suggested that ET-1-induced secretion of MCP-1 and IL-6 results in an autocrine signaling loop controlling ET-1-induced collagen accumulation in GMC, since these cytokines can increase collagen synthesis.¹¹² ET-1 can also stimulate GMC production of TNF α , ICAM-1 and VCAM-1, and thereby induce pro-inflammatory effects.¹¹⁵

Mesenchymal Stem Cells

The presence of a multipotent mesenchymal stem cell population (Figure 1) resident in human glomeruli has been reported.²¹ During injury, these cells can differentiate into myofibroblasts, which form the cellular basis of scar tissue.¹¹⁶ It is unknown whether these mesenchymal stem cells produce ET-1 or whether ET-1 has any effects on these cells.

Stromal Cells

The cells that populate the interstitial spaces surrounding developing nephrons and ureteric bud branches during kidney development are termed stromal cells (Figure 1).¹¹⁷ Forkhead family transcription factor Foxd1 is a specific marker of stromal cells and Foxd1⁺ renal stromal cells can give rise to multiple cell types within the kidney, including endothelial cells, vascular smooth muscle cells and mesangial cells.¹¹⁸ The presence of stromal cells in the adult glomerulus has not been unequivocally shown, however there could be stromal cells either existing in the mesangium or that get established after injury/recovery. Infused multipotent stromal cells from human marrow localized to glomeruli of diabetic mice and differentiated into glomerular endothelial cells.¹¹⁹ Stromal cells may differentiate into megakaryocytes - large bone marrow cells¹²⁰ which express both ET receptors.¹²¹ ET-1 modulates megakaryocyte growth.¹²¹ At present, it is unclear whether ET-1 has a role in promoting glomerular homing of stromal cells or modulating their differentiation into glomerular cells.

Immune Cells

ET-1 plays a role in the pathogenesis of infectious diseases and immune processes.^{122, 123-128} Macrophages are prominent participants in glomerular injury and proliferative glomerulonephritis¹²⁹; these cells synthesize and secrete ETs.^{130, 131} ET-1 stimulates the aggregation and accumulation of neutrophils;¹³² myeloperoxidase activity can be inhibited by blocking ET_A receptors.¹³³ In chronic kidney disease, ET-1 has been shown to play an essential role in propagating glomerular inflammation, including accumulation of leukocytes, since these processes are inhibited by ET_A receptor blockade.¹³⁴⁻¹³⁶ In a rat model of mesangial proliferative glomerulonephritis, the infiltrating macrophages were the principal cells expressing ET-1 in glomeruli on day 1 after onset of disease.⁸¹ In contrast to synthesis and secretion of ET-1 by infiltrated macrophages, ET-1 signaling and actions in immune cells which have infiltrated glomeruli have not been well studied.

Summary and Clinical Implications

Preclinical and clinical evidence suggests a causal role for the ET-1/ET_A receptor pathway in the pathogenesis of glomerular injury in proteinuric CKD.^{2, 137} Preclinical and recent

clinical data suggest that ERAs offer therapeutic efficacy even on top of currently approved anti-proteinuric drugs such as ACE-inhibitors (ACEi) and/or angiotensin receptor blockers (ARBs).¹³⁸⁻¹⁴⁴ Thus, ERAs with a reasonable clinical safety profile and with carefully monitoring of the transient fluid retention (an ERA drug class effect^{2, 137}) may become a promising approach for the treatment of patients with CKD.¹⁴⁵ Large-scale clinical trials have shown that inhibitors of the renin-angiotensin-aldosterone system (RAAS) such as ACEi or ARBs slow the progression of chronic renal disease. By largely pressure-independent mechanisms, these drugs not only delay the progression of glomerular injury, but may even reverse proteinuria in patients with chronic proteinuric nephropathies.¹⁴⁶⁻¹⁴⁹ Experimental studies suggest that this regression of glomerular injury involves podocyte repopulation.¹⁵⁰ Preclinical studies have repeatedly demonstrated that regression of glomerular and podocyte injury in models of CKD can be achieved by ERAs targeting ET_A receptors (Figure 3),^{7, 39, 76, 136, 151-153} effects that are accompanied by an improvement of proteinuria. Studies also support the notion that ERAs may act, at least in part, by interfering with glomerular inflammation.^{135, 136} Most recent experimental evidence suggests that adjuvant use of ERAs prior to renal artery balloon angioplasty may improve renal function and proteinuria and accelerate structural recovery from glomerulosclerosis due to renal artery stenosis.¹⁵⁴ Additional clinical studies are warranted to explore whether the potential benefits of ERAs as anti-proteinuric drugs translate into a clinical benefit and improve morbidity and mortality in CKD patients on top of RAAS inhibition. Such trials are currently underway.^{1, 2, 5, 137}

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Figure 1.

Artist's impression of the structure and cellular components of the glomerulus. The cell types, glomerular components, and vascular structures are indicated. Original pencil drawing by Elena Sorokina (2014).

Figure 2.

Schematic representation of structural components of the glomerulus and the actions and interactions of ET-1 (yellow) between the glomerular capillary basement membrane (GBM, orange), the glomerular endothelial cells (GEC, blue), the glomerular podocytes (grey) and the slit diaphragm (green). The drawing is based on a concept of intracellular glomerular communication adapted from Kalluri⁵³, and depicts the main ET-1 signaling mechanisms contributing to podocyte and glomerular injury as previously proposed by one of the authors⁷⁶. ET-1 is produced and released from cells on both sides of the GBM, namely glomerular endothelial cells (GEC) and podocytes and affects cellular components of the glomerular capillary. ET-1 released from GEC interacts with the GBM (1), with the slit diaphragm (2), and with podocytes (3). ET-1 release and signaling events may also occur in the reverse direction. Finally, ET-1 released from podocytes interacts with the GBM and vice versa (4). Within the podocyte, ET-1 activates ET_A receptors (ET_A-R), promoting inflammation, glomerular injury, and sclerosis through MAPKs p38 and p44/p42 pathways (5); ET also stimulates synthesis of the growth-promoter and cdk-inhibitor p21^{waf/cip1}, and pro-inflammatory NF-kappa B (6). ET-1, via ET_A receptors, also mediates disruption of the F-actin cytoskeleton (7) and promotes dysfunction of the slit diaphragm (green) involving activation of the Rho-kinase and PI₃-kinase pathways. Reproduced from Barton M. Therapeutic potential of endothelin receptor antagonists for chronic proteinuric renal disease in humans. *Biochim Biophys Acta – Molecular Basis of Disease*. 2010; 1802:1203-13, with permission of the publisher.

Figure 3.

Effects of treatment with an ET receptor antagonist on established, age-dependent development of focal-segmental glomerulosclerosis (FSGS). Shown are histologic (A, B) or electron microscopic (C-F) sections of glomeruli of aged rats with established FSGS, either untreated (left) or after 4 weeks of treatment with an ET_A receptor-selective antagonist (ERA, darusentan (right panels)). Compared with untreated rats, ERA treatment for 4 weeks resulted in partial reversal of renal aging. **A:** In untreated rats, moderate mesangial matrix expansion and hypertrophy of podocytes with enlarged nuclei, prominent nucleoles (arrow), and many intra-cytoplasmatic vesicles (open arrow) are visible, compatible with podocyte activation in response to injury. **B:** Following ERA treatment, glomerular injury was reduced, mesangial matrix expansion and podocyte hypertrophy and activation were milder compatible indicative of regression of glomerular aging (Bar, 50 μ m). Panels **C-F** show representative transmission electron micrographs of podocytes and glomerular basement membranes. **C:** Without treatment, glomerular basement membrane hypertrophy and injury and detachment of podocytes is visible. **E:** High-power micrograph indicating thickening of glomerular capillary basement membrane with podocyte detachment. Injury of podocytes is characterized by hypertrophy, inclusion of cytoplasmatic absorption droplets due to vacuolar degeneration, and diffuse effacement of foot processes. **D:** ERA treatment for four weeks was

associated with partial improved attachment of the podocyte to the basement membrane and complete regression of GBM hypertrophy of the glomerular capillary. **F:** High-power micrograph showing partial reversal of podocyte aging after ERA treatment. Treatment was associated with a reduction of podocyte injury and complete disappearance of intra-cytoplasmatic vesicles which is also evident in panel B. Reproduced from Ortmann J, Amann K, Brandes RP, Kretzler M, Münter K, Parekh N, Traupe T, Lange M, Lattmann T, Barton M. Role of podocytes for the reversal of glomerulosclerosis and proteinuria in the aging kidney after endothelin inhibition. *Hypertension* 2004; 44: 974-981, with permission of the publisher.

Figure 4.

ET signaling and actions in glomerular mesangial cells. Shown are signaling pathways involved in ET-1-mediated proliferation and contraction of GMC. Black lines indicate signaling processes, green arrows show translocation, red arrows specify inhibitory influence. ADAM, a disintegrin and metalloprotease domain secretase; BCAR3, breast cancer anti-estrogen resistance 3; βPix, PAK-interacting exchange factor β; CAM, calmodulin; caMKII, calcium-dependent protein kinase; DAG, diacyl glycerol; ET-1, endothelin-1; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FOXO3a, forkhead box O3; GMC, glomerular mesangial cell; Grb2, growth factor receptor-bound protein-2; IP3, inositol triphosphate; Mek, mitogen-activated protein kinase; p130Cas, Crk-associated substrate; p27Kip1, cyclin-dependent kinase inhibitor 1B; p38, p38 MAP kinase; p52Shc, p52 isoform of the SHC-transforming protein-1; p66Shc, p66 isoform of the SHC-transforming protein-1; PDGF, platelet-derived growth factor; PKC, protein kinase C; PLC, phospholipase C; Pyk2, protein tyrosine kinase 2; Raf, raf kinase; RAS, ras protein; Sos, son of sevenless homolog 1; Src, proto-oncogene tyrosine-protein kinase Src; TRPC, transient receptor potential canonical channel. P indicates phosphorylation, GTP indicates guanosine triphosphate.