Endothelial dysfunction in renal interstitial fibrosis

Heather M. Perry and **Mark D. Okusa**

Division of Nephrology and Center for Immunity, Inflammation, and Regenerative Medicine, University of Virginia Health System, Charlottesville, VA 22908

Abstract

Kidney disease affects millions of people worldwide and it is now widely accepted that many pathological processes may persist after AKI that can cause the progression to CKD. Tubulointerstitial fibrosis manifests soon after injury and while many cellular and molecular components of kidney fibrosis have been discovered, largely in animal models, new therapeutic strategies are still desperately needed. The renal endothelium has emerged as important in progression of fibrosis through regulation of hypoxia, inflammation, and cellular crosstalk. This review aims to highlight our current understanding of the role of the endothelium in interstitial fibrosis and to identify potential therapeutic targets.

Keywords

endothelium; kidney; myofibroblasts; pericytes; chronic kidney disease

Introduction

Progressive kidney fibrosis, a hallmark of chronic kidney disease, can be a consequence of maladaptive repair processes after acute kidney injury (AKI). The severity and type of injury dictates the ability for the kidney to recover function or progressively decline and activates the fibrotic pathway as part of the wound healing response. The clinical and pathophysiological phases of acute kidney injury (AKI) begin with early events that are initiated by endothelial dysfunction, microvascular injury, leukocyte adhesion, inflammation, oxidative injury, and tubular apoptosis/necrosis[1]. Renewal of endothelial function heralds the recovery phase of AKI and is necessary for tubule regeneration. Thus, the endothelium contributes to both the initiation of and recovery from AKI and preserving endothelial function could be key to attenuating progressive kidney damage and prevention of fibrosis.

Insults to the kidney trigger the endothelium to switch from a quiescent to an activated state which can lead to a cascade of pathways that contribute to fibrosis[2,3] (Figure 1) in mice and humans. Endothelial cell dysfunction can lead to increased vascular permeability, activation of complement, inflammation, vasoconstriction and capillary rarefaction. Microvascular congestion by leukocytes and RBCs, vasoconstriction, and capillary rarefaction can all result in tissue hypoxia. Hypoxia directly induces myofibroblast

Correspondence: Mark D. Okusa, MD, University of Virginia, PO Box 800133, Charlottesville, VA 22908, mdo7y@virginia.edu, Phone: 434-924-2187, Fax: 434-924-5848.

differentiation or may indirectly induce a pro-fibrotic response in tubule epithelial cells and/or leukocytes, all leading to progression of fibrosis[2]. Endothelial dysfunction (vasoconstriction and leukocyte adhesion molecule expression) is associated with renal tubule cell stress[4], which can lead to production of profibrotic factors. Additionally, endothelial dysfunction may direct pericytes (PC) or other interstitial cells to differentiate into myofibroblasts[5,6], promoting fibrosis. Conversely, accumulating evidence suggests that endothelial dysfunction can lead to pericyte activation[5], resulting in vascular instability and microvascular rarefaction. This review will summarize recent developments on the role of the endothelium in regulating vascular functions in health and in interstitial fibrosis following kidney injury.

Role of endothelium in pathophysiology of renal fibrosis

Hypoxia

Early studies highlighted the role of hypoxia in murine kidney fibrosis models. Vasoconstriction, in part regulated by the vascular endothelium, in response to injury can result in localized or general hypoxia in kidneys of humans and mice[3]. These hemodynamic disturbances, along with microvascular plugging of RBCs and leukocytes, lead to a concept described as the 'no-reflow' phenomenon in renal ischemia reperfusion injury (IRI), whereby areas of the peritubular capillary network exhibit a loss of blood flow during the reperfusion phase, further contributing to regional reductions in blood flow and presumed hypoxia[7]. Although total renal blood flow can return to baseline levels within 5 hrs of IRI, vascular reactiveness is altered for at least one week[8] suggesting that endothelial dysfunction may persist well after IRI and contribute to long term maladaptive processes.

Vascular rarefaction can also lead to hypoxia. Human kidney biopsies from patients with chronic tubule interstitial disease had a reduction in peritubular capillary density that was associated with tubule atrophy and increased interstitial fibrosis compared to normal kidneys[7]. Mouse models of interstitial fibrosis observed vascular rarefaction occurring as early as day 1 after severe IRI and 3 days after unilateral ureteral obstruction (UUO)[9]. Microvascular loss is apparent at 4 weeks after IRI[10], but can apparently return to sham levels 6 to 9 weeks after IRI when renal function was restored[11]. The extent and timing of vascular rarefaction likely depends on the type and severity of fibrosis model. The renal endothelium lacks regenerative capacity early after IRI, unlike the tubule epithelium, potentially due to a low level of angiogenic signals and endothelial proliferation[2,12]. Approaches to preserve the renal endothelium such as infusion of VEGF in pigs after established renovascular disease, prevented microvascular rarefaction, attenuated fibrosis & normalized renal blood flow and kidney function compared to controls[13], suggesting that targeting therapeutic interventions to promote endothelial function may be an effective strategy to reduce fibrosis.

Tissue inflammation

Endothelial cells are the gatekeepers to tissue inflammation. Hypoxia/reoxygenation, mechanical and cytokine stimuli can induce leukocyte adhesion molecule expression on the

endothelium promoting rolling, adhesion and transmigration of immune cells, and in particular innate immune cells such as neutrophils & monocytes. The precise role of the endothelium in specifically modulating adaptive immune cell infiltration and its consequence on fibrosis is unknown. Monocytes can differentiate to tissue macrophages which can promote inflammation, tubule repair and/or fibrosis[14]. Due to their cellular plasticity, the macrophage phenotype may switch depending on environmental milieu. Alternatively, resident macrophages may also have a role tissue recovery and progression of fibrosis. While many studies focus on the secreted factors by macrophages that can drive tissue fibrosis, less is understood about intracellular interactions within the renal microenvironment.

Crosstalk to pericytes

Emerging evidence demonstrates an important role for endothelial and pericyte crosstalk[15] in renal vascular homeostasis and disease. Under normal homeostatic conditions, a basement membrane separates the endothelium and pericytes, but through direct contact or indirect paracrine factors, communication is maintained between these two cell types[5]. In disease states, endothelial dysfunction may lead to pericyte detachment, proliferation and migration and vasculature lacking pericytes is unstable, prone to leakiness and can lead to microvascular rarefaction[16]. Activation of pericytes can also contribute to microvascular loss[16]. The specific cell-cell interactions and paracrine signaling pathways between endothelial cells and pericytes important for homeostasis as well as during disease states in the renal microvasculature have yet to be defined. Blockade of prosurvival factors such as VEGFR2 or PDGFRβ has been shown to reduce pericyte proliferation and differentiation, microvascular rarefaction, inflammation and fibrosis[16]. **The apparent discrepancy between these findings and that administration of VEGF protects against fibrosis**[13] **may be due to the complex signaling pathway including three VEGF receptors and the regulated expression of four distinct VEGF genes and multiple splice variants. Therefore, these studies cannot simply be explained by a binary ligand receptor interaction**.

Other kidney cells have also been shown to regulate capillary function. Atrophic epithelial cells can release profibrotic signals which may disrupt endothelial/pericyte interactions in peritubular capillaries, leading to disintegration microvascular rarefaction[17]. Endothelial crosstalk to other cells in the microenvironment, such as epithelial cells and immune cells, is largely unknown.

EndoMT

Endothelial to mesenchymal transition has gained some interest in kidney fibrosis yet; mouse models used for understanding the role of the endothelium in renal fibrosis can be non-specific, confounding results. The renal endothelium is thought to constitute a minor population to the myofibroblast pool[6]. However, the VE-cadherin-Cre (Cdh5-Cre) mouse used for lineage tracing endothelial cells in this study has been shown to have reporter expression in a subset of hematopoietic cells throughout embryonic and adult organs[18]. Many studies exploring the role of EndoMT in renal fibrosis have used the constitutive Tie2-Cre for linage tracing or deletion of specific factors in endothelial cells. For example, the

Tie2-Cre reporter colocalized with SMA after IRI[12] and inactivation of endothelial TGFβR signaling reduced fibrosis[19]. However, in the constitutive Tie2-Cre mouse, Tie2 has been shown to be non-specific for the endothelium and is expressed early in hematopoietic cell development[20,21] and potentially on pericyte precursors[22]. The lack of a truly specific marker for the endothelium for lineage tracing makes interpreting these studies difficult[23,24].

Targets to enhance endothelial recovery

Molecular mechanisms of endothelial recovery after AKI and prevention of fibrosis remain largely unknown in murine models of fibrosis. Insulin-like growth factor-1 receptor, a receptor important for cellular proliferation and differentiation, has been shown to be necessary for prevention of UUO induced fibrosis by preserving endothelial barrier integrity[25]. Administration of SS-31, a compound that preserves mitochondrial integrity, at the time of IRI protected against early endothelial injury, microvascular congestion and kidney injury. At 4 weeks after IRI, there was reduced capillary rarefaction and fibrosis in mice treated with SS-31[10] suggesting that preservation of endothelial mitochondrial integrity can prevent kidney injury and fibrosis. Recently, we used an inducible Tie2-Cre to delete sphingosine 1-phosphate 1 receptor (S1P1), a sphingolipid receptor that is essential for vascular function, in endothelial cells in a temporal manner during IRI[26]. With this approach, Cre activation is specific to the endothelium and is not activated in WBC or other kidney cells[20,26]. When the deletion of endothelial S1P1 was delayed either in the injury phase or recovery phase after a mild renal IRI, mice were unable to recover kidney function and exhibited severe injury and fibrosis within 9 days and lasted to at least 14 days. There was marked renal inflammation including neutrophils and macrophages. Furthermore, we demonstrated that S1P1 has a novel role in directly regulating endothelial leukocyte adhesion molecule expression, and suppression of these molecules is crucial to dampening inflammation and allowing for renal recovery. As FTY720, a FDA approved drug that activates S1P1, prevents endothelial dysfunction and kidney fibrosis in rats[27], these data suggest that activation of the S1P1 signaling pathway after AKI may help to prevent fibrosis.

Conclusion

The endothelium plays a critical role in maintaining normal vascular homeostasis and following injury endothelium contributes to repair processes leading to renal recovery. However following severe injury, maladaptive repair ensues leading to progressive fibrosis and loss of renal function. **Angiogenic growth factors, activating the sphingolipid receptor signaling pathway or preserving mitochondrial function may be attractive physiological and pathophysiological endothelial processes targetable for therapeutic intervention**.

Acknowledgments

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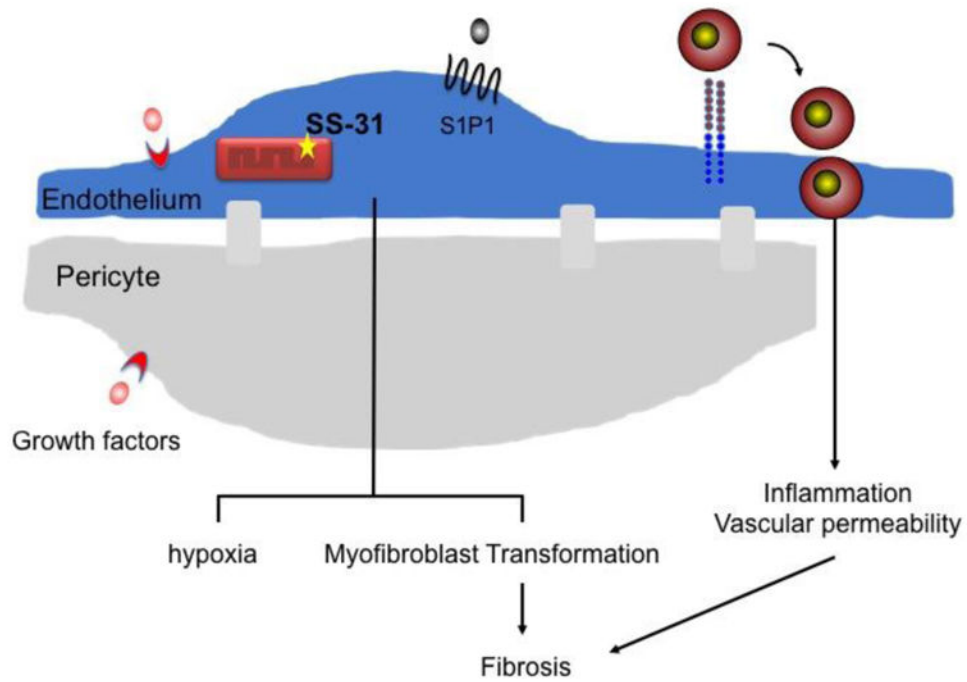
Figure 1.
Potential targets to prevent endothelial dysfunction and renal interstitial fibrosis. During progressive kidney fibrosis peritubular capillaries recruit inflammatory cells, have increased vascular permeability and vasoconstriction, and undergo rarefaction. Endothelial dysfunction may also lead to pericyte detachment, proliferation, migration and transformation to myofibroblasts. Strategies to enhance endothelial function by promoting survival or angiogenesis (VEGF121), preserving mitochondrial function (SS31), preventing leukocyte adhesion molecule expression and enhancing vascular barrier function (S1P1, IGF1R) may reduce fibrosis.