Commentary

Endothelial–Mesenchymal Transition as a Novel Mechanism for Generating Myofibroblasts during Diabetic Nephropathy

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Diabetes is the primary cause of end-stage renal disease, accounting for 40% of all cases.¹ Changes in the tissue morphology of kidneys are a hallmark of diabetic nephropathy, and renal diseases associated with type 1 diabetes exhibit morphological abnormalities such as increased glomerular basement membrane thickness, mesangium expansion, glomerular sclerosis, and interstitial fibrosis.² Early glomerular changes correlate with the onset of microalbuminuria, an indicator of early phase diabetic nephropathy.³

In the clinic, the evidence-based treatment guidelines for diabetic nephropathy include the control of hyperfiltration, microalbuminuria, systemic blood pressure, and blood glucose. Indeed, clinical trials of renin-angiotensin system (RAS) inhibitors in patients with type 1 and type 2 diabetes have demonstrated reduced renal and cardiovascular damage in advanced diabetic nephropathy with proteinuria.^{4,5}

Until recently, little was known about the effects of inhibiting RAS in patients with early diabetic nephropathy. A large randomized trial was conducted to examine the effects of systemic inhibition of RAS on diabetics at an early phase of nephropathy. Surprisingly, this trial revealed that inhibition of the RAS does not reduce the incidence of microalbuminuria or slow the decline of renal function.⁶ In addition, another recent study using RAS inhibition for antihypertensive therapy has shown RAS inhibition to be effective for decreasing microalbuminuria, but not for the improvement of renal function.⁷ Both trials suggest that the conventional therapy of targeting microalbuminuria in early diabetic nephropathy using RAS inhibitors is not sufficient for the prevention of kidney

disease. The benefits of RAS inhibition appear to only affect advanced renal disease with proteinuria, but not early renal disease.

These results suggest that glomerular damage and interstitial damage may progress independently through different mechanisms. Therefore, a targeted therapy affecting both glomerular and interstitial lesions must be considered for the treatment of diabetic nephropathy. Yet, the mechanisms that promote interstitial fibrosis, a prominent mediator of renal dysfunction, remain largely unknown.

Kidney fibrosis is associated with epithelial–mesenchymal transition (EMT), which results from different kinds of injury or inflammation.⁸ During EMT, epithelial cells lose their apical–basal polarity to form highly migratory spindle-shaped mesenchymal cells. Furthermore, they undergo biochemical changes by losing epithelial markers (such as E-cadherin and cytokeratin) and gaining mesenchymal markers (such as fibroblast specific protein-1 and α -smooth muscle actin). EMT is prominent in various stages of embryonic development and is the primary mechanism of tumor metastasis and organ fibrosis.⁹

In this issue of the *American Journal of Pathology*, Li and colleagues¹⁰ suggest that endothelial–mesenchymal transition (EndMT) is a novel mechanism for generation of myofibroblasts in early diabetic renal fibrosis. Using endothelial-lineage tracing with Tie2-cre; LoxP-enhanced green fluorescent protein transgenic mice, they identified a significant number of interstitial α -smooth muscle actin-positive cells (myofibroblasts) of endothelial origin in fibrotic kidneys from mice with streptozotocin-induced diabetic nephropathy. They also found double positive

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Figure 1. Schematic representation of the pathogenesis of early diabetic fibrosis. In the kidney, endothelial cells line the inner surface of capillary vessels in the glomeruli and interstitium. Epithelial cells form the inner surface of the tubular interstitium. Exposure to stress in the diabetic state, such as hyperglycemia, hyperfiltration, inflammation, or oxidative stress, induces biochemical changes in these cells that may cause them to undergo EMT or EndMT. Transitioning endothelial cells lose expression of epithelial or endothelial markers and acquire a mesenchymal phenotype. These mesenchymal cells derived from epithelial in the tubulointerstitium or endothelial cells in the glomerulus and tubulointerstitium contribute to progression of diabetic nephropathy.

cells in the glomerulus. These data suggest that EndMT may contribute to the early progression of diabetic nephropathy.

In an earlier study, Zeisberg et al¹¹ detected fibroblasts expressing the endothelial marker CD31 in three different mouse models of renal disease: unilateral ureteral obstructive nephropathy, streptozotocin-induced diabetic nephropathy, and a mouse model of Alport syndrome. Approximately 30% to 50% of fibroblasts formed in the kidneys of these models co-expressed the endothelial marker CD31 and the fibroblast/myofibroblast markers fibroblast specific protein-1 and/or α -smooth muscle actin. These two papers provide strong evidence to suggest an endothelial origin of many activated fibroblasts/myofibroblasts in diabetic nephropathy.

Studies of kidney fibrosis have now demonstrated that pathological fibroblasts can be derived from the bone marrow,^{12,13} tubular epithelium,¹³ and vascular endothelium.^{10,11} In a previous study using the unilateral ureteral

obstructive mouse model, lineage-tracing of proximal tubule epithelial cells with γ GT-LacZ mice revealed that 36% of all fibroblast specific protein-1 positive fibroblasts exhibited proximal tubule epithelial origin, and 12% of these fibroblasts exhibited bone marrow origin.^{13,14} Zeisberg et al¹¹ demonstrated that 36% of all fibroblast specific protein-1 positive cells co-expressed the endothelial marker CD31 in the unilateral ureteral obstructive model. These data suggest that both EMT and EndMT play critical roles in contributing to the activated fibroblasts/myofibroblasts responsible for fibrosis (Figure 1).

In contrast to EMT, which has been shown to be a key mechanism in generating both diverse cell types during development and fibroblasts in adult pathologies, EndMT in adult tissues is a recent discovery. EndMT is an essential mechanism of cardiac development and renal and cardiac fibrosis,¹⁵ as well as a source of the carcinoma associated fibroblasts in the tumor microenvironment that regulate cancer progression.¹⁶ Similar to EMT, EndMT

can be induced by members of the TGF- β family of signaling proteins. TGF- β 2 has been shown to be the essential isoform that stimulates EndMT during embryonic development¹⁷ and in cultured endothelial cells.¹⁸ TGF- β 1 has also been shown to induce EndMT in cultured adult murine cardiac endothelial cells.¹⁵ Interestingly, bone morphogenetic protein-7 inhibits EndMT both in the *in vitro* and *in vivo* settings.¹⁵

The discovery that EndMT mediates the pathogenesis of diabetic nephropathy in both interstitial fibrosis and glomerular lesions provides us an opportunity to develop drugs that target EndMT. Further studies are urgently required to explore such possibilities and the current study is a step in the correct direction.

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