Pigment epithelium-derived factor, a noninhibitory serine protease inhibitor, is renoprotective by inhibiting the Wnt pathway

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Pigment epithelium-derived factor (PEDF) expression is downregulated in the kidneys of diabetic rats, and delivery of PEDF suppressed renal fibrotic factors in these animals. PEDF has multiple functions including anti-angiogenic, anti-inflammatory and antifibrotic activities. Since the mechanism underlying its antifibrotic effect remains unclear, we studied this in several murine models of renal disease. Renal PEDF levels were significantly reduced in genetic models of type 1 and type 2 diabetes (Akita and *db/db*, respectively), negatively correlating with Wnt signaling activity in the kidneys. In unilateral ureteral obstruction, an acute renal injury model, there were significant decreases of renal PEDF levels. The kidneys of PEDF knockout mice with ureteral obstruction displayed exacerbated expression of fibrotic and inflammatory factors, oxidative stress, tubulointerstitial fibrosis, and tubule epithelial cell apoptosis, compared to the kidneys of wild-type mice with obstruction. PEDF knockout enhanced Wnt signaling activation induced by obstruction, while PEDF inhibited the Wnt pathway-mediated fibrosis in primary renal proximal tubule epithelial cells. Additionally, oxidative stress was aggravated in renal proximal tubule epithelial cells isolated from knockout mice and suppressed by PEDF treatment of renal proximal tubule epithelial cells. PEDF also reduced oxidation-induced apoptosis in renal proximal tubule epithelial cells. Thus, the renoprotective effects of PEDF are mediated, at least partially, by inhibition of the Wnt pathway. Hence, restoration of renal PEDF levels may have therapeutic potential for renal fibrosis.

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Renal fibrosis, mainly categorized as glomerulosclerosis and tubulointerstitial fibrosis, is an advanced pathologic feature of many acute kidney injuries[1] and chronic kidney diseases.[2] This irreversible process causes a progressive decline of renal functions, eventually leading to renal insufficiency. Currently, there are no effective treatments for the devastating renal fibrosis.[3]

Tubules play important roles in maintaining renal functions. In human glomerular nephritis[4] and diabetic nephropathy,[5] loss of renal functions correlates more closely with tubular damage than with glomerular changes. Proximal tubules are recognized as a significant part of tubules carrying out regulatory functions of water and salt balance, acid-base homeostasis, and the endocrine system.[6] Various kidney diseases are associated with proximal tubule disorders, such as acute kidney injuries[7] and diabetic nephropathy.[8–10] Moreover, injuries of proximal tubules alone trigger glomerulosclerosis and tubulointerstitial fibrosis.[11] During the renal fibrotic process, renal proximal tubule epithelial cells (PTECs) produce extracellular matrix[2] and act as immune cells by expressing and releasing adhesion molecules, cytokines, and chemokines.[12]

Pigment epithelium-derived factor (PEDF) is a noninhibitory member of the serine protease inhibitor family.[13] It has multiple functions[14] including antiangiogenesis,[15] especially in retinal vascular diseases such as diabetic retinopathy,[16] anti-inflammation,[17,18] and antifibrosis activities.[19–23] In human kidneys, PEDF protein is highly expressed in tubules and moderately in glomeruli.[24] The PEDF mRNA is detected in the kidneys of both fetal and adult humans.[25] Decreased PEDF expression has been demonstrated in type 1 diabetic rat kidneys with renal fibrosis.[19] Delivery of PEDF into diabetic rats suppresses the renal levels of fibrotic markers,[23] suggesting an antifibrotic activity of PEDF. However, PEDF's function in the tubules and the mechanism underlying PEDF's antifibrosis activity remain elusive.

Aberrant activation of the canonical Wnt pathway is reported in kidney diseases including obstructive nephropathy,[26,27] Adriamycin-induced nephropathy,[28] and type 1 and 2 diabetic nephropathy.[29] In an obstructive nephropathy model

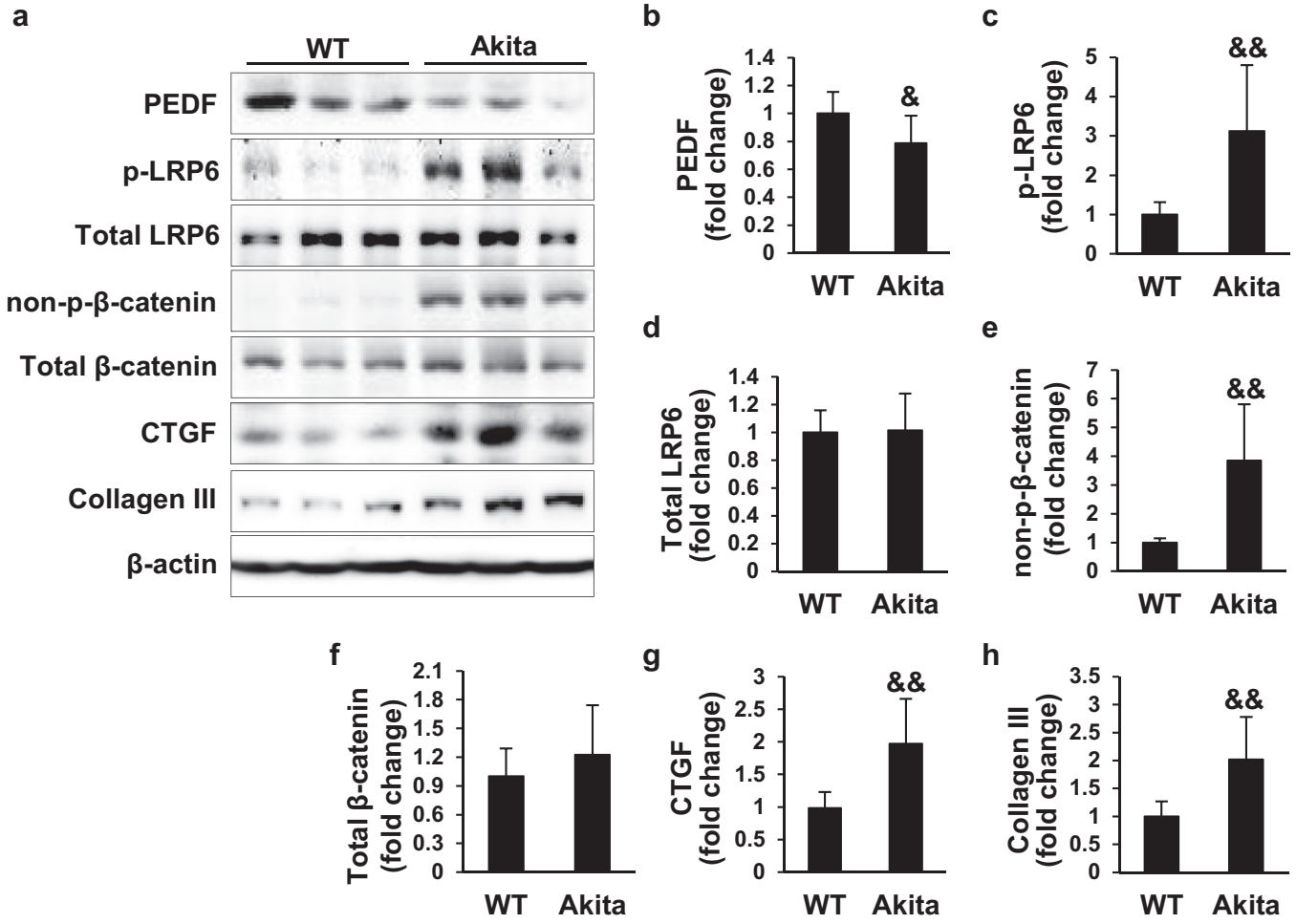

Figure 1 | Downregulated pigment epithelium-derived factor (PEDF) and activated Wnt signaling in the kidneys of Akita mice. (**a**) Western blot analysis and (**b–h**) densitometry quantification of PEDF, phosphorylated low-density lipoprotein receptor–related protein 6 (p-LRP6), total LRP6, nonphosphorylated β-catenin (non–p-β-catenin), total β-catenin, connective tissue growth factor (CTGF), and collagen type III in kidney homogenates from 3-month-old Akita mice and wild-type (WT) controls ($N = 10$). Each lane represents an individual mouse. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, Akita versus WT mice. All values are expressed as mean ± SD.

i.e., unilateral ureteral obstruction (UUO), 16 Wnt ligands, and 6 Frizzled receptors are upregulated.[26] The Wnt pathway promotes myofibroblast differentiation.[30] Persistent activation of this pathway further drives the transition from acute kidney injury to chronic kidney disease.[31] A number of Wnt target genes are also reported to contribute to renal fibrosis, including fibrotic factors such as fibronectin and fibroblast-specific protein 1.[32] Plasminogen activator inhibitor-1, another target gene of Wnt signaling, stimulates the accumulation of immune cells and myofibroblasts in the kidney.[33,34] On the other hand, inhibitors of the Wnt pathway, such as ICG-001,[31] secreted frizzled-related protein 4,[27] and dickkopf-1,[26] attenuate renal fibrosis. Interestingly, levels of β-catenin are predominantly induced in the tubules of the kidneys with UUO,[26] diabetic nephropathy,[29] Adriamycin-induced nephropathy,[35] and ischemia/reperfusion renal injury.[31]

Our previous study identified PEDF as an endogenous inhibitor of the Wnt signaling pathway[36]; it is possible that the antifibrotic effect of PEDF is mediated via inhibition of the Wnt signaling pathway. The present study has addressed the following questions: (i) What is the role of PEDF in tubulointerstitial fibrosis? (ii) Does PEDF knockout (KO) affect renal tubule epithelial cells, a group of cell types crucial for the regulation of renal inflammatory processes and tubulointerstitial fibrosis? (iii) What is the underlying molecular mechanism mediating the antifibrotic effect of PEDF?

RESULTS

Inverse correlations between PEDF levels and Wnt signaling activation in diabetic kidneys

The renal PEDF levels have not been previously examined in genetic models of types 1 and 2 diabetes mellitus. Thus, we examined PEDF protein levels in the kidneys of 3-month-old Akita mice, a type 1 diabetic model, and 6-month-old *db/db* mice, a type 2 diabetic model. Renal PEDF was significantly downregulated in these 2 diabetic models (Figures 1a and b and 2a and b), accompanied by increased levels of the fibrosis