Decreased Expression of Pigment Epithelium-Derived Factor Is Involved in the Pathogenesis of Diabetic Nephropathy

Joshua J. Wang, Sarah X. Zhang, Kangmo Lu, Ying Chen, Robert Mott, Sanai Sato, and Jian-xing Ma

Pigment epithelium-derived factor (PEDF) is a potent angiogenic inhibitor. Previous studies have shown that decreased ocular levels of PEDF are associated with diabetic retinopathy. However, the implication of PEDF expression in diabetic nephropathy has not been revealed. In the present study, we demonstrated for the first time that the expression of PEDF was decreased at both the mRNA and protein levels in the kidney of diabetic rats, whereas transforming growth factor-β (TGF-β) and fibronectin levels were increased in the same diabetic kidneys. As shown by immunohistochemistry, the decrease of PEDF expression occurs primarily in the glomeruli. In vitro studies showed that high concentrations of glucose significantly decreased PEDF secretion in primary human glomerular mesangial cells (HMCs), suggesting that hyperglycemia is a direct cause of the PEDF decrease in the kidney. Toward the function of PEDF, we showed that PEDF blocked the high-glucose–induced overexpression of TGF-β, a major pathogenic factor in diabetic nephropathy, and fibronectin in primary HMCs, suggesting that PEDF may function as an endogenous inhibitor of TGF-β expression and fibronectin production in glomeruli. Therefore, decreased expression of PEDF in diabetic kidneys may contribute to extracellular matrix overproduction and the development of diabetic nephropathy. 

Diabetic nephropathy is one of the most important microvascular complications of diabetes and occurs in 30–40% of diabetic patients (1,2). The early changes in diabetic nephropathy are characterized by the thickening of glomerular basement membrane and expanded extracellular matrix (ECM), leading to glomerular hyperfiltration and microalbuminuria (1,3). Although intensified control of hyperglycemia, blood pressure, and hyperlipidemia reduces the risks of diabetic nephropathy, it does not sufficiently prevent the diabetic patients with microalbuminuria from progression to devastating overt diabetic nephropathy, a leading cause of end-stage renal diseases (2,4,5). The exact pathogenesis of diabetic nephropathy remains largely unknown.

Several growth factors have been suggested to be involved in the pathogenesis of diabetic nephropathy—most importantly, transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF) (6,7). TGF-β has been recognized as a modulator of ECM formation. Overexpression of TGF-β in diabetic glomeruli is believed to contribute to the matrix accumulation by increasing synthesis and decreasing degradation of extracellular proteins such as fibronectin (8–11). VEGF is a major angiogenic factor and is also referred to as the vascular permeability factor, based on its ability to induce vascular hyperpermeability (12,13). Accumulating evidence indicates that VEGF and TGF-β are key pathogenic factors in early stages of diabetic nephropathy (14–19). Serum and urinary TGF-β levels have been found to correlate with the severity of microalbuminuria (20,21). Therefore, the increase of the systemic TGF-β level has been suggested as a marker for diabetic nephropathy (22).

Pigment epithelium-derived factor (PEDF) is a multifunctional serine proteinase inhibitor (23). Although PEDF was first identified in the eye, it is widely distributed in a variety of organs, such as brain, spinal cord, liver, heart, placenta, bone, pancreas, and prostate (24–26). As a potent anti-angiogenic factor, PEDF plays an important role in maintaining the homeostasis of the ocular vascular system (25–27). A recent study has shown that PEDF also reduces VEGF-induced vascular hyperpermeability (28). In diabetic retinopathy, decreased endogenous PEDF and increased VEGF levels break the delicate balance between angiogenic stimulators and inhibitors, resulting in retinal vascular hyperpermeability and retinal neovascularization (29–31). Systemic injection of PEDF or virus-mediated delivery of PEDF significantly inhibits retinal angiogenesis (32,33). Recent studies demonstrated that downregulation of PEDF may also contribute to the development of renal and prostate cancer (34,35).

In contrast to the extensive studies of PEDF in ocular tissues, the function of PEDF in the kidney has not been studied. In the present study, we have determined the PEDF expression in the normal and diabetic rat kidneys and explored the possible association between PEDF levels and pathogenesis of diabetic nephropathy.
RESEARCH DESIGN AND METHODS

Brown Norway (BN) rats were purchased from Charles River Laboratories (Wilmington, MA). Care, use, and treatment of all animals in this study were in strict agreement with the guidelines set forth in the Care and Use of Laboratory Animals by the University of Oklahoma.

Induction of experimental diabetes. BN rats (8 weeks of age) were used in the experiment. Diabetes was induced by an intraperitoneal injection of streptozotocin (STZ) (50 mg/kg in 10 mmol/l of citrate buffer, pH 4.5) (Sigma, St. Louis, MO) into BN rats after an overnight fast. Control rats received an injection of citrate buffer alone. Blood glucose levels were measured at 24 h after the injection and monitored every 3 days thereafter. Only the animals with blood glucose concentrations >350 mg/dl were considered diabetic.

Cell culture. Primary human glomerular mesangial cells (HMCs) were purchased from Cambrex Bio Science Walkersville (Walkersville, MD). The cells were cultured in mesangial cell basal medium (Cambrex) with 0.1% GA-1000 (Cambrex) and 5% fetal bovine serum (FBS) at 37°C in a humidified 5% CO2 atmosphere. Cells of passages from 6 to 10 were used in the experiments. After reaching 80% confluence, cells were exposed to medium with 5% FBS for 12 h before the treatments with high glucose or PEDF.

Isolation of glomeruli. Rats were deeply anesthetized, and the kidneys were immediately removed. The cortex was excised, cut into fine fragments, and homogenized. After being passed through consecutive stainless steel screens of 150- and 75-μm pore size, the glomeruli were suspended in 1 × PBS and collected by centrifugation at 2,000 × g for 3 min.

Quantitative PCR. The total RNA was isolated from tissues using the RNeasy Mini-Isolation Kit following the manufacturer’s protocol (Qiagen, Santa Clarita, CA). Primers (PEDF-F, 5′-aagctatgggacggccc-3′; PEDF-R, 5′-taccaacctcgtcagt-3′) were designed from mRNA sequences spanning >1 kb introns using the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3_www.cgi). RT reaction used 1.0 µg total RNA, oligo-dT primer, and MuLV reverse transcriptase at a final volume of 20 µl and conducted at 42°C for 60 min, followed by a denaturation at 95°C for 5 min. The real-time PCR used 1 µl of the RT product and 3 pmol of primers and was performed using a GeneAmp RNA PCR kit and SYBR Green PCR Master Mix (Applied Biosystems). The PCR mix was denatured at 95°C for 10 min, followed by 40 cycles of melting at 95°C for 15 s and elongation at 60°C for 60 s. Fluorescence changes were monitored after each cycle. Amiplocin size and reaction specificity were confirmed by 2.5% agarose gel electrophoresis. All reactions were performed in triplicate. The average Ct (threshold cycle) of fluorescence units was used to analyze the mRNA levels. The PEDF mRNA levels were normalized by 18s ribosomal RNA levels. Quantification was calculated as follows: mRNA levels (percent of control) = 2^(-A_{Ct,C} - A_{Ct,PEDF}) + C_{T,18s} and (ΔC_{T}) = ΔC_{T,normal sample} - ΔC_{T,STZ-induced diabetic sample}.

Western blot of PEDF. Western blot analysis was performed using a monoclonal anti-PEDF antibody (Chemicon, Temecula, CA) as described previously (31).

Immunohistochemistry study. Immunohistochemistry was performed on frozen tissue sections. Briefly, the sections were blocked with solution containing 3% BSA (Sigma) and 5% rabbit serum (Jackson Immunoresearch). After incubation with 1:800 dilution of an anti-PEDF antibody (Chemicon) or an anti-fibronectin antibody (Biodesign, ME) for 1 h, the sections were rinsed with PBS and incubated with a biotinylated rabbit anti-mouse antibody (Jackson Immunoresearch) for 1 h. After extensive washing, the sections were visualized and photographed under a fluorescent microscope (Olympus, Hamburg, Germany) and confocal microscope (Leica, Mannheim, Germany).

PEDF enzyme-linked immunosorbent assay. The protein concentration was measured with the BioRad DC protein assay (BioRad Laboratories, Hercules, CA). The amounts of PEDF in the tissue extracts, serum, and conditioned medium were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit specific for PEDF (Chemicon) according to the manufacturer’s instructions. Briefly, the tissue samples were homogenized in 1 × PBS and centrifuged (TL, Beckman) at 50,000 rpm (rotor type: TL5 100.3) for 20 min at 4°C. The serum or tissue extract was incubated with 8 mol/l urea (Sigma) for 1 h and diluted 1:200 before being applied to the plate. After incubation at 37°C for 1 h and the extensive washing, the plate was incubated with 100 µl of a biotinylated mouse anti-PEDF antibody for 1 h, followed by incubation with 100 µl streptavidin peroxidase conjugate for 1 h. After the addition of TMB/E (3,3′,5,5′-tetramethylbenzidine) for 5–10 min, the plate was read immediately at 450 nm by a Wallac-Victor3 1420 microplate reader (Perkin-Elmer Wallac). For standardization, the PEDF concentration in conditioned medium were determined using a commercial enzyme-linked immunosorbent assay kit specific for fibronectin (R&D Systems, Minneapolis, MN). Briefly, the tissue extracts were activated with 1 N HCl for 10 min, followed by neutralization with 1.2 N NaOH. The activated samples were applied to the plate precoated with soluble type II receptor and incubated at room temperature for 3 h. After extensive washing, horseradish peroxidase–conjugated anti-TGF-β antibody was added and incubated for another 1.5 h. Then the chromogen was added, and the plate was read at 450 nm. The results were expressed as picograms per milligram of total protein.

ELISA specific for fibronectin. Fibronectin protein was quantified by competitive sandwich ELISA (Assaypro, Winfield, MO). Briefly, samples were diluted and applied to the plate coated with anti-fibronectin antibody, and the same amount of biotin-labeled fibronectin was immediately added to the wells. After incubation for 1.5 h and extensive washing, the horseradish peroxidase–conjugated streptavidin was added to the wells and incubated for 30 min. Then the chromogen was added and the plate was read at 450 nm. The results were expressed as picograms per milligram of total protein.

Cell proliferation assay. The tetrazolium dye–reduction assay (MTT; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide; Sigma) was used to determine cell survival and proliferation rate according to the manufacturer’s protocol. Briefly, the primary HMCs were seeded in 12-well plates at a density of 5 × 103 cells/well 24 h before the treatments. Then the growth medium was replaced by a medium containing 1% FBS with PEDF (concentrations of 2.5–160 nmol/l). After incubation for 72 h, cells were washed with PBS, and 100 µl/well MTT solution was added and incubated at 37°C for 4 h. The formazan crystals that formed were dissolved by incubation with DMSO (1 ml/well) overnight. Absorption was measured at 550 nm, and the number of viable cells was calculated according to the standard curve. Experiments were performed in triplicate.

Evaluation of rat microalbuminuria. The 24-h urine collected from each diabetic rat and age-matched control was centrifuged at 2,000 × g for 10 min. The concentration of albumin in the supernatant was measured by ELISA according to the manufacturer’s protocol (Bethyl Laboratories, Montgomery, TX). The total amount of albumin in 24-h urine was calculated accordingly.

Statistical analysis. Statistical analysis used the Student’s t test and ANOVA with post hoc test. Statistical difference was considered significant at a P value of <0.05.

RESULTS

High-level expression of PEDF in the kidney of normal rats. PEDF was recently found to express in the kidney as well as in other organs, but its expression levels and cellular localization in the kidney have not been determined previously (35). Because the liver is regarded as the major source of systemic PEDF (36,37) and the retina is a well-known site of PEDF expression and function, we first compared PEDF levels in the kidney in those with those in the liver and retina. Western blot analysis showed that PEDF in the kidney was at a level comparable to that in the liver and was much higher than that in the retina (Fig. 1A). Quantitative analysis using ELISA confirmed the results from Western blot analysis (Fig. 1B). A significant difference was observed between the PEDF levels in the liver, kidney, and retina (F = 194.41, P < 0.0001). There is no difference between the PEDF levels in the kidney and those in the liver (249.21 ± 34.45 vs. 221.19 ± 40.38 ng/mg total protein, P > 0.05, n = 6). The PEDF level in the retina (51.21 ± 13.30 ng/mg total protein) was significantly lower than that in the kidney and liver (P < 0.01, n = 6). These results suggested that the kidney expresses high levels of PEDF.

High levels of PEDF in glomeruli. To determine the cellular localization of PEDF in the kidney, we compared the PEDF expression in the cortex and medulla in normal rat kidney. Western blot analysis showed that the PEDF levels in renal cortex were substantially higher than those in the medulla (Fig. 2A). PEDF ELISA confirmed that the PEDF levels in the cortex was 324.98 ± 51.29 ng/mg total protein, significantly higher than that in the medulla (173.58 ± 5.79 ng/mg total protein, P < 0.01, n = 6) (Fig. 2B). Immunohistochemistry using a monoclonal antibody specific for PEDF showed that PEDF was predominantly...
expressed in the glomeruli (Fig. 2-C-a), along the parietal glomerular capsule and basement membrane (Fig. 2-C-b). In the medulla, the PEDF signal was also detected at lower levels in the tubular basement membrane and interstitial tissue (Fig. 2-C-c). To further identify the cellular origin of PEDF, we immunolabeled PEDF and synaptopodin, which is accepted as a podocyte marker, in the consecutive sections of the same kidney. The results showed that the distribution pattern of synaptopodin and PEDF are similar but not identical. PEDF (Fig. 2-D-a), but not synaptopodin (Fig. 2-D-b), was found on the parietal glomerular capsule.

**Decreased expression of PEDF in the kidney of STZ-induced diabetic rats.** BN rats with STZ-induced diabetes developed polyuria and microalbuminuria at 1–6 weeks after the onset of diabetes, reflecting the impaired function of the glomeruli (Table 1). To determine if the PEDF expression change is implicated in diabetic nephropathy, we compared the PEDF levels in the kidney and serum in STZ-induced diabetic rats with that in age-matched control animals. Both Western blot analysis and specific ELISA demonstrated that the PEDF protein levels were significantly decreased in diabetic cortex and medulla, when compared with that in the nondiabetic control animals (Fig. 3A and B). The decreased PEDF expression was also detected at the mRNA level in the diabetic cortex and medulla (Fig. 3C). In the same tissue samples, however, TGF-β and fibronectin levels were significantly increased in the diabetic kidneys (Fig. 3D and E). When compared with the age-matched nondiabetic control animals, the PEDF levels in the serum were significantly decreased at the late stage of diabetes (3.54 ± 0.83 ng/mg total protein in 12-week diabetic rats vs. 8.35 ± 1.13 ng/mg in age-matched normal rats, $n = 4$, $P < 0.01$) but not at the early stage in diabetic rats (7.00 ± 2.12 ng/mg total protein in 3-week diabetic rats vs. 7.08 ± 1.17 ng/mg in normal rats, $n = 4$, $P > 0.1$), suggesting that the PEDF decrease in diabetic kidney occurs before the decrease in serum PEDF levels.
An immunohistochemistry study was performed on kidney sections from rats diabetic for 2 weeks, 4 weeks, and 6 months and nondiabetic controls. The average body weight of diabetic rats was 20–30% lower than that of age-matched nondiabetic control animals. The results showed the decrease of PEDF in the diabetic kidneys at both the early stage (2 and 4 weeks after diabetes onset; Fig. 4A and D) and late stage (6 months after diabetes onset; Fig. 4B) of diabetes. Moreover, the decrease of PEDF expression in the diabetic kidney was primarily observed in the glomeruli (Fig. 4A, B, and D), whereas the PEDF expression was not affected in the medulla of the same diabetic kidney (Fig. 4B). Western blot of PEDF in the isolated glomeruli confirmed that PEDF levels decreased in diabetic glomeruli compared with that in nondiabetic controls. At 2 weeks after diabetes onset, PEDF was decreased dramatically, whereas no detectable podocyte loss was observed (Fig. 4D), indicating that the decrease of PEDF levels in the early stage of diabetic nephropathy is not a result of podocyte loss.

**High glucose decreased PEDF secretion in primary HMCs.** To reveal the cause of decreased expression of PEDF in diabetic kidney, we determined the effect of high glucose on the expression of PEDF in primary HMCs. HMCs were cultured with different concentrations of n-glucose (Sigma) for 96 h with the same concentrations of D-mannitol as osmotic controls. The PEDF secreted into the medium was quantified by ELISA. The results showed that high glucose significantly decreased PEDF secretion in HMCs in a dose-dependent manner (F = 14.6, P = 0.013), whereas the mannitol had no significant effects on PEDF secretion (F = 0.96, P = 0.45) (Fig. 5). **PEDF blocked high-glucose-induced TGF-β overexpression in primary HMCs.** Because TGF-β is recognized as one of the major mediators of the proliferation of mesangial cells and the overproduction of ECM in diabetic nephropathy, we also examined the effects of PEDF on TGF-β secretion by HMCs. Under high-glucose conditions (30 mmol/l), the TGF-β secretion was significantly increased, compared with the normal glucose and mannitol controls (Fig. 6A). PEDF at concentrations of 40–160 nmol/l significantly downregulated TGF-β expression in a dose-dependent manner (F = 40.5, P < 0.0001). The inhibitory effects of PEDF on TGF-β expression occurred at 24 h and lasted for at least 48 h (Fig. 6B).

**PEDF blocked high-glucose-induced fibronectin secretion in primary HMCs.** In the early stage of diabetic nephropathy, the overproduction of ECM proteins, such as fibronectin, plays a critical role in the progression of diabetic nephropathy. Since fibronectin is overexpressed in patients with diabetic nephropathy, we examined the effect of PEDF on fibronectin expression in HMCs. PEDF blocked high-glucose-induced fibronectin secretion in a dose-dependent manner (Fig. 6C). The inhibitory effects of PEDF on fibronectin expression were observed at 24 h and lasted for at least 48 h (Fig. 6D). **PEDF blocked high-glucose-induced TGF-β overexpression in primary HMCs.** Because TGF-β is recognized as one of the major mediators of the proliferation of mesangial cells and the overproduction of ECM in diabetic nephropathy, we also examined the effects of PEDF on TGF-β secretion by HMCs. Under high-glucose conditions (30 mmol/l), the TGF-β secretion was significantly increased, compared with the normal glucose and mannitol controls (Fig. 6A). PEDF at concentrations of 40–160 nmol/l significantly downregulated TGF-β expression in a dose-dependent manner (F = 40.5, P < 0.0001). The inhibitory effects of PEDF on TGF-β expression occurred at 24 h and lasted for at least 48 h (Fig. 6B).

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fibronectin and collagen, is a major causative factor responsible for glomerular hyperfiltration (38). In primary HMCs, exposure to high glucose (30 mmol/l) for 48 h led to significant increases of fibronectin secretion, compared with mannitol control (Fig. 7A). At low doses (5–40 nmol/l), PEDF decreased fibronectin secretion in a dose-dependent manner in HMCs cultured in the high-glucose medium \((F = 39.98, P < 0.0001)\) (Fig. 7A). At 24 and 48 h after the addition of 40 nmol/l PEDF, the fibronectin secretion was decreased to 23 and 18% of control, respectively (Fig. 7B).

**PEDF does not affect the growth of HMCs.** The viability of glomerular mesangial cells and enlargement of the kidney are known as the major pathological changes in the early stage of diabetic nephropathy. To explore whether the decrease of PEDF expression in the glomeruli contributes to the pathogenesis of diabetic nephropathy, we studied the effects of PEDF and high glucose on mesangial cell proliferation using primary HMCs. The results (data not shown) showed that neither high glucose nor PEDF of the doses from 2.5 to 160 nmol/l altered viable cell numbers in HMCs, suggesting that PEDF’s effects on downregulation of fibronectin and TGF-β are not through the inhibition of mesangial cell proliferation.

**DISCUSSION**

PEDF is a 50-kDa glycoprotein initially identified in human retinal pigment epithelial (RPE) cells. Its function as a neurotrophic factor and angiogenic inhibitor has been well studied in ocular tissues (23–27,29–31). Its implication in diabetic retinopathy has been established (25,26,29–31). This study revealed for the first time that PEDF may function as an endogenous inhibitor of TGF-β in the kidney, and decreased PEDF expression in diabetic kidney may contribute to the development of diabetic nephropathy.
Previous studies have shown that PEDF is present in a variety of ocular tissues, such as the interphotoreceptor matrix and ganglion cell layer of the retina, epithelium of the cornea, and ciliary epithelium (23,25,39). Expression of PEDF was also found in human brain and spinal cord of the neural system and several nonneural tissues, including the liver, placenta, heart, and skeletal muscle, suggesting that PEDF's function may not be limited to ocular tissues (25). Recently, Abramson et al. (35) detected the expression of PEDF in the murine kidney. The present study confirmed the expression of PEDF in rat kidney at both the mRNA and protein levels. Moreover, we quantitatively compared the PEDF levels in the rat kidney, liver, and retina. Surprisingly, our results showed that PEDF levels in the kidney are as high as those in the liver, which is considered the major source of systemic PEDF. PEDF levels in the kidney and liver are much higher than those in the retina. The high level of PEDF in the kidney underscores its significance for renal functions.

The cortex and medulla have different structure and function in the kidney. The immunohistochemical analysis demonstrated that PEDF is predominantly present at the glomerular capsule and basement membrane in the cortex, whereas the PEDF signal was relatively weaker at the tubular basement membrane and interstitial tissue in the medulla. This finding suggests a possible role of PEDF in the regulation of glomerular functions. This cellular localization of PEDF is different from that in Abramson's report, which mentioned that PEDF was mainly expressed in tubular epithelial cells but not in the glomeruli of mice kidney. It is not clear what causes the disparity between our results and those of Abramson et al. in respect to the cellular localization of PEDF in the kidney, because the figure showing the PEDF signal in the glomeruli was not presented in their article (35). Different species used in our studies and theirs may be a possible reason for the disparity.

Functional studies have demonstrated that PEDF is a multifaceted factor with potent antiangiogenesis activity and neuroprotective function (25). PEDF inhibits endothelial cell migration induced by VEGF and fibroblast growth factor (26,27). A recent study showed that in PEDF gene knockout mice, the microvascular density in the kidney is significantly increased compared with the wild-type mice, suggesting that PEDF may play a role in the regulation of renal vasculature development and maintenance of renal homeostasis (35).

Previous studies from both diabetic patients and animal models have demonstrated that decreased PEDF levels are involved in diabetic retinopathy (29,30,40,41). Because of the close relationship between diabetic nephropathy and diabetic retinopathy, we proceeded to determine in the present study whether PEDF levels in the kidney are also decreased in a diabetic animal model. The results showed that the PEDF expression is significantly decreased in the kidney at both the mRNA and protein levels in STZ-induced diabetic rats, which have exhibited early diabetic nephropathy changes including albuminuria and polyuria. Moreover, the PEDF decrease was mainly observed in the glomeruli, whereas the PEDF levels in the tubular region

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**FIG. 6. PEDF-induced downregulation of TGF-β expression in HMC.**

A: HMCs were incubated with high glucose (30 mmol/l) for 48 h; then PEDF was added to different concentrations (2.5–160 nmol/l) and incubated for another 48 h. TGF-β in the medium was measured by ELISA. B: After incubation with high glucose (30 mmol/l) for 48 h, 40 nmol/l PEDF was added to the medium of HMCs. The medium was harvested at 24 and 48 h after the addition of PEDF. The TGF-β concentrations in the medium were determined by ELISA. The results were expressed as picograms per milligram total protein in the medium (means ± SD).
function of PEDF in diabetic kidney, we investigated the effects of PEDF on TGF-β secretion in HMCs. The results showed that PEDF significantly blocked the high-glucose-induced TGF-β overexpression, suggesting that PEDF may act as an endogenous inhibitor of TGF-β expression via a paracrine or autocrine regulation in normal kidney. This finding also suggests that the decreased PEDF levels in diabetic kidney may be responsible, at least in part, for the TGF-β overexpression.

One of the pathological functions of TGF-β in diabetic nephropathy is to promote the overproduction of ECM by mesangial cells, which is also closely correlated with microalbuminuria (1,2). Because mesangial cells are the major producer of ECM, we used primary HMCs as a model to determine if PEDF also blocks the function of TGF-β in the induction of ECM production. The results showed that high glucose (30 mmol/l) significantly increased fibronectin secretion. PEDF blocks the high-glucose-induced fibronectin overproduction in a dose-dependent manner. Further, we tested whether the effect of PEDF on fibronectin production was through the inhibition of mesangial cell proliferation. The results showed that PEDF had no effect on mesangial cell growth rate. These results suggest that PEDF blocks ECM secretion from mesangial cells without interfering with the cell proliferation.

In summary, the present study demonstrated for the first time that PEDF expression is implicated in diabetic nephropathy. PEDF may play an important role in preventing mesangial ECM overproduction and pathological growth factor upregulation in the kidney. The decreased expression or the dysfunction of PEDF may be involved in the pathogenesis of diabetic nephropathy. Therefore, PEDF may have therapeutic potential in the treatment of diabetic nephropathy.

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