Urinary Pigment Epithelium-Derived Factor as a Marker of Diabetic Nephropathy

Haibing Chen^a Zhi Zheng^b Rongxia Li^c Junxi Lu^a Yuqian Bao^a
Xiafang Ying^c Rong Zeng^c Weiping Jia^a

^aDepartment of Endocrinology and Metabolism, Shanghai Clinical Center for Diabetes, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai Diabetes Institute, Shanghai Key Laboratory of Diabetes Mellitus, ^bDepartment of Ophthalmology, Shanghai Jiaotong University Affiliated First People's Hospital of Shanghai, ^cKey Laboratory of Systems Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Key Words

Diabetic nephropathies • Pigment epithelium-derived factor • Microalbuminuria • Angiotensin II type 1 receptor antagonist

Abstract

Background: Pigment epithelium-derived factor (PEDF), a serine protease inhibitor, regulates extracellular matrix production in the kidney. We sought the association between urinary PEDF (uPEDF) and development of nephropathy among patients with type 2 diabetes (T2DM). **Methods:** Two human studies were performed in which uPEDF was determined by ELISA. These studies included (1) a cross-sectional study of T2DM (n = 228) and healthy controls (n = 49) and (2) a longitudinal study of hypertensive T2DM with microalbuminuria (MA; n = 42) treated with irbesartan for 6 months. An animal study was performed in which PEDF was measured in the kidney and urine samples of control rats, rats rendered diabetic with streptozotocin that were also fed a high-fat diet, and diabetic rats treated with irbesartan for 3 months. **Results:** Cross-sectional study: compared to controls, uPEDF was significantly higher in patients with diabetic nephropathy. uPEDF independently correlated with MA. In the MA

group, uPEDF in patients with diabetic retinopathy was significantly higher than that in patients without diabetic retinopathy. Longitudinal study: irbesartan significantly decreased uPEDF in T2DM with MA. Animal study: in diabetic rats, increased PEDF was observed in both the urine and kidney samples. uPEDF showed a significant correlation with the expression of PEDF in the kidney. Irbesartan could significantly decrease the PEDF expression in the kidneys of diabetic rats as well as uPEDF. **Conclusion:** uPEDF may serve as a novel marker for screening for nephropathy among patients with T2DM and monitoring the response to therapy.

Copyright © 2010 S. Karger AG, Basel

Introduction

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease in the western world [1]. It accounts for 54% of all renal failure patients requiring chronic dialysis [2]. Early detection can ensure timely in-

H.C., Z.Z. and R.L. contributed equally to this work.

tervention and improvement of treatment outcome. Thus far, microalbuminuria (MA) has been recognized as an early indicator of DN; however, the presence of albuminuria might not be always indicative of DN in individuals with type 2 diabetes (T2DM), as revealed by biopsy studies [3]. In fact, MA is a marker for endothelial dysfunction. MA may develop because of hypertension or/and insulin resistance [4], along with hyperglycemia, and the presence of MA in T2DM patients may also be indicative of cardiovascular disease [5]. Therefore, there is a need to identify new sensitive and specific markers for screening and assessing incipient DN and monitoring responses to therapy.

Pigment epithelium-derived factor (PEDF), a 50-kDa protein, was first identified as a neurotrophic factor in the conditioned medium of human retinal pigment epithelial cells [6, 7]. Sequence analysis of the human PEDF gene indicated that it is a member of the serine protease inhibitor (serpin) gene family [8]. PEDF plays a protective role in the development and progression of angiogenic diseases [9]. Several studies have indicated that the loss of PEDF in the eye is functionally important in the pathogenesis of proliferative diabetic retinopathy (DR) [10, 11]. Recently, Wang et al. [12] reported that a decreased PEDF expression in diabetic kidneys may contribute to the overproduction of extracellular matrix and the development of DN. Moreover, PEDF can prevent fibrogenesis in diabetic kidneys via the inhibition of transforming growth factor- β and connective tissue growth factor expression and function [13], and reduce proteinuria by suppressing increased vascular endothelial growth factor (VEGF) expression [14]. In our previous study, we demonstrated that serum PEDF (sPEDF) is independently correlated with the clinical parameters associated with the severity of DN [15]; this finding was verified by Matsuyama et al. [16]. However, an increase in the sPEDF level in T2DM patients could reflect the systemic transport of PEDF, which is primarily overproduced in the liver and adipose tissue [17]. On the other hand, urinary PEDF (uPEDF) level may probably indicate the changes in the expression of PEDF in the kidney. To the best of our knowledge, there are no data on the implication of uPEDF in nephropathy in T2DM patients.

Thus, this study aimed to investigate whether uPEDF is associated with nephropathy in T2DM patients. Our results showed that uPEDF significantly correlated with DN, and uPEDF in patients with MA with DR was significantly higher than that in patients without DR. After irbesartan treatment, T2DM patients with MA and diabetic rats showed significant decrease in the uPEDF level.

Subjects and Methods

Subjects and Study Protocol

The study was performed according to the principles of the Declaration of Helsinki and was approved by the local ethics committee. All subjects provided their informed consent.

In the present study, uPEDF was determined in the urine samples of 228 well-characterized T2DM patients and 49 healthy normoglycemic control subjects. Patients were recruited from the outpatient clinic at the Shanghai Clinical Center for Diabetes (Shanghai, China). uPEDF was expressed as a ratio relative to the creatinine level ($\mu\text{g/l}$ creatinine). Diabetic patients were categorized as having normoalbuminuria (NA) when the albumin excretion rate (AER) was persistently $<20 \mu\text{g/min}$. Patients were categorized as having MA when the AER was between 20 and $200 \mu\text{g/min}$. Patients were categorized as having DN if they had persistent albuminuria ($>200 \mu\text{g/min}$) and DR, without any other kidney or renal tract disease. Demographic and clinical data, including age, sex, duration of diabetes, weight, height, and medication, were recorded. Blood pressure was measured twice with a Hawksley sphygmomanometer after 10 min of supine rest, and the average blood pressure was calculated by using the following formula: $(\text{SBP} + 2\text{DBP})/3$. Presence of retinopathy was scored as nil, simplex, or proliferative on the basis of fundus photography. AER was determined in two consecutive 24-hour urine samples by Dade Behring Nephelometer II System (antiserum to human albumin, Siemens Healthcare Diagnostics). Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease study equation [18].

In the MA group, 42 hypertensive T2DM patients with MA were enrolled for a longitudinal intervention study, and the patients were administered angiotensin II receptor blocker (ARB) irbesartan therapy. These patients exhibited essential hypertension (diastolic blood pressure, DBP, ranging from 80 to 100 mm Hg and systolic blood pressure, SBP, ranging from 130 to 160 mm Hg) and had been prescribed antihypertensive agents other than ACE-I or ARB. After 2 weeks of washout, all the patients received irbesartan daily at doses ranging from 150 mg/day to a maximum of 300 mg/day over a 6-month period. The targeted blood pressure 3 months after the commencement of the irbesartan therapy was $<135/85$ mm Hg. Patients continued to receive their usual diabetes care.

Animals

Eight-week-old male Sprague-Dawley rats (180–200 g) from Shanghai Experimental Animal Center, Chinese Academy of Sciences, China, were used in the study. The Medical Experimental Animal Administrative Committee of Shanghai approved all the experiments. Animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All possible efforts were made to minimize animal suffering and reduce the number of animals used.

As suggested by Sahin et al. [19], fat-fed/streptozotocin (STZ)-treated rats were used as animal models for T2DM. The rats were randomly divided into the following three groups: control group, high-fat diet (HFD) plus STZ group (DM), and HFD-STZ plus irbesartan intervention group (DM + ARB). Control rats were fed with commercially available normal pellet diet (10% calories as fat; SLACCAS, China). The DM rats were fed with HFD (40% calories as fat) for a period of 2 weeks. Next, the rats were injected

intraperitoneally with a low dose of STZ (40 mg·kg⁻¹; Sigma, USA), and the original diet was continued. One week after STZ injection, we measured the blood glucose level. Rats were categorized as diabetic when the blood glucose level exceeded 16.7 mM. Most of the DM rats displayed hyperglycemia, insulin resistance, and glucose intolerance as previously reported [19]. Diabetic rats with similar degrees of hyperglycemia and body weight were randomly assigned into groups receiving either 15 mg·kg⁻¹·day⁻¹ irbesartan [20] (Sanofi-aventis, HangZhou, China) through drinking water for 3 months or no treatment at all.

Western Blot

Western blot analysis was performed using a monoclonal anti-PEDF antibody (1:500; Chemicon, Temecula, Calif., USA) as described previously [21].

Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction

Quantitative real-time reverse transcriptase-polymerase chain reaction was performed as described previously [21].

Immunohistochemistry

The kidneys of diabetic and control rats were fixed in 10% formaldehyde and used for immunohistochemically analyzing PEDF expression. In brief, after dewaxing, the sections were treated with 10 mM sodium citrate buffer (pH 6.0) in a microwave oven at low power for 10 min. Endogenous peroxidase was inactivated using 3% hydrogen peroxide in methanol for 20 min. The sections were then incubated in a protein-blocking agent for 30 min followed by incubation with a monoclonal mouse antibody to PEDF (Chemicon, Temecula) overnight at 4°C. Biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories, Burlingame, Calif., USA) was used as the secondary antibody. Sections were then incubated with horseradish peroxidase-conjugated streptavidin. Peroxidase conjugates were localized by using 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical, St. Louis, Mo., USA), which acted as a chromogen. Sections were counterstained with hematoxylin. All the analyses were performed in a blinded manner.

Enzyme-Linked Immunosorbent Assay

Urine samples were maintained at -70°C for subsequent assays. The uPEDF measurements were performed using the commercially available enzyme-linked immunosorbent assay kit (USCN Life Science and Technology Company) according to manufacturer's protocol. The detection limit of the human uPEDF assay was 0.39 ng/ml and intra- and inter-assay variations were 6.7 and 8.5%, respectively. The detection limit of the rat uPEDF assay was 0.078 ng/ml and the intra-assay variation was 6.4%. Duplicate measurements were obtained for all the samples. Serial dilutions of recombinant PEDF were included in all the assays as a standard.

Statistical Analysis

Each variable was examined for normal distribution. Data were expressed as means ± standard deviation (SD) for normally distributed variables, and as geometric means (95% confidence interval, CI) for skewed variables. Skewed variables were natural logarithm-transformed to improve normality prior to the analysis and then retransformed to their natural units to represent them

in a tabulated form. Characteristics of the subjects across the different patient groups were compared by ANOVA and analysis of covariance, and those between the controls and patient groups were compared by the t test. Comparisons between the groups before and after irbesartan treatment were made using Wilcoxon signed-ranked test, and analysis of covariance was used to exclude the impact of BP and AER on uPEDF. Pearson correlation tests, multivariable linear regression analyses, and partial correlation analyses were also performed. Partial correlation analysis was used to analyze the association between the variation of AER and uPEDF, independent of the variation of BP. All the calculations were performed using the GraphPad Prism software (GraphPad; San Diego, Calif., USA) and the Statistical Package for Social Sciences 13.0 software (Los Angeles, Calif., USA). All reported p values were two tailed, and p values <0.05 were considered statistically significant.

Results

The general characteristics and clinical parameters of the cross-sectional study are summarized in table 1. Compared with the controls, type 2 diabetic patients had higher BP and higher levels of hemoglobin A1c (HbA1c), fasting plasma glucose, and 2-hour postprandial plasma glucose and lower levels of high density lipoprotein-cholesterol. There was no significant difference with respect to sex, age, blood glucose level, and lipid counts in diabetic patients. Compared with the NA group, the DN group had longer disease duration and higher BP. eGFR in the DN group was lower than that in the control or non-DN group.

uPEDF Was Significantly Increased in DN Patients and Independently Correlated with AER

uPEDF was significantly higher in the diabetic groups than in the control group (12.3 ± 5.8 vs. 6.7 ± 2.6 µg/l creatinine; p<0.05; fig. 1a). In the diabetic groups, uPEDF in the DN group was higher than that in the MA group or in the NA group (22.9 ± 6.8 vs. 10.8 ± 4.5 µg/l creatinine or 22.9 ± 6.8 vs. 8.6 ± 3.2 µg/l creatinine; p<0.05 and 0.01, respectively). In addition, uPEDF was significantly higher in the MA group than in the NA group (10.8 ± 4.5 vs. 8.6 ± 3.2 µg/l creatinine; p<0.05), whereas there was no significant difference in the uPEDF level between the control and NA groups (fig. 1b). After adjustment for eGFR, we obtained similar results for the uPEDF level, as determined by the analysis of covariance (control, 6.8 ± 2.1 µg/l creatinine; NA, 9.0 ± 1.9 µg/l creatinine; MA, 12.3 ± 1.8 µg/l creatinine; DN, 21.0 ± 2.8 µg/l creatinine). uPEDF was higher in patients with more advanced DN.

Fig. 1. uPEDF in the control subjects and T2DM patients. **a** uPEDF in the control group and diabetic groups. uPEDF, a skewed variable, was natural logarithm-transformed to improve its normality prior to analysis. **b** uPEDF in the control group and different patient subgroups.

Table 1. General and clinical parameters of healthy control subjects and type 2 diabetic patients

	Control subjects	Type 2 diabetes		
		normoalbuminuria	microalbuminuria	macroalbuminuria
Subjects (M/F)	46 (21/25)	59 (36/23)	130 (76/54)	39 (22/17)
Age, years	46.41 ± 7.95	56.03 ± 10.15**	57.69 ± 10.25**	60.13 ± 9.26**,†
Duration of diabetes, years	–	6.97 ± 6.68	6.19 ± 4.38	10.03 ± 5.57†
SBP, mm Hg	120 ± 13	129 ± 18*	137 ± 27**	149 ± 19**,††
DBP, mm Hg	79 ± 10	79 ± 8	81 ± 10†	87 ± 11**,‡
BMI	23.41 ± 2.61	24.71 ± 2.83	25.21 ± 3.35**	24.68 ± 4.43
HbA1c, %	5.27 ± 0.25	8.34 ± 2.19**	7.81 ± 2.03**	8.47 ± 2.31**
FPG, mM	5.03 ± 0.38	8.35 ± 2.85**	8.35 ± 2.34**	8.66 ± 3.27**
PG2h, mM	5.67 ± 1.05	13.14 ± 4.00**	14.7 ± 4.83**	12.87 ± 5.38**
TC, mM	4.83 ± 0.81	4.92 ± 1.09	5.38 ± 1.22*	4.92 ± 1.59
LDL-c, mM	2.76 ± 0.78	3.03 ± 0.76	3.43 ± 0.96*	2.83 ± 1.12
TG, mM	1.54 ± 1.40	1.82 ± 1.20	2.68 ± 3.45*	2.27 ± 2.81
HDL-c, mM	1.56 ± 0.38	1.23 ± 0.35**	1.22 ± 0.32**	1.13 ± 0.28**
Cr, mM	71.17 ± 16.36	68.79 ± 15.40	73.43 ± 21.22	136.95 ± 89.80**,††,‡‡
AER, µg/min	5.05 (3.48–8.14)	7.12 (4.26–10.20)	77.35 (31.24–189.14)**,,††	721.6 (518.1–1,005)**,,††,‡‡
eGFR, ml·min ⁻¹ ·1.73 m ²)	98.15 ± 18.48	102.70 ± 21.44	98.91 ± 26.79	62.87 ± 30.66**,,††,‡‡
Hypertension	0	21 (35.59%)	108 (83%)	39 (100%)
ACEI/ARB treatment in hypertensive patients	0	13 (22.03%)	25 (19%)	21 (53.85%)
DR (non/simplex/proliferative)	–	41/18/0	70/60/0	0/23/16

Data are expressed as means ± SD or geometric means (95% CI). Data were analyzed using one-way analysis of variance. Pearson correlation analyses were used to determine the association between uPEDF and other parameters. R values refer to the Pearson correlation coefficient. BMI = Body mass index; FPG = fasting plasma glucose; PG2h = 2-hour postprandial plasma glucose; TC = total cholesterol; LDL-c = low-density lipoprotein cholesterol; TG = triglyceride; HDL-c = high-density lipoprotein cholesterol; Cr = serum creatinine.

* p < 0.05, ** p < 0.01 vs. control; † p < 0.05, †† p < 0.01 vs. normoalbuminuria; ‡ p < 0.05, ‡‡ p < 0.01 vs. microalbuminuria.

Fig. 2. uPEDF (a) and AER (b) in patients with and without DR in the MA group that did not receive RAS inhibitor treatment.

Pearson correlation tests suggested that the uPEDF level significantly correlated with SBP ($r = 0.27$, $p < 0.01$), DBP ($r = 0.19$, $p < 0.05$), HbA1c ($r = 0.18$, $p < 0.05$), highly sensitive c-reactive protein (hs-CRP; $r = 0.28$, $p < 0.01$), eGFR ($r = -0.20$, $p < 0.01$), and AER ($r = 0.42$, $p < 0.01$). To elucidate the independent relationships between uPEDF and clinical parameters, we selected uPEDF as a dependent variable and other clinical parameters as the independent variables to build a multiple linear stepwise regression equation. Only variables that were significantly ($p < 0.05$) related to uPEDF by Pearson correlation analyses were entered into the multiple linear stepwise regression analysis. The results revealed an independent correlation between uPEDF and AER ($r = 0.43$, $p < 0.01$).

uPEDF Level in Diabetic Patients with Retinopathy

In the MA group, uPEDF level was significantly higher in patients with DR than in patients without DR (15.5 ± 4.6 vs. 8.3 ± 2.3 $\mu\text{g/l}$ creatinine) who did not undergo treatment with renin-angiotensin system (RAS) inhibitor ($p < 0.05$, fig. 2a); there was no significant difference between the two groups with respect to AER (61.4 ± 39.8 vs. 51.7 ± 36.2 $\mu\text{g/min}$; fig. 2b).

PEDF Level in the Kidney Correlated with uPEDF Level in the HFD/STZ-Induced Diabetic Rats

To determine whether uPEDF was associated with the expression of PEDF in the kidneys of DN patients, we generated a nongenetic rodent model mimicking human

T2DM, in which Sprague-Dawley rats were fed an HFD for 2 weeks and then administered a single low dose of STZ followed by continued HFD for an additional week. As shown in figure 3a–d, the PEDF expression in the kidneys of diabetic rats was significantly higher than that in the kidneys of nondiabetic rats, at both protein and mRNA levels. Similarly, uPEDF level was significantly higher in the diabetic rats than in the age-matched nondiabetic control rats (0.19 ± 0.07 vs. 0.40 ± 0.12 ng/mM creatinine, $p < 0.01$).

In addition, we assayed the PEDF expression in the serum, kidney, liver and adipose tissues in diabetic rats by Western blot analysis (fig. 3e). We found that the uPEDF level was correlated with the sPEDF level, and with the PEDF expression in kidney, liver and adipose tissue (data not shown); furthermore, a multiple linear stepwise regression analysis indicated that uPEDF was independently associated with PEDF expression in the kidney. Similarly, after irbesartan treatment, we found that uPEDF was also independently associated with PEDF expression in the kidney (data not shown). It is worth noting that uPEDF significantly correlated with PEDF expression in the kidney in control rats, diabetic rats, and diabetic rats treated with irbesartan (fig. 3f). All these data suggested that uPEDF level might be associated with the expression of PEDF in the kidneys of DN patients.

uPEDF was significantly reduced by ARB. To investigate the effect of RAS intervention on the uPEDF level in DN patients, we performed a longitudinal study with

a

b

c

d

e

Table 2. Characteristics and clinical parameters before and after irbesartan intervention therapy

	Before ARB	After ARB	p
Subjects (M/F)	42 (24/18)		
Duration, years	4.3 ± 3.2		
Age, years	57.2 ± 7.4		
BMI	24.9 ± 2.4	25.3 ± 2.9	0.112
WC, cm	87 ± 5.4	88 ± 2.4	0.541
Average BP, mm Hg	103.5 ± 14.3	96.2 ± 8.0	<0.0001
HbA1c	7.2 ± 1.3	6.7 ± 0.8	0.476
TG, mM	1.9 ± 0.9	2.0 ± 0.9	0.919
HDL-c, mM	1.3 ± 0.3	1.2 ± 0.4	0.562
TC, mM	5.1 ± 0.9	5.2 ± 1.0	0.367
LDL-c, mM	3.3 ± 0.9	3.2 ± 1.0	0.461
hs-CRP, mg/l	1.83 ± 1.17	1.02 ± 0.97	0.432
AER, µg/min	74.8 ± 30.7	30.1 ± 16.2	<0.0001
eGFR, ml·min ⁻¹ ·1.73 m ²	102 ± 26	103 ± 20	0.829
Irbesartan dose, mg/day	215.6 ± 75.6		

WC = Waist circumference; TG = triglyceride; HDL-c = high-density lipoprotein cholesterol; TC = total cholesterol; LDL-c = low-density lipoprotein cholesterol.

Fig. 3. Kidney and uPEDF levels in the control rats, diabetic rats (DM), and diabetic rats treated with irbesartan (DM + ARB). **a** Immunohistochemical staining for PEDF in the three groups. **b** Real-time reverse transcriptase-polymerase chain reaction determination of PEDF mRNA in the three groups. **c** Western blot analysis of the PEDF protein expression in the three groups. Equal protein loading was confirmed using the β -tubulin antibody. **d** uPEDF in the three groups. **e** Western blot analysis of the PEDF protein expression of different tissues of diabetic rats. **f** Correlation analysis between uPEDF and PEDF expression in the kidney in control rats, diabetic rats, and diabetic rats treated with irbesartan. Data are means $\pm$ SD of 4 rats per group, and the experiments were repeated independently at least three times with similar results. * $p < 0.05$, ** $p < 0.01$ vs. control, # $p < 0.05$ vs. DM + ARB.

ARB-irbesartan treatment. Our results showed that uPEDF was significantly decreased from a baseline level of 16.0 ± 7.3 to 7.3 ± 4.4 µg/l creatinine after irbesartan treatment ($p < 0.01$; fig. 4a), and the AER concomitantly reduced from 74.8 ± 30.7 to 30.1 ± 16.2 µg/min ($p < 0.01$; $n = 42$; fig. 4b). After ARB treatment, the average blood pressure significantly decreased from 103.46 ± 14.33 to 96.15 ± 8.03 mm Hg ($p < 0.01$; fig. 4c). After adjustment for BP and AER, a significant decrease was still observed after ARB treatment (15.7 ± 3.2 vs. 6.2 ± 3.2 µg/l creatinine, $p < 0.05$). There was a significant positive correlation between the changes in the uPEDF level and the changes in the AER ($r = 0.52$, $p < 0.01$; fig. 4d), and this correlation persisted even after adjustment for the changes in the average blood pressure, as determined by partial correlation analyses ($r = 0.54$, $p < 0.05$). There was no significant difference in the general characteristics and clinical parameters of the patients (table 2).

In addition, in the HFD/STZ-induced diabetic rats, after 3 months of treatment with irbesartan, the expression of PEDF was significantly decreased in the kidneys at both mRNA and protein levels (fig. 3a–c). Similarly, there was a decrease in the level of uPEDF (fig. 3d).

Discussion

This is the first study which demonstrated that uPEDF increased significantly and correlated independently with AER in patients with DN. In diabetic rats, uPEDF positively correlated with PEDF expression in the kidney. Furthermore, we found that uPEDF level in patients with MA in the presence of DR was significantly higher than that in patients with MA in the absence of DR. In addition, after the treatment with irbesartan for 6 months, uPEDF level in the T2DM patients with MA decreased significantly, and there was a concomitant alleviation in MA. In the diabetic rats, PEDF level was also reduced in the kidney and urine by irbesartan.

PEDF is synthesized in a wide range of human tissues, including the lung, brain, kidney [12], and, in particular, the liver [17]; this might account for the high PEDF level in the blood. Our previous results indicated that in T2DM patients, sPEDF was significantly higher in the DN group than in the control and NA groups. Moreover, multiple linear regression analyses demonstrated that sPEDF independently correlated with eGFR, triglycerides, and AER, suggesting that sPEDF is probably an important determinant of the pathogenesis of DN [15]. In the present study, uPEDF was significantly elevated in the diabetic

Fig. 4. Changes in uPEDF level (a), AER (b), and average BP (c) before and after irbesartan intervention therapy, and a correlation analysis between the changes in uPEDF (Δ uPEDF) and AER (Δ AER) in DN patients with irbesartan intervention (d).

patients with DN compared with that in the control group and in patients without DN, and it increased as DN advanced. Pearson correlation tests demonstrated significant correlation between uPEDF and SBP, DBP, HbA1c, hs-CRP, eGFR, and AER. Furthermore, the multiple stepwise regression analysis revealed that uPEDF had independent correlations with AER – one of the main characteristics of DN. Therefore, uPEDF might be a sensitive indicator for incipient DN.

It has been reported that PEDF counteracts the effects of VEGF [22] and advanced glycation end products [23]. A previous study also showed that sPEDF levels may be elevated as a counter-system in the metabolic syndrome in general residents [24]. In contrast, recent studies have indicated that on the one hand, PEDF ameliorates ad-

vanced glycation end product-induced hepatic insulin resistance in vitro by suppressing Rac-1 activation [25], and on the other hand, PEDF plays a causal role in obesity-induced insulin resistance in mice in vivo [26]. We still do not know the exact reason for this discrepancy; however, taking into account such reports and our present results, it is possible that the increase in sPEDF and uPEDF levels in DN patients is a response to counteract the effects of VEGF, and AGEs.

It is noteworthy to determine whether uPEDF may be used as a specific marker for screening incipient DN. Several studies have suggested that albuminuria can be attributed with confidence to DN if DR is present [27]. In 2007, KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic

Kidney Disease [28] stated that in most patients with diabetes, chronic kidney disease should be attributable to diabetes if MA is present along with DR. In this study, we demonstrated that in T2DM patients with MA in the presence of DR, uPEDF level was significantly higher than that in patients with MA in the absence of DR, suggesting that the increased uPEDF level might be a specific indicator for incipient DN. Meanwhile, these findings should answer the question as to whether the increased levels of uPEDF in patients with MA would indicate microvascular damage and may be of predictive value for the progression of retinopathy. Estimates of the prevalence of retinopathy are usually based on direct examination of the anatomic retinal changes, whereas those of nephropathy are defined by functional abnormalities such as MA or overt proteinuria. Further longitudinal studies should be performed with repetitive samples from the same donor to determine the changes with time and its clinical significance.

The reason for an increased uPEDF level in T2DM is unclear. It is speculated that there are several reasons behind the increased urinary concentrations of PEDF. (1) Hyperfiltration of circulating PEDF because of the increased permeability of the glomerular basement membrane. Our previous and present studies revealed that uPEDF correlated with sPEDF (data not shown) and AER; moreover, PEDF is a glycoprotein with a molecular weight of 50 kDa, which is similar to albumin with a molecular weight of 65 kDa. (2) Increased production or secretion of PEDF by renal tissues in response to hyperglycemia. This hypothesis could be best confirmed by biopsy studies, but it is very difficult to perform such studies in human beings. It has been reported that a rodent model induced by high-fat chow feed plus administration of a relatively moderate amount of STZ, which simulates the natural course and metabolic characteristics of patients with T2DM, has been recognized as a T2DM model [19, 29]. Therefore, in this study, we used an HFD-fed STZ-induced diabetes model of rat to investigate the possible association between uPEDF and PEDF in the kidney. Our results indicated that there was a positive correlation between the two above-mentioned factors, suggesting that the local PEDF production in the kidney is probably partly responsible for the changes in the pattern of its urinary excretion. (3) Diabetes-induced changes in the management of the PEDF filtered load by the renal tubules; this needs to be explored.

In order to determine whether uPEDF could serve as an indicator for evaluating treatment response, we used ARB to treat hypertensive T2DM patients with MA and

diabetic rats. In the longitudinal study, after 6 months of irbesartan treatment, there was a significant decrease in uPEDF level in T2DM patients with MA and a concomitant alleviation in MA. In diabetic rats, irbesartan could also significantly decrease the PEDF levels in the urine and kidney. These findings indicated that uPEDF might be a new marker for monitoring response to therapy.

In our previous study [21], we firstly found that the inhibition of renin-angiotensin system (RAS) inhibition directly upregulated PEDF expression in bovine retinal capillary endothelial cells with or without high glucose. However, in the present study, after 6 months of irbesartan treatment, we found a significant decrease in the uPEDF level in T2DM patients with MA and a concomitant alleviation in MA; this significant decrease persisted even after adjustment for BP and AER, suggesting that the decrease in the uPEDF mediated by ARB was independent of AER. Furthermore, we found that, in the diabetic rats, the kidney PEDF expression and uPEDF level were significantly decreased after 6 months of irbesartan treatment. These results indicated that ARB may indirectly mediate kidney PEDF expression *in vivo*. In order to validate the hypothesis, an *in vitro* experiment should be performed.

In conclusion, we confirmed that uPEDF significantly and independently correlated with AER in patients with DN. uPEDF level in patients with MA with DR was significantly higher than that in patients with MA without DR. ARB led to a significant decrease in the uPEDF level in patients with T2DM and in diabetic rats. Thus, uPEDF might be used as a new sensitive and specific marker for screening and assessing incipient DN and for monitoring response to therapy; however, a prospective study is needed to test these findings.

Acknowledgments

This work was supported by Shanghai Key Laboratory of Diabetes Mellitus (08DZ2230200), grants of the National Natural Science Foundation of China (30871204) to H. Chen, and the Major Program of Shanghai Municipality for Basic Research (08dj1400601) to W. Jia, and grants of the National Natural Science Foundation Youth Fund of China (30900261) to R. Li, and the Basic Research Foundation (2006CB910700, 2006CB503900) and the CAS Project (KSCX1-YW-02) to R. Zeng.

References

- Locatelli F, Pozzoni P, Del Vecchio L: Renal replacement therapy in patients with diabetes and end-stage renal disease. *J Am Soc Nephrol* 2004;15(suppl 1):S25–S29.
- System URD: USRDS 2009 Annual Data Report: Atlas of End-Stage Renal Disease in the United States. Bethesda, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2009.
- Molitch ME, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, Steffes MW: Nephropathy in diabetes. *Diabetes Care* 2004;27(suppl 1):S79–S83.
- Lin CY, Chen MF, Lin LY, Liao CS, Lee YT, Su TC: Insulin resistance is the major determinant for microalbuminuria in severe hypertriglyceridemia: implication for high-risk stratification. *Intern Med* 2008;47:1091–1097.
- Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, Hoogwerf B, Halle JP, Young J, Rashkow A, Joyce C, Nawaz S, Yusuf S: Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and non-diabetic individuals. *JAMA* 2001;286:421–426.
- Tombran-Tink J, Johnson LV: Neuronal differentiation of retinoblastoma cells induced by medium conditioned by human RPE cells. *Invest Ophthalmol Vis Sci* 1989;30:1700–1707.
- Tombran-Tink J, Chader GG, Johnson LV: PEDF: a pigment epithelium-derived factor with potent neuronal differentiative activity. *Exp Eye Res* 1991;53:411–414.
- Steele FR, Chader GJ, Johnson LV, Tombran-Tink J: Pigment epithelium-derived factor: neurotrophic activity and identification as a member of the serine protease inhibitor gene family. *Proc Natl Acad Sci USA* 1993;90:1526–1530.
- Miyagishi D, Ohno-Matsui K, Amagasa T, Morita I: Regulation of the expression of pigment epithelium-derived factor, an anti-angiogenic factor in human oral squamous cell carcinoma cell lines. *Cancer Lett* 2003;196:77–85.
- Gao G, Li Y, Zhang D, Gee S, Crosson C, Ma J: Unbalanced expression of VEGF and PEDF in ischemia-induced retinal neovascularization. *FEBS Lett* 2001;489:270–276.
- Spranger J, Osterhoff M, Reimann M, Mohlig M, Ristow M, Francis MK, Cristofalo V, Hammes HP, Smith G, Boulton M, Pfeiffer AF: Loss of the antiangiogenic pigment epithelium-derived factor in patients with angiogenic eye disease. *Diabetes* 2001;50:2641–2645.
- Wang JJ, Zhang SX, Lu K, Chen Y, Mott R, Sato S, Ma JX: Decreased expression of pigment epithelium-derived factor is involved in the pathogenesis of diabetic nephropathy. *Diabetes* 2005;54:243–250.
- Wang JJ, Zhang SX, Mott R, Knapp RR, Cao W, Lau K, Ma JX: Salutary effect of pigment epithelium-derived factor in diabetic nephropathy: evidence for antifibrogenic activities. *Diabetes* 2006;55:1678–1685.
- Fujimura T, Yamagishi S, Ueda S, Fukami K, Shibata R, Matsumoto Y, Kaida Y, Hayashida A, Koike K, Matsui T, Nakamura K, Okuda S: Administration of pigment epithelium-derived factor (PEDF) reduces proteinuria by suppressing decreased nephrin and increased VEGF expression in the glomeruli of adriamycin-injected rats. *Nephrol Dial Transplant* 2009;24:1397–1406.
- Chen HB, Jia WP, Lu JX, Bao YQ, Li Q, Lu FD, Lu W, Yu HY, Xiang KS: Change and significance of serum pigment epithelium-derived factor in type 2 diabetic nephropathy (in Chinese). *Zhonghua Yi Xue Za Zhi* 2007;87:1230–1233.
- Matsuyama K, Ogata N, Matsuoka M, Shima C, Wada M, Jo N, Matsumura M: Relationship between pigment epithelium-derived factor (PEDF) and renal function in patients with diabetic retinopathy. *Mol Vis* 2008;14:992–996.
- Tombran-Tink J, Mazuruk K, Rodriguez IR, Chung D, Linker T, Englander E, Chader GJ: Organization, evolutionary conservation, expression and unusual Alu density of the human gene for pigment epithelium-derived factor, a unique neurotrophic serpin. *Mol Vis* 1996;2:11.
- Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med* 1999;130:461–470.
- Sahin K, Onderci M, Tuzcu M, Ustundag B, Cikim G, Ozercan IH, Sriramoju V, Juturu V, Komorowski JR: Effect of chromium on carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus: the fat-fed, streptozotocin-treated rat. *Metabolism* 2007;56:1233–1240.
- Bonnet F, Cooper ME, Kawachi H, Allen TJ, Boner G, Cao Z: Irbesartan normalises the deficiency in glomerular nephrin expression in a model of diabetes and hypertension. *Diabetologia* 2001;44:874–877.
- Zheng Z, Chen H, Ke G, Fan Y, Zou H, Sun X, Gu Q, Xu X, Ho PC: Protective effect of perindopril on diabetic retinopathy is associated with decreased vascular endothelial growth factor-to-pigment epithelium-derived factor ratio: involvement of a mitochondria-reactive oxygen species pathway. *Diabetes* 2009;58:954–964.
- Liu H, Ren JG, Cooper WL, Hawkins CE, Cowan MR, Tong PY: Identification of the antivasopermeability effect of pigment epithelium-derived factor and its active site. *Proc Natl Acad Sci USA* 2004;101:6605–6610.
- Inagaki Y, Yamagishi S, Okamoto T, Takeuchi M, Amano S: Pigment epithelium-derived factor prevents advanced glycation end products-induced monocyte chemoattractant protein-1 production in microvascular endothelial cells by suppressing intracellular reactive oxygen species generation. *Diabetologia* 2003;46:284–287.
- Yamagishi S, Adachi H, Abe A, Yashiro T, Enomoto M, Furuki K, Hino A, Jinnouchi Y, Takenaka K, Matsui T, Nakamura K, Imaizumi T: Elevated serum levels of pigment epithelium-derived factor in the metabolic syndrome. *J Clin Endocrinol Metab* 2006;91:2447–2450.
- Yoshida T, Yamagishi S, Nakamura K, Matsui T, Imaizumi T, Takeuchi M, Koga H, Ueno T, Sata M: Pigment epithelium-derived factor (PEDF) ameliorates advanced glycation end product (AGE)-induced hepatic insulin resistance in vitro by suppressing Rac-1 activation. *Horm Metab Res* 2008;40:620–625.
- Crowe S, Wu LE, Economou C, Turpin SM, Matzaris M, Hoehn KL, Hevener AL, James DE, Duh EJ, Watt MJ: Pigment epithelium-derived factor contributes to insulin resistance in obesity. *Cell Metab* 2009;10:40–47.
- Remuzzi G, Schieppati A, Ruggenenti P: Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med* 2002;346:1145–1151.
- KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. *Am J Kidney Dis* 2007;49:S12–S154.
- Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM: A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. *Metabolism* 2000;49:1390–1394.

Erratum

In the article by Chen et al. entitled 'Urinary pigment epithelium-derived factor as a marker of diabetic nephropathy' [Am J Nephrol 2010;32:47–56], two errors occurred: (1) on page 47, line 8 of the abstract, 'healthy controls (n = 49)' should read 'healthy controls (n = 46)', and (2) on page 55, line 6 of the Acknowledgments, 'Foundation Youth Fund of China (30900261) to R. Li', should read 'Foundation Youth Fund of China (30900261) and Knowledge Innovation Program of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (2008KIP313) to R. Li'.