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RESEARCH ARTICLE

Serum levels of pigment epithelium-derived factor (PEDF) are positively associated with visceral adiposity in Japanese patients with type 2 diabetes

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Abstract

Background Pigment epithelium-derived factor (PEDF) inhibits endothelial cell injury. Further, serum levels of PEDF are elevated in the metabolic syndrome. These observations suggest that PEDF may be elevated as a counter-system against vascular cell damage in the metabolic syndrome. However, little is known about the regulation of PEDF in patients with diabetes. In order to clarify the determinants of serum PEDF, here, we examined the relationship between the 1-year changes in PEDF levels and those in anthropometric and metabolic variables in type 2 diabetic patients.

Methods Eighty-six consecutive outpatients with type 2 diabetes underwent a complete history and physical examination, determination of blood chemistries, and serum levels of PEDF at baseline and 1 year after. PEDF gene expression in cultured subcutaneous or omental adipocytes were analysed by quantitative real-time reverse transcription-polymerase chain reactions.

Results Multiple regression analyses revealed that waist circumference, triglycerides, creatinine, and TNF-α were independently associated with PEDF. Further, the percent changes in serum levels of PEDF during 1-year observational periods were positively correlated with those of BMI. In addition, PEDF mRNA levels in cultured adipocytes were increased in parallel to the BMI values of subjects from whom adipocytes were derived, especially in omental adipocytes.

Conclusion These results demonstrated that serum levels of PEDF were positively associated with metabolic components and TNF-α in Japanese patients with type 2 diabetes. Our present study suggests that PEDF may be generated from adipose tissues and play some role in visceral obesity in type 2 diabetic patients. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords PEDF; diabetes; obesity; atherosclerosis

Introduction

Pigment epithelium-derived factor (PEDF), a glycoprotein that belongs to the superfamily of serine protease inhibitors, was first purified from the conditioned media of human retinal pigment epithelial cells as a factor which possesses potent neuronal differentiating activity [1]. Recently, PEDF has been shown to be a highly effective inhibitor of angiogenesis in cell culture and animal models; PEDF inhibited retinal endothelial cell (EC) growth and

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migration and suppressed ischemia-induced retinal neovascularization [2,3]. In addition, PEDF has been found in vitreous, and its levels were decreased in angiogenic eye diseases, suggesting that loss of PEDF in the eye is functionally important in the pathogenesis of proliferative diabetic retinopathy [4].

We have previously found that PEDF inhibits tumor necrosis factor-α (TNF-α)-induced, angiotensin II-induced, or advanced glycation end product-induced EC injury by suppressing NADPH oxidase-mediated reactive oxygen species generation [5–10]. These findings suggest that PEDF could also exert beneficial effects on atherosclerosis by blocking the inflammatory-proliferative responses to injuries. Further, since serum PEDF levels are elevated in proportion to the accumulation of the number of the components of the metabolic syndrome in general population, PEDF may be elevated as a counter-system against vascular cell damage in the metabolic syndrome [11]. However, little is known about the regulation and role of serum PEDF in patients with diabetes. Therefore, in this study, we examined the determinants of serum PEDF levels in Japanese subjects with type 2 diabetes and then studied the relationship between the 1-year changes in serum PEDF levels and those in anthropometric, metabolic, and inflammatory variables. We further investigated whether PEDF mRNA levels in cultured subcutaneous or omental adipocytes were increased in parallel to the body mass index (BMI) values of subjects from whom adipocytes were derived, using commercially available adipocyte RNAs.

Materials and methods

Subjects

The study involved 86 consecutive outpatients with type 2 diabetes (68.4 ± 9.6 years old, 36 male and 50 female, diabetic nephropathy $n = 22$, diabetic retinopathy $n = 19$) with a mean duration of diabetes of 9.0 ± 7.1 (mean $\pm$ standard deviation (SD)) and a current hemoglobin A_{1c} (HbA_{1c}) of $7.6 \pm 1.4\%$. The diagnosis of type 2 diabetes was determined by the criteria of the ADA reported in 1997 [12]. We excluded the patients with active inflammatory diseases, acute coronary syndromes, and cancers.

Data collection

The medical history, and smoking and alcohol habits were ascertained by a questionnaire. Smoking and alcohol consumption were classified as current habitual use or not. Height and weight were measured, and BMI (kg/m^2) was calculated as an index of presence or absence of obesity. Blood pressure (BP) was measured in the sitting position (first) and supine position (second) at 3-min intervals using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 min before BP measurement. The second BP measurement with the fifth phase diastolic pressure was used for analysis.

Blood was drawn from the antecubital vein for determinants of lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides), plasma glucose, HbA_{1c}, creatinine, uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GTP). The chemistries were measured at a commercially available laboratory (Wakamatsu Medical Research Laboratory, Kitakyushu, Japan). PEDF levels were determined with an enzyme-linked immunosorbent assay (ELISA) system [11]. Inter- ($n = 17$) and intra-assay ($n = 14$) coefficient of variations of the ELISA were 4.7 and 7.3%, respectively. Recovery of the added recombinant PEDF in serum samples was $94.2 \pm 1.7\%$ (mean $\pm$ SD). The assay linearity was shown intact with serial dilution of serum. Serum levels of monocyte chemoattractant protein-1 (MCP-1), TNF-α, and adiponectin were determined with commercially available ELISA kits (R & D systems, Minneapolis, MN, USA). These samples were processed blindly. Written informed consents were obtained from all the participants.

Human cultured adipocyte RNAs

Human cultured subcutaneous or omental adipocyte RNAs isolated from subjects with various BMI values [subcutaneous adipocytes derived from subjects with normal BMI value (22.26), overweight BMI (27.6), and obese BMI (36.51) and omental adipocytes derived from subject with obese BMI value (38.6)] were purchased from Zen-Bio, Inc., Research Triangle Park, NC, USA.

Quantitative real-time reverse transcription-polymerase chain reactions (RT-PCR)

Quantitative real-time RT-PCR was performed using SYBR green reagent (Applied Biosystems, Foster city, CA, USA) according to the manufacturer's recommendation. Primers of human PEDF and β-actin were as follows: PEDF forward primer, 5′-CGACCAACGTGCTCCTGTCT-3′, PEDF reverse primer 5′-GATGTCTGGGCTGCTGATCA-3′; β-actin forward primer, 5′-GGCGCTTTTGACTCAGGATT-3′, β-actin reverse primer 5′-GGGATGTTTGCTCCAACCAA-3′.

Statistical methods

On account of skewed distributions, the natural logarithmic (ln) transformations were performed for triglycerides, glucose, and MCP-1. Mean values with upper and lower 95% confidence intervals (CI) were exponentiated and presented as geometric mean $\pm$ SD. The medications for hypertension, hyperlipidemia, and diabetes were coded as dummy variables. Univariate analysis was performed

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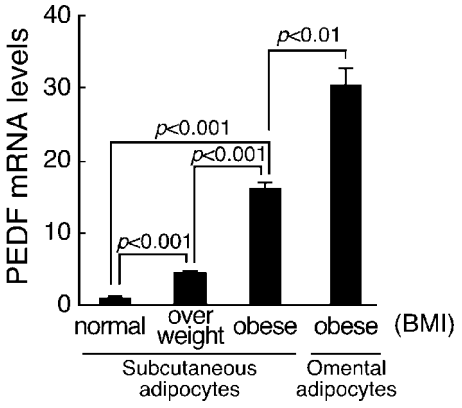

Figure 1. PEDF, leptin, and SAA mRNA levels in human cultured subcutaneous or omental adipocytes, which were commercially derived from subjects with various BMI values. Data were normalized by the intensity of β-actin mRNA-derived signals and related to the value of the normal. $n = 4–6$ per group

for determinants of serum PEDF levels. To determine independent determinants of PEDF levels, multiple stepwise linear regression analysis was performed. Relations between 1-year changes in serum PEDF levels against baseline and those in anthropometric, metabolic, and inflammatory variables were also examined by multiple linear regression analysis. All clinical statistical analyses were performed with the use of the SPSS system. In Figure 1, one-way ANOVA followed by the Scheffe F test, was performed for statistical comparisons. Statistical significance was defined as $p < 0.05$.

Results

Clinical characteristics of the patients are presented in Table 1. The mean serum levels of PEDF in this study population were 14.9 ± 4.6 μg/mL (range 7.87–28.2 μg/mL). Table 2 shows determinants of serum PEDF levels by stepforward logistic regression analysis. Univariate analysis for determinants of serum level of PEDF revealed that it was statistically and significantly correlated with BMI ($r = 0.312$, $p = 0.003$), waist circumference ($r = 0.326$, $p = 0.003$), AST ($r = 0.221$, $p = 0.041$), triglycerides ($r = 0.366$, $p < 0.001$), creatinine ($r = 0.393$, $p < 0.0001$), uric acid ($r = 0.385$, $p < 0.0001$), TNF-α ($r = 0.364$, $p < 0.001$), hyperlipidemia medication ($r = 0.245$, $p = 0.023$) and diabetes medication ($r = -0.301$, $p = 0.005$). As these significant parameters were closely correlated with each other, these variables were included and multiple linear regression analysis was performed. Finally, triglycerides ($p < 0.0001$), creatinine ($p < 0.0001$), waist circumference ($p = 0.012$), and TNF-α ($p = 0.029$) remained significant and were independently related to serum levels of PEDF ($R^2 = 0.381$).

We next examined the relationship between the 1-year changes in PEDF levels and those in BMI, AST, ALT, GTP, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, creatinine, glucose, HbA_{1c}, TNF-α, systolic, and diastolic BPs in multiple linear regression analysis. Over the 1 year, the mean serum PEDF levels decreased by 1.3 μg/mL. Further, the 1-year PEDF changes were positively and significantly associated with those of BMI ($r = 0.394$, $p < 0.001$). None of the changes in other variables were significantly correlated with the changes in PEDF.

In addition, PEDF mRNA levels in cultured adipocytes were increased in parallel to the BMI values of subjects from whom adipocytes were derived, especially in omental adipocytes (Figure 1).

Table 1. Clinical characteristics of the patients

Characteristics	$n = 86$
Age (years)	68.4 ± 9.6
Sex (%male)	36 (41.9%)
BMI (kg/m^2)	24.7 ± 4.1
Waist circumference (cm)	90.2 ± 9.3
PEDF (μg/mL)	14.9 ± 4.6
AST (IU/L)	22.9 ± 8.0
ALT (IU/L)	24.2 ± 17.0
γ-GTP (IU/L)	40.7 ± 43.1
Total cholesterol (mg/dL)	201.9 ± 32.1
Triglyceride (mg/dL)[a]	126.4 ± 15.7
HDL-cholesterol (mg/dL)	50.2 ± 12.6
LDL-cholesterol (mg/dl)	130.9 ± 30.5
Creatinine (mg/dL)	0.89 ± 0.28
Glucose (mg/dL)[a]	172.0 ± 14.4
HbA_{1c} (%)	7.6 ± 1.4
Systolic BP (mmHg)	132.2 ± 10.3
Diastolic BP (mmHg)	75.2 ± 7.2
Uric acid (mg/dL)	5.0 ± 1.4
MCP-1 (pg/mL)[a]	308.5 ± 1.3
Adiponectin (μg/mL)	3.8 ± 3.3
TNF-α (pg/mL)	1.16 ± 0.66
Frequency (%)	
Current smoking (% yes)	12 (14%)
Alcohol intake (% yes)	25 (29.1%)
DM medication (% yes)	57 (66.3%)
HT medication (% yes)	65 (75.6%)
HL medication (% yes)	24 (27.9%)

Values are mean $\pm$ SD or percentage, unless indicated otherwise.
[a]Log-transformed values were used.

Discussion

In the present study, we demonstrated for the first time that waist circumference, triglycerides, creatinine, and TNF-α were significant independent determinants of serum PEDF levels in type 2 diabetic patients and that 1-year changes in PEDF were positively correlated with those in BMI. The present findings have extended our previous observations showing that serum PEDF levels were associated with the components of the metabolic syndrome in general population and that the levels were significantly higher in patients with end-stage renal disease than in control subjects [11,13]. Therefore, given the fact that PEDF possesses anti-oxidative and anti-inflammatory properties [5–10], the present observations further support the concept that serum PEDF levels may be elevated as a counter-system against coronary risk factors in the metabolic syndrome and/or type 2 diabetes

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and that kidney may be a main organ for the clearance of PEDF in the circulation.

Univariate analysis revealed that PEDF was associated with several anthropometric and metabolic risk factors (Table 2). However, it may be interesting to note that strong well-known risk factors such as age, BP, and smoking were not related to PEDF. Multiple stepwise regression analysis revealed that factors related to visceral obesity such as waist circumference, triglycerides, and TNF-α were significantly and independently related to serum PEDF levels in type 2 diabetes (Table 2). Further, although the data of waist circumference was not available in this study, 1-year changes in serum PEDF levels were positively correlated with changes in BMI in our subjects. In addition, PEDF mRNA levels in cultured adipocytes were increased in parallel to the BMI values of subjects from whom adipocytes were derived, especially in omental adipocytes (Figure 1). Recently, it is reported that PEDF is synthesized by adipose tissue and its level is down-regulated during the differentiation process to mature adipocytes [14]. Since this expression pattern is opposite to that of adiponectin, adipocyte PEDF production may be increased in visceral obesity in contrast to the case of adiponectin [15]. Taken together, although adiponectin level is *not* inversely associated with PEDF in our subjects, the present findings suggest that immature adipocytes in visceral adipose tissues may be one of the main origins of serum PEDF in diabetes. In addition, it should be noted that liver may be another source of circulating PEDF in humans. Indeed, expression level of PEDF in the liver is found to be high in humans and mice [16,17]. Further, expression level of PEDF in porcine liver is associated with body muscularity and obesity as well [18]. Production of PEDF by the liver may also be regulated by visceral adiposity.

Table 2. Determinants of PEDF by stepforward logistic regression analysis in diabetic patients

	Univariate[a]		Multivariate[b]		
Factors	β	P	β	F	P
Age (years)	−0.069	NS			
Sex (% male)	−0.140	NS			
BMI (kg/m^2)	0.312	0.003			
Waist circumference (cm)	0.326	0.003	0.243	0.048	0.012
AST (IU/L)	0.221	0.041			
ALT (IU/L)	0.175	NS			
GTP (IU/L)	0.114	NS			
Total cholesterol (mg/dL)	−0.006	NS			
Triglycerides (mg/dL)[c]	0.366	<0.001	0.347	0.006	<0.0001
HDL-cholesterol (mg/dL)	−0.106	NS			
LDL-cholesterol (mg/dL)	−0.092	NS			
Creatinine (mg/dL)	0.393	<0.001	0.407	1.681	<0.0001
Glucose (mg/dL)[c]	−0.019	NS			
HbA_{1c} (%)	0.028	NS			
Systolic BP (mmHg)	−0.007	NS			
Diastolic BP (mmHg)	0.053	NS			
Uric acid (mg/dL)	0.385	<0.0001			
MCP-1 (pg/mL)	0.054	NS			
Adiponectin (μg/mL)	0.031	NS			
TNF-α (pg/mL)	0.364	<0.001	0.210	0.651	0.029
HT medication (% yes)	0.048	NS			
HL medication (% yes)	0.245	0.023			
DM medication (% yes)	−0.301	0.005			
Smoking (% yes)	0.123	NS			
Alcohol (% yes)	0.113	NS			
R^2			0.381		

[a]Univariate coefficients.
[b]A stepwise multivariate regression analysis was performed.
[c]Log-transformed values were used.
β: Regression coefficients.
NS, not significant.

Limitations

First, although our present data suggest the positive association of factors related to visceral adiposity with serum levels PEDF in type 2 diabetic patients, the degree of visceral obesity in our subjects was modest (mean waist circumference, 90.2 cm; mean BMI, 24.7 kg/m^2). Since the relationship of visceral fat deposition to metabolic complications vary between populations and that Asians are quite prone to visceral adiposity [19], whether PEDF levels are positively correlated with visceral obesity in other ethnic populations remains unknown. Second, this study could not assess the questions of whether elevation of PEDF was a cause or consequence of visceral obesity in type 2 diabetes. Moreover, clinical and pathophysiological significance of elevated serum PEDF levels in diabetes remains to be clarified. Future longitudinal and interventional studies are needed to solve these issues. Finally, although we have showed here that gene expression level of adipocyte PEDF is higher in obese people, and higher in omental tissues than subcutaneous ones, whether these regulations are primary or secondary remains unknown. Further study is needed to clarify how adipocyte PEDF gene is regulated and whether it is correlated with leptin and serum amyloid A, two adipokines known to be positively associated with BMI [20,21].

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Conflict of interest

None declared.

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