Inhibitory effects of adiponectin on platelet-derived growth factor-induced mesangial cell migration

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Abstract

Adiponectin, an adipocyte-derived hormone, has been involved in metabolic syndrome, a known risk factor for the development of chronic kidney disease (CKD). Recent studies have demonstrated that plasma adiponectin levels are elevated when kidney function declines in patients with CKD. Excessive mesangial cell (MC) turnover is one of the important features of CKD. The aim of the present study is to elucidate the effects of adiponectin on platelet-derived growth factor (PDGF)-induced cell migration and intracellular signaling pathways, in cultured rat MCs (RMCs). PDGF-induced RMC migration was significantly inhibited by the pretreatment of adiponectin. Adiponectin alone had no effect on RMC migration. Big mitogen-activated protein (MAP) kinase 1 (BMK1), p38 MAP kinase, and Akt were activated by PDGF stimulation in a time- and concentration-dependent manner in

RMC. Adiponectin alone did not affect BMK1, p38 MAP kinase, and Akt phosphorylations in RMC. PDGF-induced BMK1 and p38 MAP kinase phosphorylations were significantly attenuated by the pretreatment of adiponectin in RMCs. On the other hand, the phosphorylation of Akt by PDGF was not diminished by the pretreatment of adiponectin. Adiponectin had no effects on PDGF-receptor autophosphorylation by PDGF. We also confirmed that PDGF-induced RMC migration was significantly suppressed by siBMK1 transfection or SB203580, a p38 MAP kinase inhibitor. From these findings, it is implied that the elevated plasma adiponectin levels in patients with CKD might play a compensatory role aimed at counteracting renal dysfunction related to MC disorders.

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Introduction

Metabolic syndrome (MS) is known to be associated with obesity, type 2 diabetes mellitus, and hypertension, and the coexistence of these diseases leads to cardiovascular disease (CVD; Sowers *et al.* 2001, Lorenzo *et al.* 2003, Ninomiya *et al.* 2004, Weiss *et al.* 2004). In addition, it was reported that MS was a significant risk factor for the development of chronic kidney disease (CKD; Chen *et al.* 2004, Kurella *et al.* 2005, Tozawa *et al.* 2007). It was also showed that adipocytes were capable of releasing numerous vasoactive factors, as well as other cells that present within fat tissues, leading to CKD and CVD in MS (Chu *et al.* 2001, Yaturu *et al.* 2007). Adiponectin is a novel adipocyte-derived hormone, which has been proposed to play an important role in the regulation of energy homeostasis and insulin sensitivity (Kadowaki *et al.* 2006). Plasma adiponectin levels are inversely correlated with obesity (Arita *et al.* 1999, Asayama *et al.* 2003), coronary artery disease (Hotta *et al.* 2000), and insulin resistance (Weyer *et al.* 2001, Pellme *et al.* 2003). The effects of adiponectin against atherosclerosis have been demonstrated

in previous studies (Matsuda *et al.* 2002, Ouedraogo *et al.* 2006). In *in vitro* studies, adiponectin has been reported to inhibit platelet-derived growth factor (PDGF)- or heparin-binding epidermal growth factor-like growth factor-induced vascular smooth muscle cell (VSMC) migration (Arita *et al.* 2002). Other studies confirmed that adiponectin knockout mice showed severe neointimal thickening and increased proliferation of VSMC in response to mechanically vascular injury (Kubota *et al.* 2002, Matsuda *et al.* 2002). It was reported that plasma adiponectin levels are elevated when renal function is impaired – as early as CKD stage III – and particularly in proteinuric patients (Guebre-Egziabher *et al.* 2005). Another study demonstrated that plasma adiponectin levels were markedly elevated in patients with end-stage renal disease (Stenvinkel *et al.* 2004). It has been considered that the rise in plasma adiponectin that occurs during renal function deterioration represents an adaptive response to the altered metabolic profile associated with a high cardiovascular risk in CKD patients. However, the role of elevated plasma adiponectin levels in patients with CKD against renal dysfunction has not been elucidated.

Cell migration and cell proliferation are fundamental responses of mesangial cells (MCs) to glomerular injury and contribute to the hypercellularity observed in a number of glomerular diseases. Increased glomerular MC turnover is a hallmark of CKD (Striker *et al.* 1989). MC migration caused by various vasoactive substances is known to be critical for the development of glomerular sclerosis and also for the ECM organization process (Kagami *et al.* 1999, Kohno *et al.* 1999, Ishizawa *et al.* 2004, Takeuchi *et al.* 2006). In addition, the initial phase of the anti-Thy 1 nephritis model in animals characterized by mesangiolysis is followed by migration and proliferation of MCs (Bagchus *et al.* 1990). PDGF has been recognized as a major mitogen and one of the most important growth factors, which mediates multiple cellular activities such as cell proliferation and extracellular matrix protein synthesis in a variety types of cells including MCs (Floege *et al.* 1993, Isaka *et al.* 1993). Furthermore, the hemodialysis patients showed a significant increase in plasma concentration of PDGF (De Marchi *et al.* 1996). Previous studies have shown that PDGF-induced cell migration is mediated by mitogen-activated protein (MAP) kinase family members, phosphatidylinositol 3-kinase (PI3K)/Akt and many other kinases (Kagami *et al.* 2004, Harper *et al.* 2007). In addition to the classical MAP kinases, i.e. extracellular signal-regulated kinase 1/2 (ERK1/2), JNK, and p38, recently, big MAP kinase 1 (BMK1)/ERK5 was identified as a new MAP kinase family member that is activated by MAP kinase/ERK kinase 5 (MEK5; Lee *et al.* 1995). Recently, we reported that BMK1 is activated in the glomeruli of diabetic rats and that a high concentration of glucose stimulates BMK1 activation and the proliferation of MCs (Suzaki *et al.* 2004). PDGF concentration was elevated in the plasma, and its mRNA expression was increased in adipose tissues in obese mice (Pang *et al.* 2008). Also diabetic obese rats showed increased PDGF protein expression and MC number in glomerulus (Gross *et al.* 2004). Therefore, in MS, PDGF plays an important role in kidney disease including MC disorder. Although it was reported that adiponectin attenuated PDGF-induced VSMC migration (Arita *et al.* 2002), there has been no report on whether adiponectin affects the activation of MCs. We hypothesized that elevated adiponectin in patients with CKD was involved in the progression of renal injury, such as migration and proliferation in MCs. The objective of the present study was to investigate the effects of adiponectin on PDGF-induced cell migration and intracellular signaling pathway in cultured rat MCs (RMCs).

Materials and Methods

Chemicals

PDGF-BB and SB203580, a p38 MAP kinase inhibitor, were purchased from Sigma. Murine adiponectin, recombinant protein was purchased from Biovendor Laboratory Medicine, Inc. (Modrice, Czech Republic). Anti-phospho-big MAP

kinase 1 (BMK1; Thr218/Tyr220) antibody, which recognizes the activated form of BMK1 (Lee *et al.* 1995), anti-BMK1 antibody, anti-phospho-p38 MAP kinase (Thr180/Tyr182) antibody, anti-p38 MAP kinase antibody, anti-PDGF receptor β -antibody, phospho-Akt (Ser473) antibody, and Akt antibody were from Cell Signaling Technology, Inc. (Beverly, MA, USA). Antibody for phosphotyrosine (clone 4G10) was from Upstate Biotechnology, Inc. (Lake Placid, NY, USA). All other chemicals were of reagent grade, obtained from commercial sources, and used without further purification.

Cell culture and transfection

RMCs were isolated from intact glomeruli of 4- to 6-week-old male Sprague–Dawley rats and maintained in 18% fetal bovine serum (FBS) and RPMI 1640 as described previously (Ishizawa *et al.* 2004). For the experiments, cells from passages 5 to 9 were used after 24–48 h of serum depletion. For small interfering RNA (siRNA) assay, RMCs were transiently transfected with lamin A/C siRNA as a control or BMK1-specific siRNA, a cocktail of three siRNAs designed by B-Bridge International, Inc. (Mountain View, CA, USA), by Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. Briefly, 5 μ l Lipofectamine 2000 was diluted in 250 μ l Opti-MEM I Reduced Serum Medium (Invitrogen), incubated for 5 min at room temperature, and mixed with a variable amount of BMK1-specific siRNA (final concentrations: 50 nM). After 30-min incubation at room temperature, the mixture was added to each well of RMCs, which were plated into six-well plates. After 24 h, cells were washed and fresh medium that contained 10% FBS was added. Thirty-six hours after transfection, cells were treated with or without PDGF and/or adiponectin.

RMC migration assays

To assess cell migration, a modified Boyden chamber assay was performed with minor modification to the method described previously (Ishizawa *et al.* 2004). The assay was performed using Transwell chambers (6.5 mm, model 3422; Costar, Cambridge, MA, USA) with an 8 μ m pore polycarbonate membrane. The underside of the polycarbonate membrane was coated with 5 μ g/ml collagen I rat tail (3.78 mg/ml, model 35-4236; Collaborative Biochemical Products, Bedford, MA, USA) overnight at 4 °C. Then the lower chamber was blocked with RPMI 1640/0.1% BSA for 30 min at room temperature. Quiescent control cells or siRNA-transfected cells were harvested, washed, and suspended in serum-free RPMI 1640. Cells were added to the upper chamber of the Transwell at 5×10^5 cells in 100 μ l/well. A total of 600 μ l serum-free RPMI 1640 was added to the lower chamber and then pretreated with or without adiponectin or SB203580 in the lower chamber for 30 min at 37 °C followed by treatment with or without PDGF in the lower chamber, and cells were allowed to migrate at 37 °C. After 6 h of incubation, the nonmigratory

cells were removed from the upper surface of the membrane by scraping off with cotton swabs. Membrane was fixed with methanol, stained with Diff-Quik solution (Baxter, McGaw Park, IL, USA), and allowed to air-dry at room temperature. Then, the membrane was excised from the plastic supports with a sharp scalpel and mounted on a glass slide using Permount (Sigma Chemical). Cells that had migrated from the upper to the lower side of the membrane were counted at 400 $\times$ magnification in 10 microscope fields per filter.

Preparation of cell lysate for immunoprecipitation and immunoblotting

Quiescent control cells or siRNA-transfected cells were treated with or without PDGF, adiponectin, and SB203580 at the indicated concentrations for the indicated periods of time. Cells were pretreated with adiponectin or SB203580 at the indicated concentration for 30 min. Then the cells were stimulated with 10 ng/ml PDGF for 5 min for p38 MAP kinase phosphorylation and 10 min for BMK1 and Akt phosphorylations. Then, incubation media were discarded, and the cells were lysed with cell lysis buffer (20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM sodium orthovanadate, 1 μ g/ml leupeptin, and 1 mM phenylmethylsulfonyl fluoride). After freeze-thawing, lysed cells were transferred to microcentrifuge tubes, sonicated (Handy Sonic UR-20 P; Tomy Seiko Co., Ltd, Tokyo, Japan) on ice, and centrifuged at 20 000 g for 20 min at 4 $^{\circ}$ C. The protein concentrations of the supernatants were measured with a protein assay kit (Bio-Rad) and stored at -80° C until performing immunoprecipitation and immunoblotting.

Immunoprecipitation and immunoblotting

For immunoprecipitation, lysates containing equal amounts of protein were incubated with anti-PDGF receptor β -antibody overnight and then incubated with protein A/G PLUS-agarose beads for 2 h on a roller system at 4 $^{\circ}$ C. After immunoprecipitation, samples were subjected to immunoblot assay using anti-phosphotyrosine antibody (clone 4G10). For western blot analysis, cell lysates were subjected to SDS-PAGE, and proteins were transferred to nitrocellulose membranes (Hybond-ECL; Amersham Pharmacia Biotech), as described previously. The membranes were blocked for 1 h at room temperature with 5% BSA. The blots were then incubated for 12 h with 4G10 antibody, anti-phospho-specific BMK1, anti-phospho-specific p38 MAP kinase, and anti-phospho-specific Akt followed by incubation for 1 h with a secondary antibody (HRP conjugated). Immunoreactive bands were visualized using enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech) and were quantified by densitometry in the linear range of film exposure using an Epson Perfection 2580 Photo Scanner (Epson America, Inc., Long Beach, CA, USA) and imageJ 1.37v software.

Statistical analysis

Values are reported as the means $\pm$ s.d. from experiments performed on five different occasions. Two-way ANOVA was used to determine the significance among groups, after which a modified *t*-test with Bonferroni's *post hoc* test was used for comparisons between individual groups. A value of $P < 0.05$ was considered to be statistically significant.

Figure 1 PDGF-induced RMC migration. (A) Concentration-dependent increase in PDGF-induced RMC migration. Cells were treated with indicated concentration of PDGF for 6 h. And then, modified Boyden chamber assay was performed as described under Materials and Methods (values are the means $\pm$ s.d., $n=5$, $*P < 0.05$). (B) Adiponectin concentration-dependent suppression of PDGF-induced RMC migration. Cells were treated with indicated concentration of adiponectin 30 min prior to treatment with PDGF (10 ng/ml) for 6 h (values are the means $\pm$ s.d., $n=5$, $*P < 0.05$ versus PDGF 10 ng/ml without adiponectin).

Results

Adiponectin inhibited PDGF-induced RMC migration

We confirmed that PDGF increased RMC migration in a concentration-dependent manner (0.1–10 ng/ml; Fig. 1A) by modified Boyden chamber assay as described in the Materials and Methods section. We also discovered that stimulation with adiponectin alone had no effects on RMC migration at any

physiological doses (0.1, 0.3, 1, 3, and 10 µg/ml; data not shown). To examine whether adiponectin affect PDGF-induced RMC migration, growth-arrested RMCs were treated with or without adiponectin (1, 3 and 10 µg/ml) 30 min prior to stimulation with PDGF (10 ng/ml, 6 h). As shown in Fig. 1B, adiponectin pretreatment significantly suppressed PDGF-induced RMC migration (3 and 10 µg/ml).

Adiponectin attenuated PDGF-induced phosphorylation of BMK1 and p38 MAP kinase, but not Akt

To evaluate the relative magnitude of BMK1, p38 MAP kinase, and Akt activities by PDGF, RMCs were exposed to PDGF (10 ng/ml) for the indicated periods of time. PDGF rapidly activated p38 MAP kinase (peak at 5 min) and then gradually declined (Fig. 2A). BMK1 and Akt were activated within 10 min by PDGF stimulation (peak at 5 and 10 min respectively; Fig. 2A). The phosphorylations of these kinases showed PDGF concentration-dependent increase at each peak periods of time (Fig. 2B). As shown in Fig. 3, exposure with adiponectin alone had no effects on the phosphorylation of BMK1, p38 MAP kinase, and Akt in RMCs. However, pretreatment of adiponectin (3 and 10 µg/ml) significantly inhibited PDGF-induced phosphorylation of BMK1 and p38 MAP kinase, but not Akt. From these results, it was suggested that the inhibitory effects of adiponectin on PDGF-induced RMC migration was involved in the suppression of BMK1 and p38 MAP kinase phosphorylations. Furthermore, we confirmed that adiponectin did not suppress PDGF receptor autophosphorylation by PDGF stimulation (Fig. 4). These findings indicated that the inhibition of PDGF-induced kinase activities by adiponectin was the intracellular event in RMCs.

PDGF-induced RMC migration was suppressed by siBMK1 transfection or SB203580

To confirm that PDGF-induced RMC migration was mediated by BMK1 and p38 MAP kinase activations, siBMK1 transfection technique or pretreatment with SB203580, a p38 MAP kinase inhibitor, was used for the

Figure 2 (A) Time courses and (B) concentration response of PDGF-induced BMK1, p38 MAP kinase, and Akt phosphorylations in RMCs. Cells were treated with 10 ng/ml PDGF for the indicated periods of time (A) and were stimulated with the indicated concentrations of PDGF for 5 min for BMK1 and p38 MAP kinase, and 10 min for Akt (B). The cell lysates (50 µg protein) at the indicated time points were subjected to western blot analysis. No significant differences in the amounts of total BMK1, p38 MAP kinase, and Akt were observed in western blot analysis with anti-BMK1, anti-p38 MAP kinase, and anti-Akt antibodies respectively. Upper panels show the representative blots. Lower graphs show the results of densitometric analysis. Closed circle, BMK1; triangle, p38 MAP kinase; open circle, Akt (A); open column, BMK1; gray column, p38 MAP kinase; solid column, Akt (B). Values were normalized by arbitrarily setting the densitometry of control cells (time=0, without PDGF) to 1.0.

inhibition of BMK1 and p38 MAP kinase respectively as described in Materials and Methods. The cells were transfected with BMK1 siRNA (5, 50 nM) and lamin siRNA (50 nM) as a negative control. The expression of BMK1 protein was significantly suppressed in RMCs treated with BMK1 siRNA, not lamin siRNA (Fig. 5A). In siBMK1-transfected RMCs, PDGF-induced BMK1 phosphorylation was significantly decreased, but phosphorylations of p38 and Akt were not affected, compared with PDGF-stimulated mock-transfected control cells (Fig. 5B). Lamin siRNA transfection had no effects on phosphorylations of BMK1, p38, and Akt. The pretreatment with 1 μ M SB203580 significantly suppressed PDGF-induced p38 MAP kinase phosphorylation in RMCs. (Fig. 5C). It was reported that SB203580 is not specific to p38 and that the higher concentration of SB203580 can also block Akt activation (IC₅₀ 3–5 μ M; Lali *et al.* 2000). However, it was confirmed that 1 μ M SB203580 had effects neither on PDGF-induced BMK1 nor on Akt phosphorylation in our present sets of experiments (Fig. 5C).

Figure 3 Effects of adiponectin on PDGF-induced BMK1, p38 MAP kinase, and Akt phosphorylations in RMCs. Cells were pretreated with indicated concentration of adiponectin 30 min prior to treatment with PDGF (10 ng/ml). The cell lysates (50 μ g protein) at the indicated time points were subjected to western blot analysis. No significant differences in the amounts of total BMK1, p38 MAP kinase, and Akt were observed in western blot analysis with anti-BMK1, anti-p38 MAP kinase, and anti-Akt antibodies respectively. Upper panels show the representative blots. Lower graphs show the results of densitometric analysis. Open column, BMK1; gray column, p38 MAP kinase; solid column, Akt values were normalized by arbitrarily setting the densitometry of control cells (without PDGF) to 100% (values are the means $\pm$ s.d., $n=5$, * $P<0.05$ versus PDGF 10 ng/ml without adiponectin).

Figure 4 Effects of adiponectin on PDGF-induced PDGF receptor tyrosine phosphorylation in RMCs. Cells were treated with adiponectin 30 min prior to treatment with PDGF (10 ng/ml). Tyrosine phosphorylation of PDGF receptor was measured by immunoprecipitation assay with phosphotyrosine-specific antibody, 4G10 as described in Materials and Methods. Upper panel shows the representative blot. Lower graph shows the results of densitometric analysis. The cell lysates (50 μ g protein) at the indicated time points were subjected to western blot analysis. Values were normalized by arbitrarily setting the densitometry of control cells (without PDGF) to 100% (values are the means $\pm$ s.d., $n=5$). PDGFR, PDGF receptor; IP, immunoprecipitation; IB, immunoblotting.

As shown in Fig. 5D, both siBMK1 transfection and SB203580 pretreatment significantly inhibited PDGF-induced RMC migration in part. In addition, the combination of siBMK1 transfection and SB203580 almost completely inhibited RMC migration to the control level. Lamin siRNA transfection had no effects on PDGF-induced RMC migration. From these results, it was suggested that the combination of siBMK1 transfection and SB203580 plays a synergistic inhibitory effect on PDGF-induced RMC migration.

Discussion

In the present study, we demonstrated that adiponectin inhibited PDGF-induced RMC migration through the suppression of BMK1 and p38 MAP kinase activations. Furthermore, adiponectin selectively inhibited BMK1 and p38 MAP kinase pathway, not Akt pathway, stimulated by PDGF in RMCs. From these results, it was suggested that adiponectin can attenuate the renal dysfunction related with MC disorders.

PDGF is a mitogen that causes MC migration, which plays an important role in the changes in glomerular morphology in CKD. We have confirmed in this study that PDGF causes RMC migration in concentration-dependent manner

Figure 5 Effects of siBMK1 transfection and SB203580 on PDGF-induced RMC migration. Cells were transfected with 50 nM siBMK1 or pretreated with 1 μ M SB203580 for 30 min, followed by 10 ng/ml PDGF stimulation as described in Materials and Methods. (A) BMK1 expression was measured by western blotting analysis. Cells were transfected with BMK1 siRNA and lamin siRNA as a negative control. No significant differences in the amount of β -actin were observed in lysates by immunoblotting with anti- β -actin antibody. (B) PDGF-induced BMK1, p38 MAP kinase, and Akt phosphorylation in RMCs. No significant differences in the amount of β -actin were observed in lysates by immunoblotting with anti- β -actin antibody. Panels show the representative blots. The cell lysates (50 μ g protein) at the indicated time points were subjected to western blot analysis. Upper panels show the representative blots. Lower graphs show the results of densitometric analysis. Values were normalized by arbitrarily setting the densitometry of control cells (time=0, without PDGF) to 1.0 (values are the means $\pm$ s.d., $n=5$, $*P<0.05$ versus the values of PDGF stimulation in mock-transfected cells). Open column, BMK1; gray column, p38 MAP kinase; solid column, Akt (C) PDGF-induced p38 MAP kinase phosphorylation in RMCs. No significant differences in the amount p38 MAP kinase were observed in lysates by immunoblotting with anti-p38 MAP kinase antibody. Panels show the representative blots. The cell lysates (50 μ g protein) at the indicated time points were subjected to western blot analysis. Upper panels show the representative blots. Lower graphs show the results of densitometric analysis. Values were normalized by arbitrarily setting the densitometry of control cells (time=0, without PDGF) to 1.0 (values are the means $\pm$ s.d., $n=5$, $*P<0.05$ versus PDGF 10 ng/ml without SB203580). Open column, BMK1; gray column, p38 MAP kinase; solid column, Akt (D) PDGF-induced RMC migration. Values were normalized by arbitrarily setting the fold increases in the migration of control cells (without PDGF treatment) to 1.0 (values are the means $\pm$ s.d., $n=5$, $*P<0.05$ versus PDGF 10 ng/ml alone).

demonstrating increased MC proliferation and glomerular PDGF expression in the five out of six nephrectomized rats, which were well-established models of CKD (Floege *et al.* 1992). These findings suggest that PDGF-induced RMC disorders in the glomeruli may contribute to glomerular

injury leading to CKD. As shown in Fig. 1B, we clarified that PDGF-induced RMC migration was significantly suppressed by pretreatment with adiponectin. It was reported that adiponectin associated with PDGF, and that adiponectin blocked the binding between PDGF and its receptor (Arita *et al.* 2002). However, as we have shown in Fig. 4, adiponectin had no effect on PDGF receptor autophosphorylation by PDGF stimulation. These results suggest that the inhibitory effects of adiponectin on PDGF-induced kinase activities are the intracellular event in RMCs. To the best of our knowledge, this is the first report about the inhibitory effects of adiponectin on PDGF-induced RMC migration. These findings indicate that adiponectin might have beneficial effects against renal dysfunction including MC disorders. In some studies, it was reported that plasma adiponectin levels were elevated in patients with CKD (Stenvinkel *et al.* 2004, Guebre-Egziabher *et al.* 2005). It is suggested that the elevated plasma adiponectin levels can work as a compensatory mechanism to attenuate the progression of glomerular dysfunction in the patients with CKD.

To explore these inhibitory mechanisms of adiponectin on cell migration, we examined the effect of adiponectin on the PDGF-induced cellular signaling involved in RMC migration. It has been reported that PDGF-induced MC migration is mediated by MAP kinases, PI3K/Akt, and many other kinases (Kagami *et al.* 2004, Harper *et al.* 2007). As shown in Fig. 2A and B, BMK1, p38 MAP kinase, and Akt were activated by PDGF in RMCs in a time- and concentration-dependent manner. We also revealed that adiponectin attenuated PDGF-induced BMK1 and p38 MAP kinase phosphorylations, but not Akt in RMCs (Fig. 3). Therefore, we confirmed that siBMK1 transfection or SB203580 suppressed PDGF-induced RMC migration (Fig. 5C). These findings indicate that PDGF-induced RMC migration is mediated by BMK1 and p38 MAP kinase activations. In several studies, it was shown that the activation of BMK1 led to cell proliferation, morphogenesis, anti-apoptosis, and so on (Karihaloo *et al.* 2001, Pi *et al.* 2004, Barros & Marshall 2005). We have already demonstrated that BMK1 is activated in the glomeruli of diabetic rats and that a high concentration of glucose stimulates RMC proliferation via BMK1 activation (Suzaki *et al.* 2004). Moreover, we have also reported that PDGF-induced BMK1 activation leads to VSMC migration (Izawa *et al.* 2007). These reports support our new findings that BMK1 activation mediates PDGF-induced cell migration in RMCs. Meanwhile, p38 MAP kinase also has a critical role in various cellular functions including apoptosis. It was reported that increased p38 MAP kinase signaling was a feature of human and experimental diabetic nephropathy (Adhikary *et al.* 2004). We have already reported that SB203580, a p38 MAP kinase inhibitor, suppressed cell migration induced by vascular endothelial growth factor in human umbilical vein endothelial cells (Ali *et al.* 2005). In addition, consistent with our present findings, it has been reported that PDGF-induced p38 MAP kinase activation regulated cell migration in VSMCs

(Zhan *et al.* 2003, Yamaguchi *et al.* 2004). We have already demonstrated that adiponectin inhibited IGF-1-induced cell migration through suppression of ERK1/2 activation, which might be implicated in AMPK activation in VSMCs (Motobayashi *et al.* 2009). However, detailed mechanisms including signaling crosstalk have not yet been studied in RMCs. Thus, to further elucidate mechanisms of adiponectin, effects on various cellular responses by PDGF through the suppression of BMK1 and p38 MAP kinase have importance.

In conclusion, we have demonstrated the inhibitory effects of adiponectin on PDGF-induced MC migration. These findings suggest that the elevated plasma adiponectin levels in patients with CKD might play a compensatory role aimed at counteracting renal dysfunction. Further studies to elucidate the precise mechanisms by which adiponectin exerts potential therapeutic effects on CKD via the suppression of MC disorder are currently under way.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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