Regulation of Blood Pressure and Phosphorylation of β1-integrin in Renal Tissue in a Rat Model of Diabetic Nephropathy

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Objective: Induction of hypertension by diabetic nephropathy (DN) may be dependent on increased renin secretion from juxtaglomerular cells (JGC). To reveal that the mechanisms of cell adhesion and cell motility associated with β1-integrin phosphorylation contribute to pressure sensing in JGC, we tested the β1-integrin phosphorylation levels in renal tissue and the relationship between β1-integrin phosphorylation and the expression of renin.

Methods: The DN rat model was generated by intravenous injection of streptozotocin (STZ, 60 mg/kg body weight). Immunohistochemistry and an imaging analysis were performed to detect and evaluate the β 1-integrin phosphorylation levels in renal tissue. Quantitative real-time polymerase chain reaction was also performed to evaluate renin mRNA levels.

Results: We found that the serine-785 and threonine-788/789 sites of β1-integrin are specifically phosphorylated in macula densa and JGC, respectively, and that changes in their expression during the progression of DN are associated with the production of renin. Phosphorylation of these β1-integrins increased or decreased with changes of the renin expression during the progression of DN. In particular, phosphorylation of threonine-788/789 was negatively correlated with the expression of renin.

Conclusion: These findings suggest that the phosphorylation of β1-integrin may contribute to the regulatory mechanism of renin production in JGC.

Key words: diabetic nephropathy, integrin, phosphorylation of β1-integrin, renin

INTRODUCTION

It is widely known that diabetic nephropathy (DN) and hypertension aggravate each other [1–6]. Diabetes mellitus (DM) impairs the myogenic response [7, 8] and transforming growth factor (TGF) response [9], which are functions that autoregulate renal blood flow, resulting in the dilatation of afferent arteries, inducing glomerular hyperfiltration and glomerular capillary hypertension [3]. Hypertension causes oxidative stress and inflammation in the kidney, leading to the development of DN [4]. The activation of the renin-angiotensin-aldosterone system (RAAS) [10], upregulation of endothelin-1 (ET-1) [11, 12], upregulation of reactive oxygen species [13, 14], and downregulation of nitric oxide (NO) [15, 16], with worsening of the renal function further contribute to blood pressure elevation, leading to a vicious cycle of hypertension and glomerular damage.

Renin is the rate-limiting enzyme that is involved in the regulation of blood pressure by the RAAS, and is the key to the vicious cycle of renal function decline and hypertension. Studies of juxtaglomerular cells (JGC) have revealed several mechanisms of renin secretion, including the following: (a) stimulation of renin secretion via β-adrenergic receptors by excitation of renal sympathetic nerves sensing decreased blood flow and blood pressure; (b) regulation of renin secretion by prostaglandin-E2/adenosine/adenosine triphosphate (ATP)/NO released from the macula densa sensing the concentration of NaCl in primary urine; (c) regulation by humoral factors such as angiotensin II; (d) feedback regulation of renin secretion by blood pressure sensing by pressure receptors in JGC (baroreceptors) [17–19]. Perfusion experiments with isolated glomeruli have shown that the JGC themselves sense blood pressure and blood flow and regulate renin secretion [20, 21]. However, the independent mechanism of JGC, which sense blood pressure changes in afferent arterioles by baroreceptors and regulate renin production and secretion, has not been fully clarified.

β1-integrin is a molecule that functions in cells and cell-substrate adhesion by dimerizing with various subtypes of α-integrins. For example, α3β1-integrin is known to be mainly expressed in podocytes and is thought to play an important role in the adhesion of podocytes to the glomerular basement membrane [22], and its loss of function is thought to cause podocyte depletion [23, 24]. As a mechanism, it is known that the structure of integrin dimers is changed by phosphorylation and dephosphorylation of intracellular domains. In particular, phosphorylation of the

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Table 1 Blood glucose and albuminuria of rats after DN onset

Blood glucose (mg/dl)				
	1 month	2 months	3 months	6 months
Control (n = 3)	153.3 ± 13.4	155.3 ± 13.0	161.0 ± 18.3	158.7 ± 23.7
STZ-rat (n = 4)	511.4 ± 80.1	532.7 ± 101.3	542.4 ± 97.9	563.9 ± 122.6
Albuminuria (μg/24h)				
	1 month	2 months	3 months	6 months
Control (n = 3)	58.5 ± 46.0	65.1 ± 46.8	59.0 ± 40.7	65.1 ± 40.4
STZ-rat (n = 4)	204.7 ± 35.4	532.0 ± 89.3	1163.7 ± 279.7	2874.5 ± 1826.4

threonine (Thr) 788/789 site is known to regulate cell adhesion and cell motility [25–27]. Recent reports have defined this site of β1-integrin as functioning as a phospho-switch, activating/inactivating integrin dimers and regulating the functions of integrin, such as the strength of cell adhesion [28].

We hypothesized that the mechanisms of cell adhesion and cell motility associated with β1-integrin phosphorylation contribute to pressure sensing in JGC (i.e., affect the production and secretion of renin involved in blood pressure changes in afferent arterioles by baroreceptors). As β1-integrin has multiple phosphorylation sites, we tested the expression of β1-integrin phosphorylation in renal tissue and the relationship between β1-integrin phosphorylation and the expression of renin.

MATERIALS AND METHODS

Animals

Five-week-old male Sprague-Dawley rats (CLEA Japan Inc., Tokyo, Japan) were intravenously injected with streptozotocin (60 mg/kg), and individuals with blood glucose levels of ≥ 400 mg/dl after 1 week were used as models of diabetic nephropathy for the following experiments. The renal injury parameters, including 24-h urinary albumin and blood glucose were assessed. The animal experiment protocol was approved by the Animal Facility Use Committee (Permit No.: 194019) and conducted in accordance with the Tokai University Animal Experiment Regulations.

Immunohistochemistry

Kidneys were collected from rats in normal and diabetic stages. The specimens were fixed and embedded in paraffin to produce sections of 4 μm in thickness. The sections were deparaffinized and blocked by soaking in 1% blocking reagent in PBS (Invitrogen, Carlsbad, CA, USA). Mouse anti-renin monoclonal antibody (MAA889Ra21; Cloud-Clone Corp., Houston, TX, USA), mouse anti-neuronal nitric oxide synthase (nNOS) monoclonal antibody (MAB24162; R&D SYSTEMS, Minneapolis, MN, USA), rabbit anti-phosphorylated integrin β1 polyclonal antibodies (Thr788-phospho-integrin β1: PA5-38256 Thermo Fisher Waltham MA USA, Thr789-phospho-integrin β1: PA5-36779 Thermo Fisher, Ser785-phospho-integrin β 1: 44–870G Thermo Fisher, Tyr783-phospho-integrin β1: orb186316 Biorbyt San Francisco CA USA, and Tyr795-phospho-integrin β1: STJ90656 St John's Laboratory Ltd London UK). After washing with PBS containing 0.05% Tween 20, anti-mouse/rabbit IgG antibody conjugated with horseradish peroxidase was applied, and reactions were visualized with 3,3-diaminobenzidine (DAB). Alternatively, goat anti-mouse IgG antibody and goat anti-rabbit IgG antibody conjugated with Alexa Fluor 488 or Alexa Fluor 594 were used as second antibodies for detection by fluorescence microscopy.

Quantitative real-time polymerase chain reaction

Total RNA was collected from kidney tissue using an RNaqueous RNA Purification Kit (Thermo Fisher) and converted to cDNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed with the cDNA as described previously [29]. The reaction mixture was prepared as described in the instruction manual of the kit (TaqMan Gene Expression Assays; Thermo Fisher) containing primers and probes for rat renin (Assay ID: Rn00561847_m1) and 18S ribosomal RNA as an endogenous control. PCR was performed on an ABI StepOne Plus (Thermo Fisher). Data were analyzed by the comparative Ct method, and the amount of renin mRNA was expressed relative to the amount of endogenous control.

Image analysis

Quantification of the antibody response using fluorescence microscopy images was performed using the Qupath image analysis software program (v0.2.3) [30]. Areas of red and green fluorescence in double fluorescent staining images (renin and Thr788/789-phospho-β1-integrin, nNOS and Ser785-phospho-β1-integrin, as described above) were measured separately. Results were represented as average ± SD. The Mann–Whitney U test was performed to determine the significance of differences. Pearson's correlation coefficient was calculated to evaluate the relationship between two groups.

RESULTS

Detection of phosphorylation of the intracellular region of β1-integrin in DN kidney

The onset of DN was confirmed by levels of blood glucose and albuminuria of STZ rats (Table 1).

To investigate the phosphorylation of the intracellular region of β1-integrin in the DN kidney, antibodies specific for the phosphorylation of β1-integrin at four sites (Tyr783, Ser785, Thr788/789, Tyr795) were applied to kidney sections of streptozotocin-treated rats (STZ rats) to examine the distribution and phosphorylation level of β1-integrin. Fig. 1 shows the detection by DAB. Anti-phosphorylated Tyr783 antibody specifically reacted with proximal tubular epithelial cells and the parietal layer cells of the Bowman's capsule wall at the urine pole, whereas no strong reaction was observed in the distal tubular epithelial cells, glomeruli, or

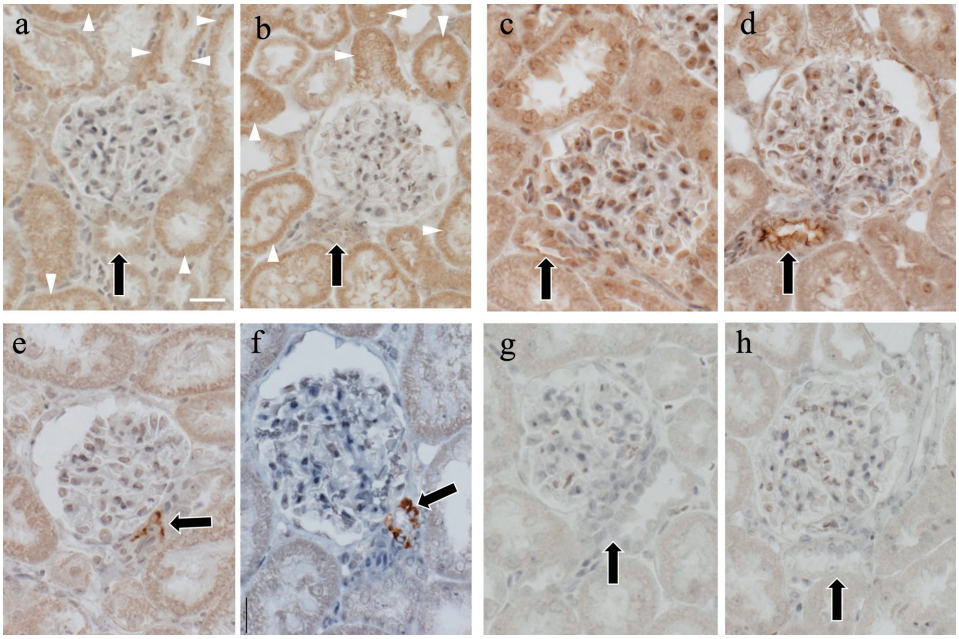

Fig. 1 Anti-phospho β1-integrin antibody reactions in the kidney of normal and STZ rats
DAB staining, hematoxylin counterstaining. a, c, e, g: Kidney sections from normal rats. b, d, f, h: kidney sections from STZ rats. a, b. Anti-Tyr783-phospho β1-integrin antibody. Arrow: JGA, Arrowhead: proximal tubules. c, d. Anti-Ser785-phospho β1-integrin antibody. Arrow: JGA. e, f. Anti-Thr788/789-phospho β1-integrin antibody. Arrow: afferent tubule, g, h. Anti-Tyr795-phospho β1-integrin antibody. Arrow: JGA. Scale bar: 20 μm.

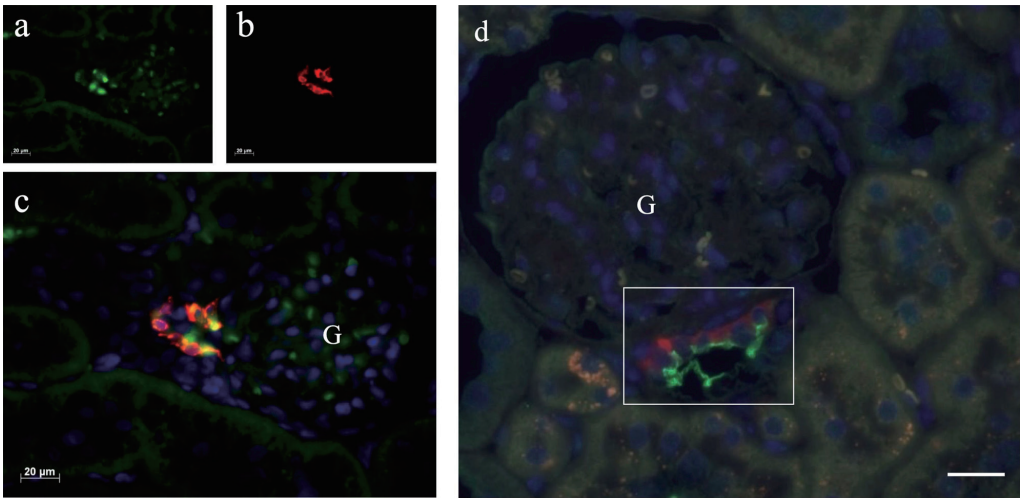

Fig. 2 Detection of phosphorylated β1-integrin in the JGA
Sections of STZ rat kidney, DAPI nuclear staining (blue). a. Fluorescent antibody against Thr788/789-phospho β1-integrin (green). b. Fluorescent antibody against renin (red). c. Merging of a and b, indicating the co-expression of renin and phosphorylated integrin β1. Autofluorescence of erythrocytes was detected as a weak green signal. d. Merging of fluorescent antibodies against nNOS (red) and Ser785-phospho β1-integrin (green); nNOS and phosphorylated β1-integrin are co-expressed in macula densa cells, but the sites at which they are expressed are different. The distal tubule containing the macula densas is surrounded by a white rectangle.

juxtaglomerular apparatus (JGA) in either normal or STZ rats (Fig. 1a, b). On anti-phosphorylated Ser785 antibody staining, there was no strong reaction in the normal kidney; however, a very strong reaction was observed on the luminal side of the distal tubular epithelial cells of the JGA area in STZ rats (Fig. 1c, d, arrows). Weak reactions were also seen in tubular epithelial cells and cells within the glomerulus with DAB staining, but these reactions were not seen with fluorescent detection, which may be an artifact of DAB staining. Both the anti-phosphorylated Thr788 antibody and the anti-phosphorylated Thr789 antibody reacted strongly and specifically with cells surrounding the entrance to the glomerulus of the afferent arteriole cells in both normal and STZ rats (Fig. 1e, f, arrows). The arrangement of these cells suggested that they may be JGC. Anti-phosphorylated Tyr795 antibody did not show a strong reaction in both normal and STZ rats (Fig. 1g, h). In the following experiments, we further examined the phosphorylation of β1-integrins at Ser785 and Thr788/789, for which a particularly strong response and/or changes of expression were specifically seen in the JGA.

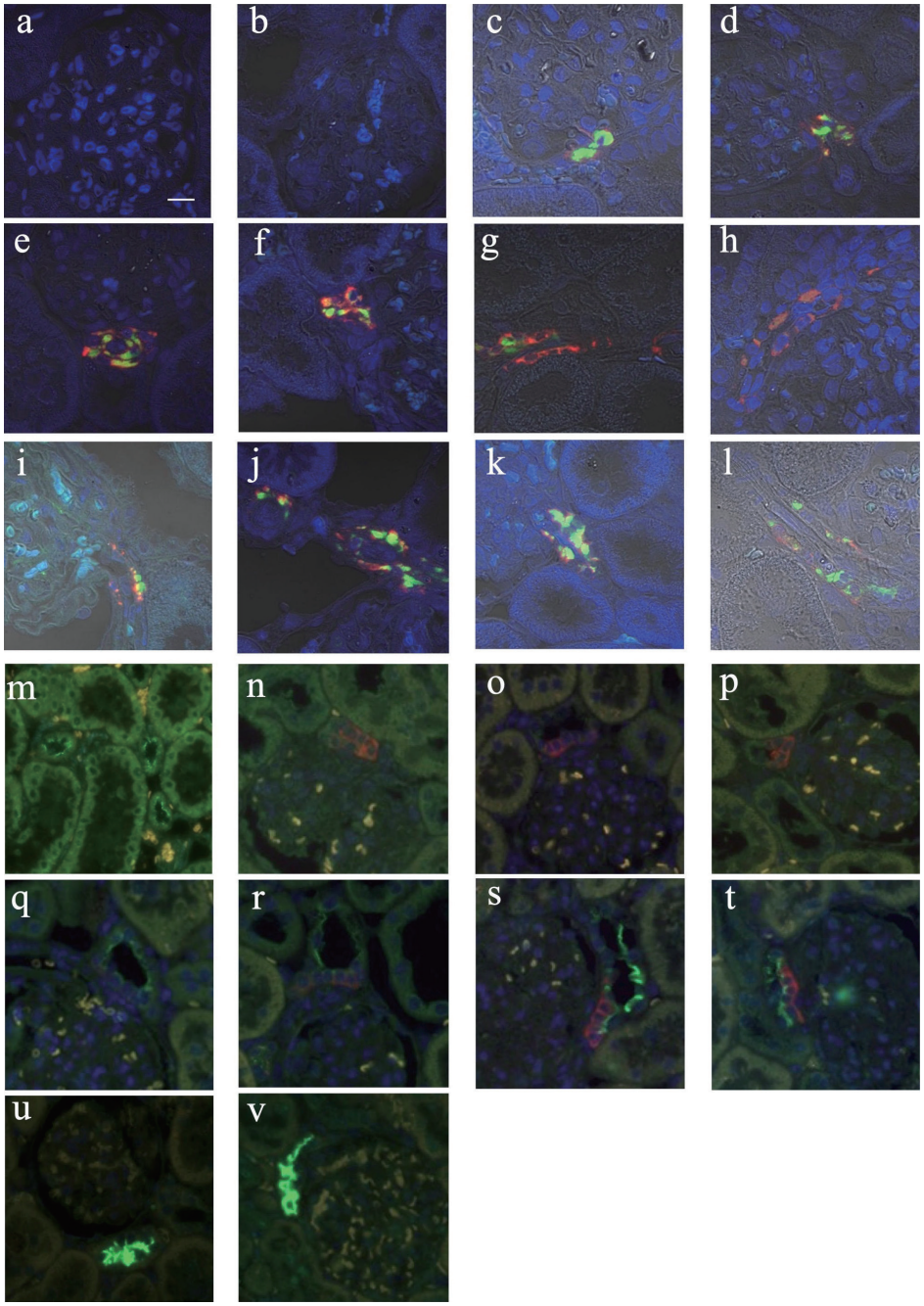

Fig. 3 Changes in phosphorylated β1-integrin during DN
Fluorescent double staining with phosphorylated β1-integrin and specific markers for kidneys of STZ rats at various stages of DN. Double fluorescent staining with fluorescent antibodies against renin (red) and Thr788/789-phospho β1-integrin (green) (c-l) and with fluorescent antibodies against nNOS (red) and Ser785-phospho β1-integrin (green) (m-r). a, b. Controls without antibody staining; c, d, m, n. Normal controls. e, f, o, p. One month after the onset of DN. g, h, q, r. Two months after the onset of DN. i, j, s, t. Three months after the onset of DN. k, l, u, v. Six months after the onset of DN. Bar: 20 μm.

Identification of phosphorylated cells of β1-integrin in the juxtaglomerular apparatus

To identify the phosphorylated cells of β1-integrin Thr788/789 that were specifically detected around the afferent arteries, double staining was performed using antibodies against renin, a specific marker of JGC, and it was confirmed that both were detected in the same cells (Fig. 2a-c). The signal of Thr788/789-phospho β1-integrin was almost always detected in cells with the signal of renin. In JGC, renin was often detected uniformly throughout the cytoplasm, except for the nucleus; however, the distribution of Thr788/789-phospho β1-integrin was not uniform within the cells, and tended to be distributed near to the afferent arterioles.

Next, to identify cells in the macula densa region where Ser785-phospho β1-integrin was detected, double staining was performed with antibodies against nNOS, a specific marker of macula densa cells. As a result, Ser785-phospho β1-integrin was detected in the distal tubular epithelial cells in this region, including the macula densa cells (Fig. 2d). Macula densas are formed where the distal tubule contacts the glomerular vascular pole, and nNOS is distributed in the cyto-

plasm excluding the nucleus of these macula densa cells. Ser785-phospho β1-integrin was predominantly distributed in the luminal surface layer and near the luminal half of intercellular space among the distal tubular epithelial cells in this region, including macula densa cells.

Changes in the phosphorylation of β1-integrin in the juxtaglomerular apparatus during the progression of DN

We investigated the changes in phosphorylation of Thr788/789 and Ser785 sites of β1-integrin in the JGA during the progression of DN in STZ rats.

We followed the changes in the expression of Thr788/789-phospho β1-integrin and renin in kidneys collected during the course of DN, and found that relatively few JGC co-expressed Thr788/789 phosphorylated β1-integrin and renin of normal control (Fig. 3c, d); however, the number of JGC increased in 1 month after the onset of the disease (i.e., from 3.09 ± 1.43 cells/glomerulus in pre-DN to 5.58 ± 2.50 cells/ glomerulus after the month of onset of DN [$p < 0.01$]). The number of JGC started to increase, the expression of renin became stronger, and phosphorylation of the Thr788/789 sites of β1-integrin became weaker (Fig. 3e, f). This trend was more clearly seen in 2 months after the onset, when the phosphorylation of Thr788/789 of β1-integrin was very low and the strong expression of renin was detected in many JGC (Fig. 3g, h); however, by 3 months after the onset, the phosphorylation of the Thr788/789 sites of β1-integrin was again detected in the JGC, while the expression of renin became weaker (Fig. 3i, j). Finally, by 6 months of disease, phosphorylation of Thr788/789 sites of β 1-integrin was restored in almost all JGC; however, the expression of renin was decreased (Fig. 3k, l).

On the other hand, the expression of Ser785-phospho β1-integrin and nNOS during the progression of DN showed different images. On the luminal side, the expression of Ser785-phospho β1-integrin of normal control was very weak and was detected in many distal tubular epithelial cells (Fig. 3m), while nNOS was found in the macula densa cells (Fig. 3n). At 1 month after the onset of DN, Ser785-phospho β 1-integrin, which was previously found in many distal tubular epithelial cells, disappeared, and instead, the weak expression was detected in distal tubular cells of the macula densa region. There was almost no change in the nNOS expression at this time-point (Fig. 3o, p). On the luminal side, the expression of Ser785-phospho β1-integrin in the distal tubular cells in the macula densa region became clear at 2 months after the onset. The expression of nNOS was also observed (Fig. 3q, r). The expression of Ser785-phosphorylated β1-integrin began to rapidly increase at 3 months after the disease onset (Fig. 3s, t) and continued to increase until at least 6 months after the disease onset, whereas nNOS gradually decreased from 3 months after the disease onset and finally became undetectable (Fig. 3u, v).

The fluorescence intensity of phosphorylation on Ser785 increased significantly from 3 months after the onset of DN, and that of Thr788/789 phosphorylation decreased until 2 months from the onset of DN, but then increased (Fig. 4a). Renin showed the opposite pattern of phosphorylation of Thr788/789 of β 1-integrin, peaking in 2 months after the onset of DN and then decreasing. The nNOS expression decreased slightly within 2 months after the onset of DN, but recovered at 3 months and significantly decreased at 6 months.

To further investigate the relationship between the phosphorylation of Thr788/789 of β1-integrin and the renin expression observed during the progression of DN, we compared the expression of renin mRNA and the number of cells that were positive for Thr788/789 phosphorylated β1-integrin at each stage of DN. The renin mRNA levels of STZ rats were measured by real-time PCR, and the numbers of cells that were positive for Thr788/789-phospho β1-integrin were converted to the number per glomerulus included in the field of view. Both were represented as a ratio to those of sham animals (Fig. 4b). Similar to the results of the image analysis, the renin mRNA levels gradually increased after the onset of DN, reaching a peak at 2 months, but decreased and returned to the original level in 6 months. On the other hand, the number of JGC in which phosphorylation of Thr788/789 sites of β1-integrin was detected gradually decreased after the onset of DN, reaching a nadir at 2 months, but then increased and finally returned to the original level. There was a negative correlation between these changes (Pearson's correlation coefficient = -0.61, $p < 0.05$).

To confirm the above results and detect the phosphorylation levels of β1-integrin, we tried protein blot analysis, however, we could not detect the signal. Perhaps the primary reason is that the proportion of JGC in the kidney is very low and the level of phospho β1-integrin is below the limit of detection. Alternatively, when kidney is homogenized, β1-integrin may be dephosphorylated due to strong phosphatase activity, despite the inclusion of inhibitors.

These results indicate that β1-integrin in the JGA reaches a major turning point in 2 months after the onset of DN (Fig. 5). This change seems to be accompanied by a change in blood pressure, and since the JGA regulates the secretion of renin, a blood pressure regulator from JGC, it was suggested that the phosphorylation of β1-integrin may contribute to the mechanism that regulates renin production in JGC.

DISCUSSION

In this study, we detected — for the first time — the phosphorylation of Ser785 and Thr788/789 of β1-integrin specific to the JGA, indicating that the phosphorylation/dephosphorylation of these sites of β 1-integrin may be part of the blood pressure regulation mechanism during the process of DN.

There are various reports on the effects of phosphorylation of Thr788/789 of β1-integrin on cell adhesion and motility [25–27], and recent reports suggest that this site of β1-integrin functions as a phospho-switch to activate/deactivate integrin dimers and regulate integrin functions, such as cell adhesion strength [28]. In JGC, this mechanism may contribute to the production and secretion of renin, a function unique to this type of cell.

On the other hand, it has been reported that the phosphorylation of Ser785 of β1-integrin is essential for cytoskeletal reorganization during invasion and metastasis of macrophages and tumor cells [31], and

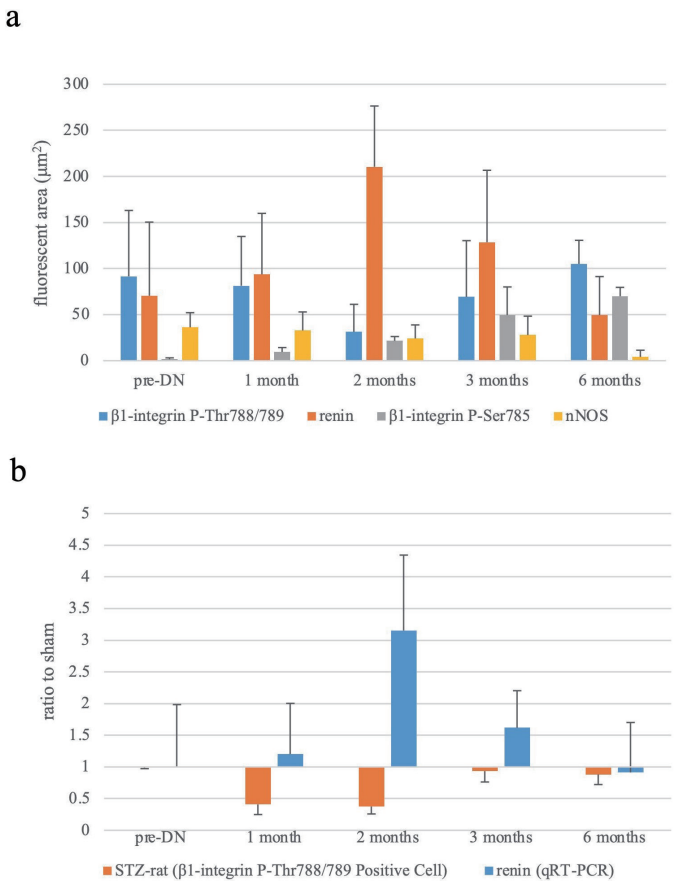

Fig. 4 Quantification of changes in phosphorylated β1-integrin during the progression of DN
a. The area of fluorescence (μm²) was measured from fluorescent microscopy images of antibody staining at each stage of DN and graphed. Images of a total of 108 glomeruli in 31 sections of kidney were examined for renin and Thr788/789-phospho β1-integrin measurement, and a total 101 glomeruli in 31 sections of kidney were examined for nNOS and Ser785-phospho β1-integrin. To avoid a selection bias, glomeruli with a fluorescent signal were randomly selected. b. The expression ratio of renin mRNA measured by qRT-PCR in the kidney at each stage of DN (STZ rat/Sham, blue) and numbers of cells positive for anti-Thr788/789-phospho β1-integrin antibody per glomerulus observed in the same stage kidney sections (STZ rat/Sham, red).

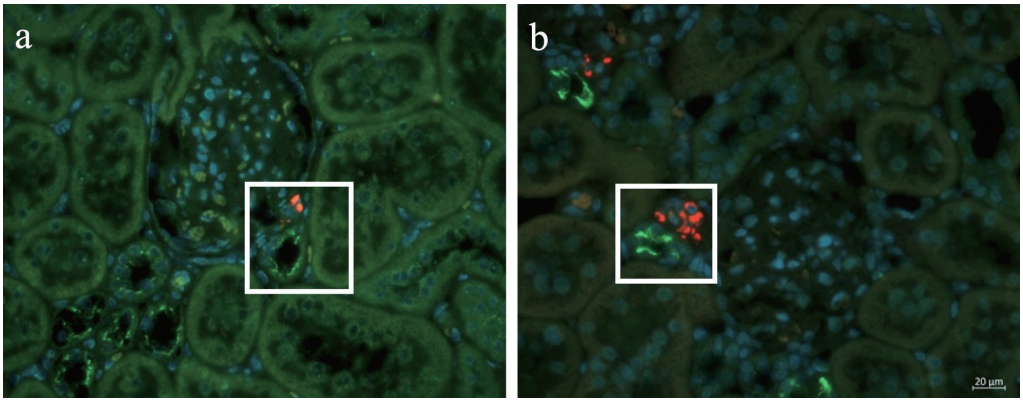

Fig. 5 Changes in phosphorylated β1-integrin in the JGA of the STZ rat kidney.
Double fluorescence staining of STZ rat kidney with fluorescent antibodies against Thr788/789-phospho β1-integrin (red) and Ser785-phospho β1-integrin (green). a. Two months after the onset of DN. b. Three months after the onset of DN. The JGA is surrounded by a white frame. bar: 20 μm

its expression in distal tubular epithelial cells in the macula densa region may promote morphological changes of distal tubular cells in this region, including the macula densa cells. It is known that macula densa cells, upon sensing changes in NaCl concentration in primary urine after DN onset, absorb water, which increases the cell swelling pressure, and cytoskeletal changes due to Ser785 phosphorylation of β1-integrin may affect the transmission of that swelling pressure to the afferent arteriole to protect the glomerulus by regulating blood flow. Furthermore, macula densa cells are in contact with JGC and secrete factors to regulate renin secretion, and the reconstitution of the cytoskeleton may regulate the transmission rates of

these factors. The rapid increase in phosphorylation of the Ser785 site of β1-integrin with the progression of DN may indicate its involvement in the activation of these functions.

Everett *et al*. reported that renin production is reduced in advanced diabetes mellitus [32]. They counted the number of renin-positive cells per JGA in rats with the spontaneous onset of diabetes and reported that the number peaked at 2 months after the onset of diabetes, which is consistent with the findings in the present study. In STZ rats, blood pressure continued to increase after the onset of diabetes, but from 2 months after the onset of diabetes, renin production began to decrease, and blood pressure gradually decreased accordingly. During this period, the Thr788/789 phosphorylation level of β1-integrin showed a negative correlation with the change in renin production, but the causal relationship between β1-integrin phosphorylation and renin production was unknown. The present results suggest that the activation of integrin dimers by phosphorylation is required for the suppression of renin production, and the inactivation of the phosphor-switch after the onset of DN in STZ rats may prevent the suppression of renin production in response to elevated blood pressure. The predominance of Protein kinase C ε and phosphatase activity in DN is predicted to be time-dependent.

The mechanism by which activated integrin dimers inhibit renin production may be the modulation of pressure sensing by JGC. JGC are able to sense blood flow and pressure in afferent arterioles by themselves. The intercellular adhesion factor Connexin (Cx)40 is essential for the baroreceptor function in JGC, because knockout mice of this molecule are unable to inhibit renin secretion, and Cx40 is therefore regarded as the baroreceptor of JGC [33]. On the other hand, the Thr788/789 phosphorylation of β1-integrin is known to alter cell-substrate adhesion in many types of cells, and the resulting change in membrane tension may be involved in the regulation of the renin expression by altering the pressure sensing threshold of baroreceptors on the cell membrane. It is quite possible that both Cx and integrin are closely related to each other on the cell membrane as adhesion factors. In fact, a recent study of mechanosensory osteocytes reported that mechanical stress-activated integrin α5β1 interacts with Cx43 to induce the opening of hemichannels [34]. A similar interaction between activated integrin and Cx40 may contribute to the regulation of pressure sensing in JGC. Although the partner α-integrin in JGC has not been determined thus far, we found that the change in the renal β1-integrin expression during the DN process was accompanied by that of α3-, α5- and αv-integrins (data not shown). As integrin α3β1 is known to be specific to podocytes [35], α5- and/or α v-integrins may contribute to the functions of the JGA.

In conclusion, our findings suggest that the phosphorylation of β1-integrin may contribute to the regulatory mechanism of renin production in JGC. Therefore, a further analysis of the regulatory mechanisms, including integrin phosphorylation/dephosphorylation during the progression of DN may lead to the elucidation of the mechanism of baroreceptor-mediated blood pressure regulation and the development of new DN therapies targeting this mechanism.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this article.

REFERENCES

1) Giunti S, Barit D, Cooper ME. Mechanisms of diabetic nephropathy: role of hypertension. Hypertension. 2006; 48: 519–26.
2) Bloomgarden ZT. Blood pressure and diabetic nephropathy. Diabetes Care. 2010; 33: e30–5.
3) Carmines PK. The renal vascular response to diabetes. Curr Opin Nephrol Hypertens. 2010; 19: 85–90.
4) Lopes de Faria JB, Silva KC, Lopes de Faria JM. The contribution of hypertension to diabetic nephropathy and retinopathy: the role of inflammation and oxidative stress. Hypertens Res. 2011; 34: 413–22.
5) Van Buren PN, Toto R. Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis. 2011; 18: 28–41.
6) Cheung BM, Li C. Diabetes and hypertension: is there a common metabolic pathway? Curr Atheroscler Rep. 2012; 14: 160–6.
7) Hayashi K, Epstein M, Loutzenhiser R, Forster H. Impaired myogenic responsiveness of the afferent arteriole in streptozotocin-induced diabetic rats: role of eicosanoid derangements. J Am Soc Nephrol. 1992; 2: 1578–86.
8) Hayashi K, Kanda T, Homma K, Tokuyama H, Okubo K, Takamatsu I, *et al*. Altered renal microvascular response in Zucker obese rats. Metabolism. 2002; 51: 1553–61.
9) Vallon V, Blantz RC, Thomson S. Homeostatic efficiency of tubuloglomerular feedback is reduced in established diabetes mellitus in rats. Am J Physiol. 1995; 269: F876–83.
10) Price DA, De'Oliveira JM, Fisher ND, Williams GH, Hollenberg NK. The state and responsiveness of the renin-angiotensin-aldosterone system in patients with type II diabetes mellitus. Am J Hypertens. 1999; 12: 348–55.
11) Donatelli M, Colletti I, Bucalo ML, Russo V, Verga S. Plasma endothelin levels in NIDDM patients with macroangiopathy. Diabetes Res. 1994; 25: 159–64.
12) Koyama H, Tabata T, Nishzawa Y, Inoue T, Morii H, Yamaji T. Plasma endothelin levels in patients with uraemia. Lancet. 1989; 1: 991–2.
13) Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001; 414: 813–20.
14) Rajagopalan S, Kurz S, Münzel T, Tarpey M, Freeman BA, Griendling KK, *et al*. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. J Clin Invest. 1996; 97: 1916–23.
15) Prabhakar SS. Role of nitric oxide in diabetic nephropathy. Semin Nephrol. 2004; 24: 333–44.
16) Fitzgerald SM, Brands MW. Nitric oxide may be required to prevent hypertension at the onset of diabetes. Am J Physiol Endocrinol Metab. 2000; 279: E762–8.
17) Friis UG, Madsen K, Stubbe J, Hansen PB, Svenningsen P, Bie P, *et al*. Regulation of renin secretion by renal juxtaglomerular cells. Pflugers Arch. 2013; 465: 25–37.
18) 18. Persson PB. Renin: origin, secretion and synthesis. J Physiol. 2003; 552: 667–71.
19) Schweda F, Friis U, Wagner C, Skott O, Kurtz A. Renin release. Physiology (Bethesda). 2007; 22: 310–9.
20) Bock HA, Hermle M, Brunner FP, Thiel G. Pressure dependent modulation of renin release in isolated perfused glomeruli. Kidney Int. 1992; 41: 275–80.

21) Salomonsson M, Skøtt O, Persson AE. Influence of intraluminal arterial pressure on renin release. Acta Physiol Scand. 1991; 141: 285–6.
22) Sawada K, Toyoda M, Kaneyama N, Shiraiwa S, Moriya H, Miyatake H, *et al*. Upregulation of α3β1-integrin in podocytes in early-stage diabetic nephropathy. J Diabetes Res. 2016; 2016: 9265074.
23) Cosio FG. Cell-matrix adhesion receptors: relevance to glomerular pathology. Am J Kidney Dis. 1992; 20: 294–305.
24) Rahilly MA, Fleming S. Differential expression of integrin alpha chains by renal epithelial cells. J Pathol. 1992; 167: 327–34.
25) Nilsson S, Kaniowska D, Brakebusch C, Fässler R, Johansson S. Threonine 788 in integrin subunit beta1 regulates integrin activation. Exp Cell Res. 2006; 312: 844–53.
26) Fransvea E, Mazzocca A, Antonaci S, Giannelli G. Targeting transforming growth factor (TGF)-betaRI inhibits activation of beta1 integrin and blocks vascular invasion in hepatocellular carcinoma. Hepatology. 2009; 49: 839–50.
27) Stawowy P, Margeta C, Blaschke F, Lindschau C, Spencer-Hänsch C, Leitges M, *et al*. Protein kinase C epsilon mediates angiotensin II-induced activation of beta1-integrins in cardiac fibroblasts. Cardiovasc Res. 2005; 67: 50–9.
28) Grimm TM, Dierdorf NI, Betz K, Paone C, Hauck CR. PPM1F controls integrin activity via a conserved phospho-switch. J Cell Biol. 2020; 219: e202001057.
29) Tatsumi R, Komaba H, Kanai G, Miyakogawa T, Sawada K, Kakuta T, *et al*. Cinacalcet induces apoptosis in parathyroid cells in patients with secondary hyperparathyroidism: histological and cytological analyses. Nephron Clin Pract. 2013; 124: 224–31.
30) Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, *et al*. QuPath: Open source software for digital pathology image analysis. Sci Rep. 2017; 7: 16878.
31) Destaing O, Planus E, Bouvard D, Oddou C, Badowski C, Bossy V, *et al*. β1A integrin is a master regulator of invadosome organization and function. Mol Biol Cell. 2010; 21, 4108–19.
32) Everett AD, Scott J, Wilfong N, Marino B, Rosenkranz RP, Inagami T, *et al*. Renin and angiotensinogen expression during the evolution of diabetes. Hypertension. 1992; 19: 70–8.
33) Wagner C, de Wit C, Kurtz L, Grünberger C, Kurtz A, Schweda F. Connexin40 is essential for the pressure control of renin synthesis and secretion. Circ Res. 2007; 100: 556–63.
34) Batra N, Burra S, Siller-Jackson AJ, Gu Sumin, Xia X, Weber GF, *et al*. Mechanical stress-activated integrin α5β1 induces opening of connexin 43 hemichannels. Proc. Natl. Acad. Sci. USA. 2012; 109: 3359–64.
35) Sawada K, Toyoda M, Kaneyama N, Shiraiwa S, Moriya H, Miyatake H. Upregulation of α5β1-integrin in podocytes in early-stage diabetic nephropathy. J Diabetes Res. 2016; 2016: 9265074.