

Latent Transforming Growth Factor- β Binding Protein-1, a Component of Latent Transforming Growth Factor- β Complex, Accelerates the Migration of Aortic Smooth Muscle Cells in Diabetic Rats Through Integrin- β_3

Tetsuto Kanzaki and Masako Otabe

Aortic smooth muscle cells (SMCs) of diabetic animals have unique properties, including the overexpression of transforming growth factor- β (TGF- β) type II receptor, fibronectin, and platelet-derived growth factor β -receptor. TGF- β 1 is produced and secreted as latent high-molecular weight complex consisting of mature TGF- β 1, latency-associated peptide (LAP), and a latent TGF- β 1 binding protein (LTBP-1). LAP has an important function in the latency of TGF- β complex, but the role of LTBP-1 is not known in diabetic angiopathy. SMC migration from the medial layer to the intimal layer of an artery is an initial major process of the formation of intimal thickening of an artery. Migration activities of SMCs from diabetic rat with 1–500 pg/ml of LTBP-1 increased significantly compared with that without LTBP-1. LTBP-1 at 10–500 pg/ml stimulated the migration of diabetic SMCs more than SMCs from control rat. An anti-integrin- β_3 antibody reduced LTBP-1-stimulated migration of diabetic SMCs to 51% compared with no antibody, but it did not reduce that of control SMCs. Furthermore, cross-linking experiments show that LTBP-1 binds integrin- β_3 in diabetic SMCs much more than in control SMCs in coincidence with the increase of integrin- β_3 in diabetic aorta by immunohistochemistry. Taken together, these observations suggest that LTBP-1 plays a critical role in intimal thickening of diabetic artery through the acceleration of SMC migration via integrin- β_3 . *Diabetes* 52:824–828, 2003

We have previously reported that cultured aortic smooth muscle cells (SMCs) and medial layers of arteries of diabetic rats and rabbits express more platelet-derived growth factor (PDGF) β -receptor, transforming growth factor- β (TGF- β)

From the Department of Internal Medicine, Kohnodai Hospital, National Center of Neurology and Psychiatry, Kohnodai, Ichikawa, Japan.

Address correspondence and reprint requests to Tetsuto Kanzaki, Department of Internal Medicine, Kohnodai Hospital, National Center of Neurology and Psychiatry, 1-7-1, Kohnodai, Ichikawa City 272-8516, Japan. E-mail: kanzaki@intmed02.m.chiba-u.ac.jp.

Received for publication 2 June 2002 and accepted in revised form 2 December 2002.

8-Cys, 8 cysteine; BCI, balloon catheter injury; DMEM, Dulbecco's modified Eagles medium; EGF, epidermal growth factor; FBS, fetal bovine serum; LAP, latency-associated peptide; LTBP, latent TGF- β 1 binding protein; PDGF, platelet-derived growth factor; RGD, arginine-glycine-aspartate; SMC, smooth muscle cell; TGF- β , transforming growth factor- β .

type II receptor, and fibronectin than those of nondiabetic animals (1–3). This means that the diabetic condition has led to a change in SMC properties. In addition, SMC-dominant intimal thickening was enhanced in diabetic animals compared with nondiabetic animals at 2–4 weeks after balloon catheter injury (BCI) of the carotid arterial wall, by which PDGF is increased in the wall (3,4), suggesting that the changes in SMC properties by the overexpression of PDGF β -receptor play a critical role in arterial intimal thickening in diabetic animals. It has been reported that fibronectin regulated by TGF- β is essential in phenotypic changes of arterial SMC, e.g., an increase of PDGF β -receptor expression (5). The above results indicate that TGF- β is one of the important factors that induce characteristic changes of arterial SMCs in diabetes.

TGF- β has been found to have multiple biological effects, such as stimulating extracellular matrix formations, inhibiting the growth of cells, suppressing immune reactions, etc. (6). Moreover, TGF- β is secreted from producer cells as latent high-molecular weight complexes (large latent complexes) (7,8). The large latent complex of TGF- β 1 is composed of mature TGF- β 1, latency-associated peptide (LAP) noncovalently bound to mature TGF- β 1, and a latent TGF- β 1 binding protein (LTBP-1) that is linked to LAP via one or more disulfide bonds. It has been reported that TGF- β 1 stimulates intimal thickening of arteries by the enhancement of extracellular matrix synthesis (9,10) and that LAP has an important function in the latency of TGF- β complex by noncovalent association with mature TGF- β (11). In contrast with the fact that TGF- β 1 and LAP are the same gene products, LTBP is a completely separate gene product (12), which means that LTBP has a different metabolism than TGF- β .

LTBP-1 is a glycoprotein that occurs in various sizes (125–210 kDa), probably due to proteolytic processing or alternative splicing (13,14). A total of 60–70% of the LTBP-1 structure is composed of two different types of cysteine-rich repeat sequences, one being 16–18 epidermal growth factor (EGF)-like repeats and the other 3–4 copies of 8 cysteine (8-Cys) repeats unique to this protein (13). LTBP-1 is a member of a superfamily of proteins that contain EGF-like repeats, 8-Cys repeats, LTBP-1, -2, -3, and -4, and fibrillin-1 and -2 (13–19). Fibrillins and LTBPs, except LTBP-3, also display one to two arginine-glycine-

aspartate (RGD) sequences for putative cell adhesion (16,20). In an *in vitro* system, LTBP-1 has been shown to have a crucial role in the assembly and secretion of TGF- β 1 complex (21) and in the association of latent TGF- β 1 complex with extracellular matrix through the 8-Cys domain of LTBP-1 and subsequent activation of TGF- β 1 (22,23). Furthermore, by experiments using antibody to LTBP-1 (Ab39), Dalles et al. (24) reported that Ab39 inhibits bone development and formation in rat fetus, and Nakajima et al. (25) showed that it inhibits the endothelial-mesenchymal transformation in atrio-ventricular endocardial cushion tissue formation. These results suggest that LTBP-1 acts as one of the regulators in embryonic development. In an arterial wall, LTBP-1 stimulates the migration activities of cultured rat SMCs *in vitro*, and it mainly appears in the intimal layer of BCI artery (26). It appears that LTBP-1 is significantly involved in arterial intimal thickening through the acceleration of SMC migration from the medial to intimal layer, but the role of LTBP-1 in diabetic arteries is not known.

In the present study, we report the function of LTBP-1 in diabetic SMCs and the mechanism of its action. The significances of LTBP-1 and latent TGF- β 1 complex in the mechanism of arterial intimal thickening on diabetes are discussed further.

RESEARCH DESIGN AND METHODS

Chemicals. Sources of materials were as follows: streptozotocin, Sigma Chemical (St. Louis, MO); fetal bovine serum (FBS) (Lot No. US177246), Invitrogen (Carlsbad, CA); PDGF-BB, R&D Systems (Minneapolis, MN); 125 I, New England Nuclear (Boston, MA); and Bis (sulfosuccinimidyl) suberate, Pierce (Rockford, IL). LTBP-1 was purified from *Escherichia coli* transfected with its cDNA (LTBP-1, short form), and its purity was estimated by SDS-PAGE, silver staining, and immunoblotting using antibody to LTBP-1. The molecular weight of this recombinant LTBP-1 was ~180 kDa.

Antibody. Mouse monoclonal antibody to rat integrin- β_3 (F11) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal antibody against the free form of LTBP-1 purified from human platelets (Ab39) was a gift from Dr. Kohei Miyazono (Department of Molecular Pathology, Tokyo University, Tokyo, Japan). Ab39 cross-reacts with rat, and its specificity has been reported (27,28).

Induction of diabetes. Diabetes was induced by the injection of 60 mg/kg streptozotocin into male Wistar rats. Control and diabetic rats were fed a standard diet for 4 weeks. The plasma glucose levels were as follows: control 4.51 ± 1.46 mmol/l ($n = 10$), diabetic 23.3 ± 3.31 mmol/l ($n = 10$). The body weights were 380 ± 18.7 g ($n = 10$) for the control and 276 ± 33.6 g ($n = 10$) for the diabetic. The experiments were repeated in 10 different pairs.

Cell culture. SMCs were explanted from the thoracic aortas of male Wistar rats essentially by the method of Fischer-Dzoga et al. (29). Primary culture and subculture were carried out in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% FBS. Cells were subcultured at a ratio of 1:2 and used at the second through fourth passages for the following experiments.

Migration assay of SMCs. The migration of SMCs was assayed by a modification of Boyden's chamber method using a polycarbonate filter (Neuro Probe, Gaithersburg, MD) with pores of 8.0- μ m diameter (30). Cultured SMCs were trypsinized and suspended at a concentration of 5.0×10^5 cells/ml in DMEM with 1 mg/ml BSA. Then the SMC suspension was placed in the upper chamber and DMEM containing 1 mg/ml BSA with LTBP-1 or PDGF-BB in the lower chamber. In some experiments, F11 was added to the upper chamber. The chamber was incubated at 37°C in 5% CO₂ and 95% air for 4 h. The filter was removed and SMCs on the upper side of the filter were scraped off. Then SMCs that had migrated to the lower side of the filter were fixed in methanol, stained with hematoxylin and eosin, and counted by light microscopy ($\times 100$) for quantitation of migration. Migration activity was expressed as the mean number of migrated cells seen in five different fields (F).

Cross-linking LTBP-1 with rat SMC. Iodination of LTBP-1 was carried out by the mild chloramine T method as described by Frolik et al. (31). The cross-linking experiment was performed as previously described (32). In brief, confluent SMCs in 75 cm² culture flasks were washed with binding buffer (PBS containing 0.9 mmol/l CaCl₂, 0.49 mmol/l MgCl₂, and 1 mg/ml BSA), preincu-

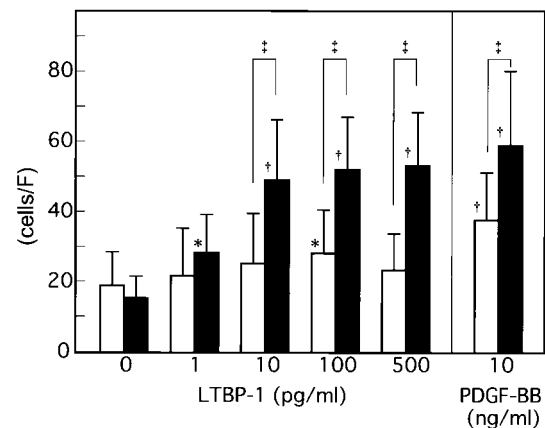


FIG. 1. Effects of LTBP-1 on the migration of SMCs in control and diabetic rats. Migration activities with the addition of various concentrations of LTBP-1 and PDGF-BB were assayed in SMCs from control (□) and diabetic (■) rats by modified Boyden chamber method. Values are expressed as the mean \pm SD of 10 different pairs of experiments. * $P < 0.05$, † $P < 0.01$ vs. SMCs with no addition; ‡ $P < 0.01$ for control and diabetic SMCs (with the same amount of factors).

bated on ice in the same buffer with excess unlabeled LTBP-1 or 20 μ g IgG of F11 for 30 min, and then incubated on ice with 100 pmol/l of 125 I-LTBP-1 for 3 h. Cells were washed, and cross-linking was done in the binding buffer without BSA together with 0.25 mmol/l Bis for 30 min on ice. Supernatants from solubilized cells (cell lysates) containing the same amounts of protein were subjected to analysis by SDS gel electrophoresis using 4% polyacrylamide gel, followed by autoradiography.

Immunohistochemical study of integrin- β_3 and LTBP-1 in aortas. The aortas were snap-frozen and stored at -80°C . They were sectioned serially at 6- μ m thickness and fixed in acetone. For the identification of integrin- β_3 and LTBP-1, we used 100 \times diluted F11 and 200 \times diluted Ab39, respectively. The staining of sections was followed by the method of ABC peroxidase immunohistochemistry described by Waltenberger et al. (27).

Statistical methods. The significance of differences was evaluated by two-tailed Student's *t* test.

RESULTS

Effects of LTBP-1 on the migration of rat SMCs in control and diabetes. First, we examined the effects of LTBP-1 on the migration and proliferation of diabetic SMC. Migration activity of control SMCs without addition of LTBP-1 was 19.4 ± 9.66 cells/F. Migration activities of control SMCs with the addition of 100 pg/ml LTBP-1 and 10 ng/ml PDGF-BB increased significantly to 28.0 ± 12.5 and 37.7 ± 12.7 cells/F, respectively, compared with those without LTBP-1 and PDGF-BB (Fig. 1). Migration activity of diabetic SMCs without LTBP-1 was 15.4 ± 6.10 cells/F, and those with the addition of 1, 10, 100, and 500 pg/ml LTBP-1 and 10 ng/ml PDGF-BB increased significantly to 27.9 ± 11.0 , 48.6 ± 16.9 , 51.4 ± 15.3 , 52.8 ± 15.4 , and 58.7 ± 21.0 cells/F, respectively (Fig. 1). Furthermore, 10, 100, and 500 pg/ml LTBP-1 and 10 ng/ml PDGF-BB significantly stimulated migration activities of diabetic SMCs compared with control SMCs (Fig. 1). However, 1–500 pg/ml LTBP-1 had no effects on the proliferation of rat SMCs in both control and diabetes (data not shown).

Effects of anti-integrin- β_3 on diabetic SMC migration by LTBP-1 and PDGF-BB. It has been reported that integrin- β_3 has an important role in SMC migration (33). To clarify the participation of integrin- β_3 in the accelerated migration of diabetic SMCs, migration activities were examined using anti-integrin- β_3 antibody (F11).

Migration activity of diabetic SMCs with the addition of

TABLE 1
Effects of F11 on diabetic SMC migration by LTBP-1 and PDGF-BB

(μg IgG/ml)	F11	Control IgG
LTBP-1		
0		57.6 \pm 14.9
0.2	29.8 \pm 7.01*	53.5 \pm 12.9
1	31.6 \pm 8.22*	57.0 \pm 12.0
PDGF-BB		
0		61.3 \pm 14.9
0.2	70.6 \pm 10.1	59.5 \pm 9.19
1	69.8 \pm 7.50	69.0 \pm 7.93

Data are means \pm SD. Migration activities were assayed in diabetic SMCs with the addition of 100 pg/ml LTBP-1 (or 10 ng/ml PDGF-BB) and 0.2 and 1.0 μg IgG/ml F11 or control IgG. * $P < 0.05$ vs. no addition of F11.

100 pg/ml LTBP-1 was 57.6 \pm 14.9 cells/F, whereas that with no LTBP-1 was 22.2 \pm 6.47 cells/F. LTBP-1-induced migration activities decreased significantly to 29.8 \pm 7.01 and 31.6 \pm 8.22 cells/F with the addition of 0.2 and 1.0 μg IgG/ml F11, respectively, compared with no addition (Table 1). There were no changes in migration activities with the addition of control (nonimmune) IgG instead of F11 (Table 1). Neither F11 nor control IgG had any effect on the PDGF-BB-induced migration activities of diabetic SMCs (Table 1).

Effects of anti-integrin- β_3 on control SMC migration by LTBP-1 and PDGF-BB. Migration activity of control SMCs with the addition of 100 pg/ml LTBP-1 was 25.5 \pm 4.17 cells/F, and that without LTBP-1 was 14.8 \pm 4.17 cells/F. Neither LTBP-1- nor PDGF-BB-induced migration activities of control SMCs were changed by the addition of F11 and control IgG (Table 2).

Binding of LTBP-1 with control and diabetic SMCs. To determine the mechanism of the accelerated migration in diabetic SMC due to LTBP-1, the bindings of LTBP-1 with control and diabetic SMCs were examined by cross-linking experiments. A 350- to 400-kDa band observed in cell lysates from diabetic SMCs cross-linked with LTBP-1 was stronger than that from control SMCs (Fig. 2, lanes 2 and 5), and such a band was not observed in cell lysate from diabetic SMCs preincubated with F11 (Fig. 2, lane 6), but a weak band was in cell lysate from control SMCs preincubated with F11 (Fig. 2, lane 3). These bands were not detected in cell lysates from both control and diabetic SMCs added with excess unlabeled LTBP-1 at binding (Fig.

TABLE 2
Effects of F11 on control SMC migration by LTBP-1 and PDGF-BB

(μg IgG/ml)	F11	Control IgG
LTBP-1		
0		25.5 \pm 4.17
0.2	27.8 \pm 4.26	28.8 \pm 8.13
1	29.7 \pm 6.74	28.8 \pm 6.24
PDGF-BB		
0		41.5 \pm 12.6
0.2	49.6 \pm 17.9	53.5 \pm 15.4
1	51.5 \pm 18.5	57.5 \pm 16.2

Data are means \pm SD. Migration activities were assayed in control SMCs with the addition of 100 pg/ml LTBP-1 (or 10 ng/ml PDGF-BB) and 0.2 and 1.0 μg IgG/ml F11 or control IgG.

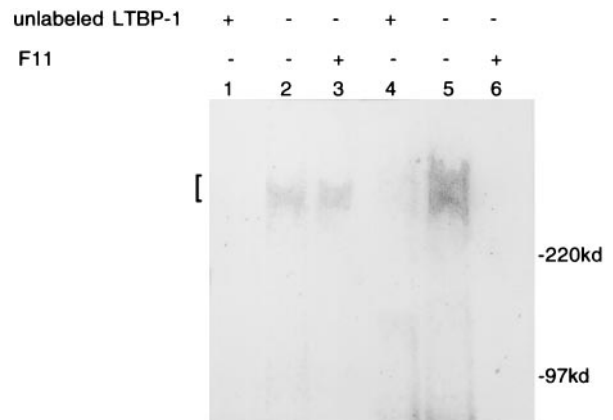


FIG. 2. Binding of LTBP-1 with control and diabetic SMCs. SMC was preincubated with excess of unlabeled LTBP-1 (lanes 1 and 4) or with F11 (lanes 3 and 6). Then, control SMC (lanes 1, 2, and 3) and diabetic SMC (lanes 4, 5, and 6) were cross-linked with ^{125}I -LTBP-1. Molecular size is indicated on the right and “[” shows the specific binding complex of LTBP-1 with SMC detected in lanes 2, 3, and 5. The experiments were performed with five different pairs of control and diabetic SMCs, and typical results are shown.

2, lanes 1 and 4). These results suggested that bands of 350–400 kDa are specific for LTBP-1 in both control and diabetic SMCs, and is also specific for integrin- β_3 in diabetic SMC. The molecular weight of a band of SMC cross-linked with LTBP-1 is 350–400 kDa and that of the LTBP-1 used in this experiment is 180 kDa, indicating that LTBP-1 associates with a 170- to 220-kDa cell surface protein of SMC, which is probably integrin- β_3 in diabetes. **Expression of integrin- β_3 and LTBP-1 in control and diabetic rat aorta.** To clarify the presence of integrin- β_3 and LTBP-1 in rat aorta, their distributions were studied by immunohistochemistry. No medial layer of rat aorta was stained with nonimmune serum (Fig. 3A). The staining with integrin- β_3 of the medial layer in diabetic aorta (Fig. 3C) was much stronger than that in control aorta (Fig. 3B). However, extracellular spaces of medial layers in both control and diabetic aortas were strongly stained with

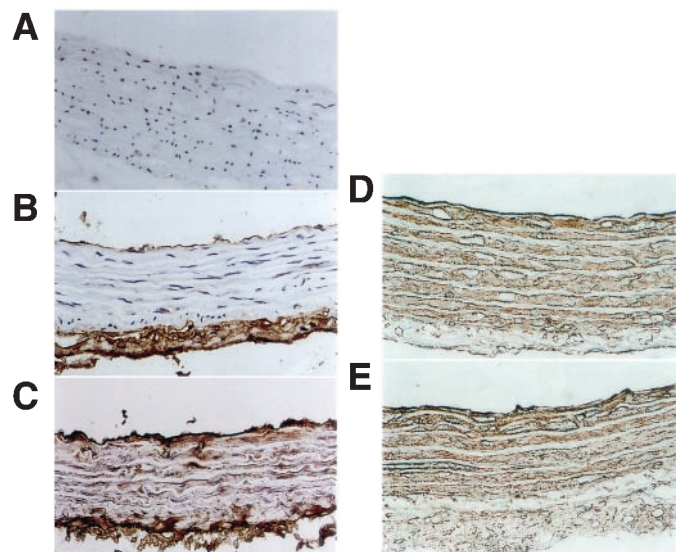


FIG. 3. Immunohistochemical study of integrin- β_3 and LTBP-1 in aortas from control and diabetic rats. Nonimmune serum was used in aorta of diabetic rat (A), F11 was applied in aortas of control (B) and diabetic (C) rat, and Ab39 was in those of control (D) and diabetic (E) rat.

antibody to LTBP-1 (Fig. 3D and E). SMC is a major cellular component in aortic medial layer, suggesting that SMCs of diabetic aorta express both LTBP-1 and integrin- β_3 , but SMCs of control aorta express LTBP-1 and not integrin- β_3 .

DISCUSSION

The present results showed that 10–500 pg/ml LTBP-1 stimulated the migration of diabetic SMCs in comparison to control SMCs in vitro, and integrin- β_3 was significantly involved in the SMC migration augmented by LTBP-1 in diabetic rats. Checker-board analysis revealed that LTBP-1-stimulated rat SMC migration was unidirectional in both control and diabetes, indicating that these SMC migrations were due to chemotaxis and not chemokinesis (data not shown).

The mechanism of cell migration has not been fully understood. However, it is known that the migration process consists of four major steps (34). First, there is the forward motility of the membrane at the front of the cell, which is called “protrusion.” Then, adhesion is required for protrusion to be converted into movement along the substrate. Next occurs the process leading to forward movement of the nucleus and cell body, which is termed “traction.” Finally, the last step is “de-adhesion” and “retraction” of the rear region of the cell. This means that cell-matrix adhesion and detachment are critical processes during cell migration. In recent studies, it has been reported that SMC migration is blocked by $\alpha_v\beta_3$ -integrin-blocking antibodies or RGD peptides in vitro and that the inhibition of $\alpha_v\beta_3$ -integrin retards the development of arterial intimal thickening and restenosis after vascular injury (33,35–39). Our data show that the LTBP-1-induced migration of diabetic SMCs is mainly due to integrin- β_3 , but that of control SMCs is not; by coincidence, integrin- β_3 increases in diabetic aorta compared with control by immunohistochemistry. These results suggest that the mechanism of migration is different between control and diabetic SMCs. Bendeck et al. (40) reported that newborn SMCs of humans express $\alpha_v\beta_3$ -integrin but that adult SMCs do not. Liaw et al. (35) demonstrated that most human aortic SMCs expressed little or no $\alpha_v\beta_3$ -integrin, but that those isolated from an infant did. Hoshiga et al. (41) reported that $\alpha_v\beta_3$ -integrin was strongly expressed in SMCs of intimal thickening and plaque of atherosclerotic coronary artery from a recipient human heart, but this expression was less in the media of coronary artery. Stouffer et al. (42) showed that integrin- β_3 expression was detected in arterial SMCs 1 week after BCI of a baboon brachial artery, but not in uninjured artery. In total, these studies demonstrate that $\alpha_v\beta_3$ -integrin accompanies changes in the SMC phenotype.

The molecular mechanism of increased expression of integrin- β_3 is not known in diabetic SMCs. But Janat et al. (43) and Basson et al. (44) reported that PDGF-BB stimulates the expression of integrin- β_3 . This means that PDGF β -receptor, which binds to PDGF-BB, is an important factor to increase integrin- β_3 on SMCs. We have already reported that diabetic SMCs expressed PDGF β -receptor much more than control SMCs (2–4). This overexpression of PDGF β -receptor probably results in the increased expression of integrin- β_3 on diabetic SMCs.

Our study showed that PDGF-BB-induced SMC migration was not inhibited by anti-integrin- β_3 antibody. Kappert et al. (45) reported that PDGF-directed migration of rat and human SMCs through gelatin-coated membranes involved not only $\alpha_v\beta_3$ -integrin but also $\alpha_v\beta_5$ -integrin by experiments using blocking antibodies to these integrins. Moreover, Liu et al. (46) showed that integrin- $\alpha_v\beta_3$ is involved in the migration of rat vascular adventitial fibroblasts stimulated by basic fibroblast growth factor but not by PDGF. However, Choi et al. (47) reported that PDGF-induced human SMC migration is mediated by $\alpha_v\beta_3$ -integrin. The reason for the divergent results regarding the role of $\alpha_v\beta_3$ -integrin in PDGF-induced migration remains to be determined.

SMC migration from the medial to the intimal layer of an artery is an initial major process of the formation of arterial intimal thickening (48). Our data demonstrated that migration of diabetic SMCs due to LTBP-1 was accelerated compared with that of control SMCs in vitro, suggesting that LTBP-1 plays a critical role in the intimal thickening of diabetic artery through the acceleration of SMC migration. As we previously described, the latent TGF- β_1 complex consists of TGF- β_1 , LAP, and LTBP-1. In the diabetic artery and SMCs, TGF- β_1 is one of the essential factors for the development of the characteristic changes of arterial SMCs, such as an increase in fibronectin and PDGF β -receptor (5). LAP has an important role in the latency of the TGF- β_1 complex (11). It is conceivable that each component of the latent TGF- β_1 complex influences the initiation, progression, and regression of arterial intimal thickening in diabetic artery. In summary, LTBP-1 accelerated the migration of diabetic SMCs through integrin- β_3 in comparison to that of control SMCs, and it appears to play an essential role in the intimal thickening of diabetic artery.

ACKNOWLEDGMENTS

This study was supported in part by the Naito Foundation, the Hamaguchi Foundation for the Advancement of Biochemistry, and the Takeda Science Foundation (to T.K.).

We thank Dr. Kohei Miyazono (Department of Molecular Pathology, Tokyo University, Tokyo, Japan) for kindly providing Ab39. We also appreciate Dr. Shiro Ueda (Chiba University, Graduate School of Pharmaceutical Sciences, Chiba City, Japan) for useful discussion and Dr. Akihiro Haruno (Taiho Pharmaceutical, Hanno City, Japan) for help with the statistical analysis.

REFERENCES

1. Kanzaki T, Shiina R, Saito Y, Zardi L, Morisaki N: Transforming growth factor- β receptor and fibronectin expressions in aortic smooth muscle cells in diabetic rats. *Diabetologia* 40:383–391, 1997
2. Kawano M, Koshikawa T, Kanzaki T, Morisaki N, Saito Y, Yoshida S: Diabetes mellitus induces accelerated growth of aortic smooth muscle cells: association with overexpression of PDGF β -receptors. *Eur J Clin Invest* 23:84–90, 1993
3. Tamura K, Kanzaki T, Tashiro J, Yokote K, Mori S, Ueda S, Saito Y, Morisaki N: Increased atherogenesis in Otsuka Long-Evans Tokushima fatty rats before the onset of diabetes mellitus: association with overexpression of PDGF β -receptors in aortic smooth muscle cells. *Atherosclerosis* 149:351–358, 2000
4. Kanzaki T, Shinomiya M, Ueda S, Morisaki N, Saito Y, Yoshida S: Enhanced arterial intimal thickening after balloon catheter injury in diabetic animals accompanied by PDGF β -receptor overexpression of aortic media. *Eur J Clin Invest* 24:377–381, 1994

5. Hedin U, Thyberg J: Plasma fibronectin promotes modulation of arterial smooth-muscle cells from contractile to synthetic phenotype. *Differentiation* 33:239–246, 1987
6. Roberts AB, Sporn MB: The transforming growth factor- β s. In *Peptide Growth Factors and Their Receptors*. Part I. Sporn MB, Roberts AB, Eds. Berlin, Springer-Verlag, 1990, p. 419–472
7. Pircher R, Lawrence DA, Jullien P: Latent β -transforming growth factor in nontransformed and Kirsten sarcoma virus-transformed normal rat kidney cells, clone 49F. *Cancer Res* 44:5538–5543, 1984
8. Pircher R, Jullien P, Lawrence DA: β -transforming growth factor is stored in human platelets as a latent high molecular weight complex. *Biochem Biophys Res Commun* 136:30–37, 1986
9. Kanzaki T, Tamura K, Takahashi K, Saito Y, Akikusa B, Oohashi H, Kasayuki N, Ueda M, Morisaki N: In vivo effect of TGF- β 1: enhanced intimal thickening by administration of TGF- β 1 in rabbit arteries injured with a balloon catheter. *Arterioscler Thromb Vasc Biol* 15:1951–1957, 1995
10. Nabel EG, Shum L, Pompili VJ, Yang Z-Y, San H, Shu HB, Libtay S, Gold L, Gordon D, Derynck R, Nabel GJ: Direct transfer of transforming growth factor β 1 gene into arteries stimulates fibrocellular hyperplasia. *Proc Natl Acad Sci U S A* 90:10759–10763, 1993
11. Bottinger EP, Factor VM, Tsang ML-S, Weartherbee J, Kopp J, Qian S, Wakefield L, Roberts A, Thorgeirsson S, Sporn M: The recombinant proregion of transforming growth factor β 1 (latency-associated peptide) inhibits active transforming growth factor β 1 in transgenic mice. *Proc Natl Acad Sci U S A* 93:5877–5882, 1996
12. Stenman G, Sahlin P, Olofsson A, Geurts van Kessel A, Miyazono K: Assignment of the gene encoding the latent TGF- β 1-binding protein (LTBP1) to human chromosome 2, region p12–q22. *Cytogenet Cell Genet* 66:117–119, 1994
13. Kanzaki T, Olofsson A, Moren A, Wernstedt C, Hellman U, Miyazono K, Claesson-Welsh L, Heldin C-H: TGF- β 1 binding protein: a component of the large latent complex of TGF- β 1 with multiple repeat sequences. *Cell* 61:1051–1061, 1990
14. Tsuji T, Okada F, Yamaguchi K, Nakamura T: Molecular cloning of the large subunit of transforming growth factor type β masking protein and expression of the mRNA in various rat tissues. *Proc Natl Acad Sci U S A* 87:8835–8839, 1990
15. Moren A, Olofsson A, Stenman G, Sahlin P, Kanzaki T, Claesson-Welsh L, ten Dijke P, Miyazono K, Heldin C-H: Identification and characterization of LTBP-2, a novel latent transforming growth factor- β -binding protein. *J Biol Chem* 269:32469–32478, 1994
16. Yin W, Smiley E, Germiller J, Mechem RP, Florer JB, Wenstrup RJ, Bonadio J: Isolation of a novel latent transforming growth factor- β binding protein gene (LTBP-3). *J Biol Chem* 270:10147–10160, 1995
17. Giltay R, Kostka G, Timpl R: Sequence and expression of a novel member (LTBP-4) of the family of latent transforming growth factor- β binding proteins. *FEBS Lett* 411:164–168, 1997
18. Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY: Partial sequence of a candidate gene for the Marfan syndrome. *Nature* 352:334–337, 1991
19. Zhang H, Apfelroth SD, Hu W, Davis EC, Sanguineti C, Bonadio J, Mechem RP, Ramirez F: Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices. *J Cell Biol* 124:855–863, 1994
20. Mangasser-Stephan K, Gressner AM: Molecular and functional aspects of latent transforming growth factor- β binding protein: just a masking protein? *Cell Tissue Res* 297:363–370, 1999
21. Miyazono K, Olofsson A, Colosetti P, Heldin C-H: A role of the latent TGF- β 1-binding protein in the assembly and secretion of TGF- β 1. *EMBO J* 10:1091–1101, 1991
22. Olofsson A, Ichijo H, Moren A, ten Dijke P, Miyazono K, Heldin C-H: Efficient association of an amino-terminally extended form in human latent transforming growth factor-beta binding protein with the extracellular matrix. *J Biol Chem* 270:31294–31297, 1995
23. Unsold C, Hyttiaainen M, Bruckner-Tuderman L, Keski-Oja J: Latent TGF- β binding protein LTBP-1 contains three potential extracellular matrix interacting domains. *J Cell Sci* 114:187–197, 2001
24. Dalles SL, Miyazono K, Skerry TM, Mundy GR, Bonewald LF: Dual role for the latent transforming growth factor- β binding protein in storage of latent TGF- β in the extracellular matrix and as structural matrix protein. *J Cell Biol* 131:539–549, 1995
25. Nakajima Y, Miyazono K, Kato M, Takase M, Yamagishi T, Nakamura H: Extracellular fibrillar structure of latent TGF- β binding protein-1: role in TGF- β -dependent endothelial-mesenchymal transformation during endocardial cushion tissue formation in mouse embryonic heart. *J Cell Biol* 136:193–204, 1997
26. Kanzaki T, Shiina R, Saito Y, Oohashi H, Morisaki N: Role of TGF- β 1 binding protein in vascular remodeling. *Biochem Biophys Res Commun* 246:26–30, 1998
27. Waltenberger J, Akyurek ML, Aurivillius M, Wanders A, Larsson E, Fellstrom B, Funa K: Ischemia-induced transplant arteriosclerosis in the rat: induction of peptide growth factor expression. *Arterioscler Thromb Vasc Biol* 16:1516–1523, 1996
28. Taipale J, Saharinen J, Hedman K, Keski-Oja J: Latent transforming growth factor beta-1 and its binding protein are components of extracellular matrix microfibrils. *J Histochem Cytochem* 44:875–889, 1996
29. Fisher-Dzoga K, Jones RM, Vesselinovich D, Wissler RW: Ultrastructural and immunohistochemical studies of primary cultures of aortic medial cells. *Exp Mol Pathol* 18:162–176, 1973
30. Ooyama T, Fukuda K, Oda H, Nakamura H, Hikita Y: Substratum-bound elastin peptide inhibits aortic smooth muscle cell migration in vitro. *Arteriosclerosis* 7:593–598, 1987
31. Frolik CA, Wakefield LM, Smith DM, Sporn MB: Characterization of a membrane receptor for transforming growth factor- β in normal rat kidney fibroblasts. *J Biol Chem* 259:10995–11000, 1984
32. Ichijo H, Momose F, Miyazono K: Biological effects and binding properties of transforming growth factor- β on human oral squamous cell carcinoma cells. *Exp Cell Res* 187:263–269, 1990
33. Slepian MJ, Massia SP, Dehadashi B, Frits A, Whitesell L: β_3 -integrins rather than β_1 -integrins dominate integrin-matrix interactions involved in postinjury smooth muscle cell migration. *Circulation* 97:1818–1827, 1998
34. Mitchison TJ, Cramer L: Actin-based cell motility and cell locomotion. *Cell* 84:371–379, 1996
35. Liaw L, Skinner MP, Raines EW, Ross R, Cheresch DA, Schwartz SM, Giachelli CM: The adhesive and migratory effects of osteopontin are mediated via distinct cell surface integrins: role of $\alpha_5\beta_3$ in smooth muscle cell migration to osteopontin in vitro. *J Clin Invest* 95:713–724, 1995
36. Matsuno H, Stassen JM, Vermeylen J, Deckmyn H: Inhibition of integrin function by a cyclic RGD-containing peptide prevents neointima formation. *Circulation* 90:2203–2206, 1994
37. Panda D, Kundu GC, Lee BI, Peri A, Fohl D, Chackalaparampil I, Mukherjee BB, Li XD, Mukherjee DC, Seides S, Rosenberg J, Stark K, Mukherjee AB: Potential roles of osteopontin and $\alpha_5\beta_3$ integrin in the development of coronary artery stenosis after angioplasty. *Proc Natl Acad Sci U S A* 94:9308–9313, 1997
38. Srivatsa SS, Fitzpatrick LA, Tsao PW, Reilly TM, Holmes DR, Schwartz RS, Mousa SA: Selective $\alpha_5\beta_3$ integrin blockade potently limits neointimal hyperplasia and lumen stenosis following deep coronary arterial stent injury: evidence for the functional importance of integrin $\alpha_5\beta_3$ and osteopontin expression during neointima formation. *Cardiovasc Res* 36:408–428, 1997
39. Coleman KR, Braden GA, Willingham MC, Sane DC: Vitaxin, a humanized monoclonal antibody to the vitronectin receptor ($\alpha_5\beta_3$), reduces neointimal hyperplasia and total vessel area after balloon injury in hypercholesterolemic rabbits. *Circ Res* 84:1268–1276, 1999
40. Bendeck MP, Irvin C, Reidy M, Smith L, Mulholland D, Horton M, Giachelli CM: Smooth muscle cell matrix metalloproteinase production is stimulated via $\alpha_5\beta_3$ integrin. *Arterioscler Thromb Vasc Biol* 20:1467–1472, 2000
41. Hoshiga M, Alpers CE, Smith LL, Giachelli CM, Schwartz SM: $\alpha_5\beta_3$ integrin expression in normal and atherosclerotic artery. *Circ Res* 77:1129–1135, 1995
42. Stouffer GA, Hu Z, Sajid M, Li H, Jin G, Nakada MT, Hanson SR, Runge MS: β_3 integrins are upregulated after vascular injury and modulate thrombospondin- and thrombin-induced proliferation of cultured smooth muscle cells. *Circulation* 97:907–915, 1998
43. Janat MF, Argraves WS, Liau G: Regulation of vascular smooth muscle cell integrin expression by transforming growth factor β 1 and by platelet-derived growth factor-BB. *J Cell Physiol* 151:588–595, 1992
44. Basson CT, Kocher O, Basson MD, Asis A, Madri JA: Differential modulation of vascular cell integrin and extracellular matrix expression in vitro by TGF- β 1 correlates with reciprocal effects on cell migration. *J Cell Physiol* 153:118–128, 1992
45. Kappert K, Blaschke F, Meehan WP, Kawano H, Grill M, Fleck E, Hsueh WA, Law RE, Graf K: Integrins $\alpha_5\beta_3$ and $\alpha_5\beta_1$ mediate VSMC migration and are elevated during neointima formation in the rat aorta. *Basic Res Cardiol* 96:42–49, 2001
46. Liu G, Eskin SG, Mikos AG: Integrin $\alpha_5\beta_3$ is involved in stimulated migration of vascular adventitial fibroblasts by basic fibroblast growth factor but not platelet-derived growth factor. *J Cell Biochem* 83:129–135, 2001
47. Choi ET, Engel L, Callow AD, Sun S, Trachtenberg J, Santoro S, Ryan US: Inhibition of neointimal hyperplasia by blocking $\alpha_5\beta_3$ integrin with a small peptide antagonist GpenGRGDSPCA. *J Vasc Surg* 19:125–134, 1994
48. Ross R: The pathogenesis of atherosclerosis—an update. *N Engl J Med* 314:488–500, 1986