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Transforming growth factor-β receptor and fibronectin expressions in aortic smooth muscle cells in diabetic rats

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Summary Smooth muscle cells in arteries of diabetic rats and rabbits have unique properties including the overexpression of platelet-derived growth factor (PDGF) β-receptor compared with controls. Fibronectin, one of the increased components of extracellular matrices in diabetic arteries, plays an important role in the phenotypic change of smooth muscle cells from the contractile to the synthetic type with the expression of the PDGF β-receptor. Moreover, fibronectin synthesis is regulated by transforming growth factor-β (TGF-β). In this study, we report on the expression of TGF-β receptors in diabetic smooth muscle cells, by immunohistochemistry, cross-linking of ^{125}I-TGF-β1 to cells and quantitative reverse transcription-polymerase chain reaction. We also report on the effects of TGF-β1 on fibronectin synthesis of diabetic smooth muscle cells by use of ELISA and immunoprecipitation, in order to clarify the role of TGF-β-fibronectin pathway in forming characteristic changes of diabetic smooth muscle cells. Cultured aortic smooth muscle cells of diabetic rats expressed TGF-β type II receptor about 8.7 times that of controls at the protein level and 5.7 times at the mRNA level, whereas the expression of the type I receptor did not differ between the two types of smooth muscle cells. These changes were accompanied by increased fibronectin synthesis in diabetic smooth muscle cells in response to TGF-β1. Furthermore, protein expression of fibronectin, and mRNA and protein of TGF-β type II receptor were increased in the diabetic aorta compared with the control aorta in vivo, implying the importance of the TGF-β-fibronectin pathway for the unique biology of smooth muscle cells in the diabetic artery. [Diabetologia (1997) 40: 383–391]

Keywords Transforming growth factor-β (TGF-β), TGF-β receptor, fibronectin, aortic smooth muscle cells, diabetes mellitus, smooth muscle cell phenotype.

We have previously reported that cultured aortic smooth muscle cells (SMC) and medial layers of arteries of diabetic rats and rabbits express more platelet-derived growth factor (PDGF) β-receptor than those of non-diabetic animals [1, 2]. In addition, diabetic SMC grow much faster than control SMC specifically in reponse to A-B heterodimer and B-B homodimer of PDGF, suggesting that the overexpression of PDGF β-receptor in SMC is a causative element in the accelerated growth of diabetic SMC. In an in vivo study, SMC-dominant intimal thickening was enhanced in diabetic rabbits compared with non-diabetic rabbits at 2 weeks after balloon catheter injury of the carotid arterial wall [2]. Taken together, the above results suggest that the change in growth properties of SMC by the overexpression of PDGF β-receptor plays a

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Abbreviations: SMC, Aortic smooth muscle cells; TGF-β, transforming growth factor-β; PDGF, platelet-derived growth factor; CM, conditioned medium; DMEM, Dulbecco's modified Eagle's medium; BSA, bovine surum albumin; PBS, phosphate buffered saline; RT-PCR, reverse transcription-polymerase chain reaction; OLETF, Otsuka Long-Evans Tokushima Fatty.

critical role in the formation of diabetic macroangiopathy.

Very little is known about the regulation of the PDGF β-receptor, but this receptor is expressed in the synthetic type of SMC but not in the contractile type [3]. Hedin et al. [3] reported that fibronectin was one of the important factors in the phenotypic change of SMC from contractile to synthetic. They showed that SMC lost contractile phenotype very rapidly and changed into synthetic phenotype expressed with the PDGF β-receptor if seeded onto a fibronectin coated surface, but rather slowly if laminin was used as a coating protein [4]. Fibronectin provides better adhesion and spreading for SMC than laminin [4]. Cell-extracellular matrix linkages play an important role in the process of adhesion, suggesting that the alteration of cell-matrix interelationship might serve as a signal that leads to phenotypic changes of SMC, e.g., PDGF β-receptor expression. Indeed, the fibronectin contents of arterial media in diabetic patients were significantly increased compared with control subjects [5], suggesting that fibronectin is closely related to characteristic changes of diabetic SMC. Furthermore, it is well known that fibronectin is one of the major components in extracellular matrices and that its synthesis and degradation are regulated by transforming growth factor (TGF-β) [6]. It is also reported that the function of TGF-β is transduced by its receptors, that is, TGF-β type I and type II receptors [7, 8]. Taken together, it is conceivable that TGF-β, its receptors and fibronectin play crucial roles in the characteristic changes of diabetic SMC.

In this study, we report on a unique phenotype of diabetic SMC, namely the overexpression of receptors for TGF-β as well as the oversynthesis of fibronectin by TGF-β1 both in in vitro and in vivo systems.

Materials and methods

Chemicals. Sources of materials were as follows: streptozotocin, Sigma Chemical Co. (St. Louis, Mo., USA); TGF-β1, King Brewing Co. (Kakogawa, Japan); Bis (sulfosuccinimidyl) suberate, Pierce (Rockford, Ill., USA); Gelatin-Sepharose, Pharmacia LKB (Uppsala, Sweden); ^{125}I-TGF-β1, ^{35}S-methionine, ^{35}S-cysteine and α-^{32}P-dCTP, New England Nuclear (Boston, Mass., USA). Antibodies and cDNAs of TGF-β type I and II receptors were kindly donated by Dr. K. Miyazono (Department of Biochemistry, The Cancer Institute, Tokyo, Japan) and monoclonal antibodies to human ED-A fibronectin (Ist-9) were from Dr. L. Zardi.

Induction of diabetes. Diabetes was induced by an injection of 60 mg/kg of streptozotocin into male Wistar rats. Control and diabetic rats were fed a standard chow for 4 weeks. The plasma glucose levels were: control, 4.61 ± 0.24 mmol/l (***n*** = 4); diabetic, 15.7 ± 2.35 mmol/l (***n*** = 4). The body weights were: control, 384 ± 28.8 g (***n*** = 4); diabetic, 292 ± 21.0 g (***n*** = 4). The experiments were repeated in four different pairs.

Culture of aortic SMC. The thoracic aorta was removed from rats under sterile conditions. Primary culture and subculture were carried out in Dulbecco's modified Eagles's medium (DMEM) containing 10 % fetal bovine serum as described previously [9]. SMC at the third to fifth passages were used for fibronectin assay or cross-linking of ^{125}I-TGF-β1 to SMC to examine the TGF-β receptor.

Assay of fibronectin. Confluent SMC in 6-well plates (Corning, New York, USA) were incubated with 2 ml of serum free DMEM for 48 h, and then were changed to the fresh DMEM containing 1 mg/ml bovine serum albumin and 0–10 ng/ml TGF-β1. After 24 h of incubation, the medium was collected (conditioned medium (CM)). Fibronectin was assayed in CM of SMC by an ELISA using human fibronectin antibody. Preliminary experiments showed that this human fibronectin antibody reacted with both human and rat fibronectin.

Analysis of fibronectin synthesis in control and diabetic SMC. To detect fibronectin synthesis, fibronectin in medium and cell lysates from ^{35}S-methionine and cysteine labelled SMC was examined as described by Ignotz and Massague [10]. Briefly, confluent SMC were incubated with serum free DMEM with 0–10 ng/ml TGF-β1 for 20 h and then labelled with ^{35}S-methionine and cysteine in methionine and cysteine-free DMEM with 0–10 ng/ml TGF-β1 at 37 °C for 3 h. After 3 h labelling, the medium and the supernatants of SMC were collected. Gelatin-Sepharose suspension (50 μl) was added to the medium and supernatants, followed by incubation at 4 °C with constant agitation overnight. The gelatin-Sepharose beads were recovered by centrifugation and washed three times with 0.5 % Triton X-100, 0.15 mol/l NaCl, 25 mmol/l Tris-HCl, pH 7.4. Fibronectin was eluted from the beads by boiling in SDS-sample buffer containing 4 % SDS, 0.2 mol/l Tris-HCl, pH 8.8, 0.5 mol/l sucrose, 0.1 % bromphenol blue and 2 % β-mercaptoethanol and was analysed by SDS-PAGE, followed by fluorography.

Detection of TGF-β receptor by cross-linking. Cross-linking experiments were performed as previously described [11]. In brief, cells in 6-well plates were washed with binding buffer (phosphate buffered saline containing 0.9 mmol/l $CaCl_2$, 0.49 mmol/l $MgCl_2$ and 1 mg/ml bovine serum albumin) and incubated on ice in the same buffer with 100 pmol/l ^{125}I-TGF-β1 in the presence or absence of excess unlabelled TGF-β1 for 3 h. Cells were washed, and cross-linking was done in the binding buffer without bovine serum albumin together with 0.25 mmol/l Bis for 15 min on ice. Supernatants from solubilized SMC containing the same amounts of protein were subjected to analysis by SDS gel electrophoresis using 6 % polyacrylamide gel, followed by autoradiography.

Immunohistochemical study of fibronectin and TGF-β receptors. The arteries were snap-frozen and stored at –80 °C. These samples were sectioned serially at 6 μm thickness and fixed in acetone.

For the identification of fibronectin, we used an undiluted mouse monoclonal antibody (Ist-9) that recognizes ED-A sequence of fibronectin, the specificity of which has been reported [12, 13]. Rabbit affinity-purified antibodies against synthetic peptides corresponding to the intracellular juxtamembrane parts of the receptors were used for the identification of TGF-β type I and II receptors at a concentration of 3 μg/ml [14, 15]. These antibodies crossreact with rat, and the specificity has been reported [15]. The staining of sections was followed by the ABC peroxidase immunohistochemstry method described by Waltenberger et al. [16].

Detection of TGF-β receptor by reverse transcription-polymerase chain reaction. Reverse transcription-polymerase chain reaction (RT-PCR) was used to detect the mRNA of TGF-β receptors. Total RNA was purified from control and diabetic SMC by the LiCl-urea method [17]. RT-PCR was perfomed as described by Tsuchida et al. [18]. Briefly, first strand cDNA was prepared from total RNA (2 μg) from the medial layers of rat carotid arteries by using first strand cDNA synthesis kit (Pharmacia LKB). Reaction mixture (10 μl) was removed for PCR. Reactions were performed in 10 mmol/l Tris-HCl, pH 8.3, containing 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 0.2 mmol/l each of dATP, dGTP, dCTP and dTTP, 20 pmol of sense and antisense primers and 2.5 units of Taq polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA). PCR primers were prepared at nucleotide sequences 511–531 (sense), 1329–1350 (antisense) of human TGF-β type I receptor [14], at 868–893 (sense), 1348–1371 (antisense) of rat TGF-β type II receptor [18] and at 1–18 (Exon 1)(sense), 1014–1032 (Exon 3)(antisense) of human β-actin [19]. PCR conditions were as follows: 30 cycles for TGF-β receptors or 25 cycles for β-actin at 94 °C (1 min), 60 °C (2 min) and 72 °C (3 min), followed by 10 min at 72 °C. After PCR amplifications, aliquots of each amplification mixture were separated by 1.5 % of agarose gel electrophoresis and visualized by ethidium bromide staining.

The protocol of RT-PCR was designed to measure the level of TGF-β receptors relative to the expression of an endogenous internal standard gene (β-actin) [19, 20]. To quantify the mRNA expressions, TGF-β receptors and β-actin cDNAs generated in the same RT reaction were amplified in separate tubes containing increasing volumes of the RT reaction (0.75, 1.5, 3.0 and 6.0 μl) to document amplification in the linear region for each cDNA [19].

Southern blot analysis. Agarose gels were soaked sequentially in 0.5 mol/l NaOH/1.5 mol/l NaCl for 1 h and neutralized in 1 mol/l Tris-HCl, pH 8.0/1.5 mol/l NaCl for 1 h, and then transferred by blotting to a Hybond N^+ membrane (Amersham). Southern hybridization was performed in 50 % formamide, 5 × SSC (1 × SSC contains 15 mmol/l sodium citrate and 150 mmol/l NaCl, pH 7.4), 5 × Denhardt's solution, 0.1 % SDS, 50 mmol/l sodium phosphate buffer (pH 6.5), and 0.1 mg/ml salmon sperm DNA at 42 °C overnight with a human cDNA of TGF-β type I or II receptor containing the amplified region without primer area, labelled with the Megaprime DNA labelling system (Amersham) as a probe. The membrane was washed three times for 20 min each in 0.1 × SSC, 0.1 % SDS at 42 °C, dried, and exposed to Amersham Hyperfilm MP.

Statistical methods

The significance of differences was evaluated by ANOVA. Results are given as mean ± SD.

Results

Immunohistochemical study of fibronectin in aortas from control and diabetic rats. Medial layers from diabetic rats stained positive with a fibronectin antibody (Ist-9), whereas the staining of control rats was very weak (Fig. 1), suggesting that diabetic SMC in vivo expressed more fibronectin than controls.

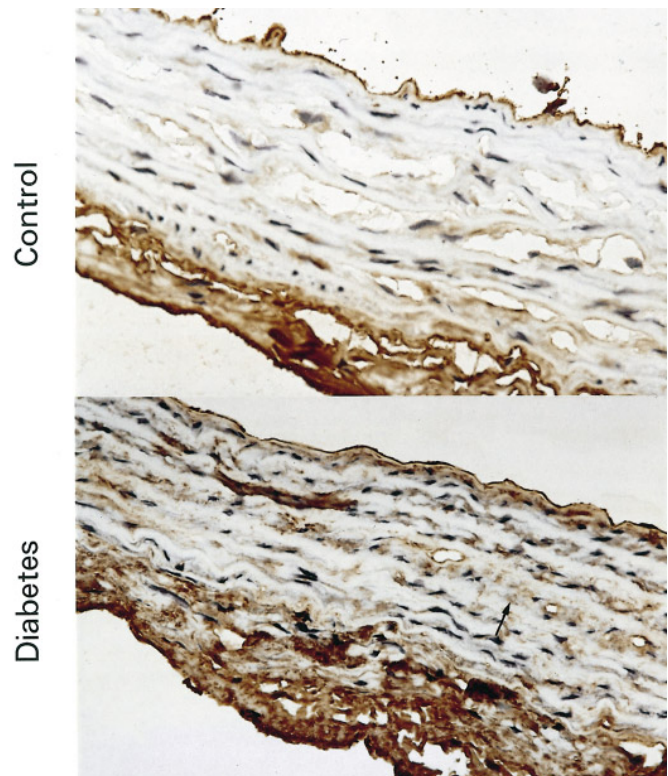

Fig. 1. Immunohistochemical study of fibronectin in aortas from control and diabetic rats. Immunohistochemistry was performed in aortas from control and diabetic rats using anti-fibronectin antibody (Ist-9) as indicated in Materials and methods

Table 1. Effects of TGF-β1 on the amount of fibronectin in control and diabetic SMC

Control SMC				
Experiment	TGF-β1 (ng/ml)			
	0	1	5	10
1	129	118	130	144 ng/ml
2	72.0	91.7	93.8	99.4
3	121	120	134	145
4	83.1	88.8	124	128
Mean ± SD	101 ± 27.9	104 ± 16.6	120 ± 18.2	129 ± 21.2
Diabetic SMC				
Experiment	TGF-β1 (ng/ml)			
	0	1	5	10
1	88.2	140	181	233 ng/ml
2	59.1	97.5	127	194
3	157	197	281	342
4	95.4	147	209	298
Mean ± SD	100 ± 38.0	145 ± 40.9	199 ± 64.1	266 ± 66.0

Effects of TGF-β1 on the amount and synthesis of fibronectin in control and diabetic SMC. To determine the mechanism of increased fibronectin in diabetic SMC, we examined the effect of TGF-β1 on the amount and synthesis of fibronectin in diabetic SMC

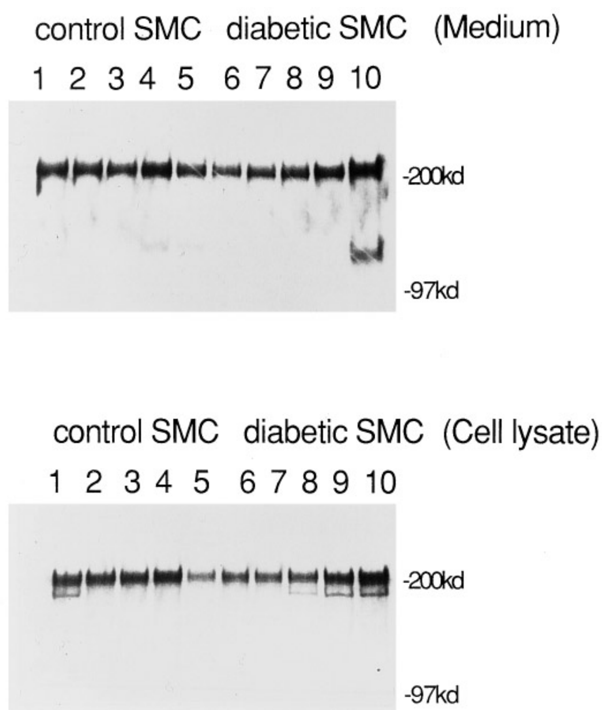

Fig. 2. Effects of transforming growth factor (TGF)-β1 on fibronectin synthesis of control and diabetic smooth muscle cells (SMC). Analysis of fibronectin in medium and cell lysates from control SMC (lanes 1–5) and diabetic SMC (lanes 6–10) was performed as described in Materials and methods. Medium and cell lysates in lanes 1 and 6 were without treatment of TGF-β1, those in lanes 2 and 7 were treated with 10 pg/ml of TGF-β1, those in lanes 3 and 8 with 100 pg/ml of TGF-β1, those in lanes 4 and 9 with 1 ng/ml of TGF-β1 and those in lanes 5 and 10 with 10 ng/ml. The experiments were done with four different pairs of control and diabetic SMC, and typical results are shown. Molecular size markers are shown on the right

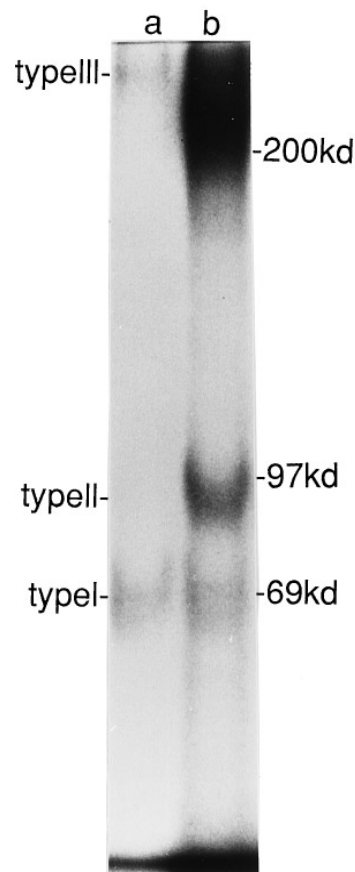

Fig. 3. Binding of ^{125}I-transforming growth factor (TGF)-β1 to control and diabetic smooth muscle cells (SMC). Cross-linking experiments were performed with four different pairs of control and diabetic SMC as described in Materials and methods, and typical results are shown. Molecular size is indicated on the right and specific binding components on the left. a: control SMC; b: diabetic SMC

by ELISA in CM and the immunoprecipitation method of metabolically labelled SMC, respectively.

The basal amount of fibronectin in CM was 101 ± 27.9 ng/ml ($n = 4$) in control SMC and 100 ± 38.0 ng/ml ($n = 4$) in diabetic SMC (Table 1). The fibronectin contents in CM from control SMC treated with 1, 5 and 10 ng/ml of TGF-β1 were 104 ± 16.6 ng/ml ($n = 4$), 120 ± 18.2 ng/ml ($n = 4$) and 129 ± 21.2 ng/ml ($n = 4$), respectively (Table 1). The fibronectin contentsCM from diabetic SMC treated with 1, 5 and 10 ng/ml of TGF-β1 were 145 ± 40.9 ng/ml ($n = 4$), 199 ± 64.1 ng/ml ($n = 4$) and 266 ± 66.0 ng/ml ($n = 4$), respectively (Table 1). These data suggested that cultured diabetic SMC, but not control SMC, specifically responded to exogenous TGF-β1.

To confirm the above results, fibronectin synthesis was measured in metabolically labelled SMC. Bands around 220 kDa corresponded to fibronectin, which was confirmed by the earlier experiment using an anti-fibronectin antibody [10]. The basal intensity of the fibronectin bands in the medium and lysates of control SMC was slightly higher than those of diabetic SMC. The intensities of the fibronectin bands in the medium and lysates of control SMC treated with TGF-β1 were the same as those without TGF-β1 except that those treated with 10 ng/ml TGF-β1 were decreased (Fig. 2). However, those of diabetic SMC were increased dose-dependently with added TGF-β1 (Fig. 2). The intensities of the fibronectin band in the medium and lysates were increased 4.5-fold and 4.1-fold, respectively, with diabetic SMC treated with 10 ng/ml of TGF-β1 compared with that without TGF-β1 treatment. These results were consistent with the data on fibronectin content as shown in Table 1, suggesting that the TGF-β receptor and/or the post-receptor signal tranduction system was altered in diabetic SMC.

Expressions of TGF-β type I and II receptors in control and diabetic SMC in vitro. As the function of TGF-β1 is mediated by TGF-β receptors,

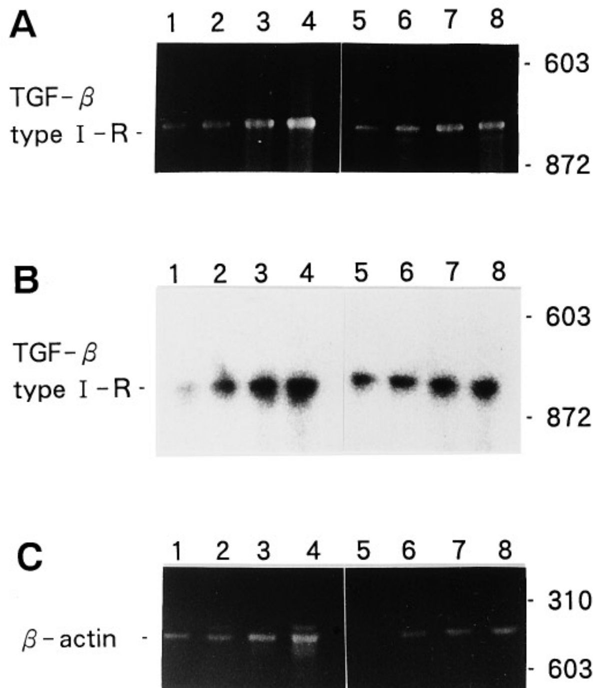

Fig. 4A–C. mRNA expressions of TGF-β type I receptor in control and diabetic SMC. RT-PCR (**A**: TGF-β type I receptor, **C**: β-actin) and Southern blot analysis (**B**: TGF-β type I receptor) were carried out with control SMC (lanes 1,2,3 and 4) and diabetic SMC (lanes 5,6,7 and 8) as described in Materials and methods. Volumes of first strand cDNA solutions: 0.75 μl (lanes 1 and 5), 1.5 μl (lanes 2 and 6), 3.0 μl (lanes 3 and 7) and 6.0 μl (lanes 4 and 8). Experiments performed with four different pairs of control and diabetic SMC, and typical results are shown. Right: DNA size markers; left: specific PCR products

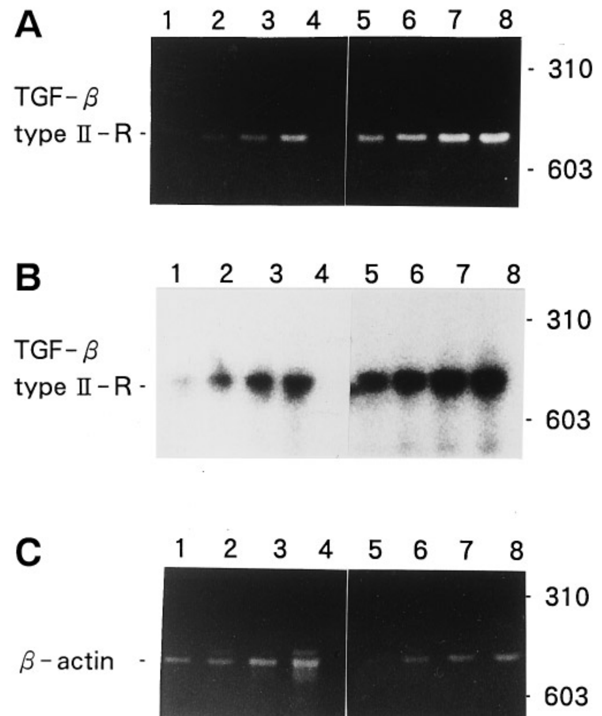

Fig. 5A–C. mRNA expressions of TGF-β type II receptor in control and diabetic SMC. RT-PCR (**A**: TGF-β type II receptor, **C**: β-actin) and Southern blot analysis (**B**: TGF-β type II receptor) were carried out with control SMC (lanes 1,2,3 and 4) and diabetic SMC (lanes 5,6,7 and 8) as described in Materials and methods. Volumes of first strand cDNA solutions: 0.75 μl (lanes 1 and 5), 1.5 μl (lanes 2 and 6), 3.0 μl (lanes 3 and 7) and 6.0 μl (lanes 4 and 8). The experiments were done with four different pairs of control and diabetic SMC, and typical results are shown. Right: DNA size markers; left: specific PCR products

cross-linking experiments were performed in order to determine the TGF-β receptor expression in SMC.

Three major bands were detected in both control and diabetic SMC. Bands of 66, 94 and 200–300 kDa corresponded to TGF-β type I, II and III receptors, respectively (Fig. 3). The intensities of the 94 and 200–300 kDa bands were 8.7 and 13.0-fold higher in diabetic SMC than in control SMC, but that of the 66 kDa band was the same in the two types of cells (Fig. 3). These results suggested that the expression of TGF-β type II and III receptors, but not that of type I receptor was increased at the protein level in diabetic SMC.

It has been reported that cross-linking experiments are able to detect TGF-β binding proteins including TGF-β receptors and other similar receptors [11]. To confirm that the TGF-β receptor is increased in diabetic SMC, we checked the expression of mRNA of TGF-β type I and II receptors.

mRNAs of TGF-β type I and II receptors, which could not be detected in control and diabetic SMC by Northern blot analysis (data not shown), were identified by RT-PCR. The expected 839 bp and 503 bp PCR products were present on agarose gel and were confirmed as TGF-β type I and II receptors, respectively, by Southern blot analysis.

The intensities of the bands of TGF-β type I and II receptors and β-actin in control and diabetic SMC increased linearly with the amounts of RT reaction subjected to amplification (Figs. 4 and 5). The ratio of TGF-β type I receptor intensity to β-actin in control SMC was almost the same as that in diabetic SMC (Fig. 4 and Table 2), indicating that the mRNA expression of TGF-β type I receptor was not different between control and diabetic SMC. However, the ratio of TGF-β type II receptor intensity to β-actin in control SMC was 0.75–0.95, but that in diabetic SMC was 4.8–4.9, the differences between control and diabetic SMC were significant ($p < 0.01$) by ANOVA. Then, the ratio of TGF-β type II receptor intensity to β-actin in diabetic SMC was 5–7 (5.7, average of various concentrations of cDNA) fold higher than that in control SMC (Fig. 5 and Table 2), reflecting that the high expression of TGF-β type II receptor mRNA in diabetic SMC compared with control SMC.

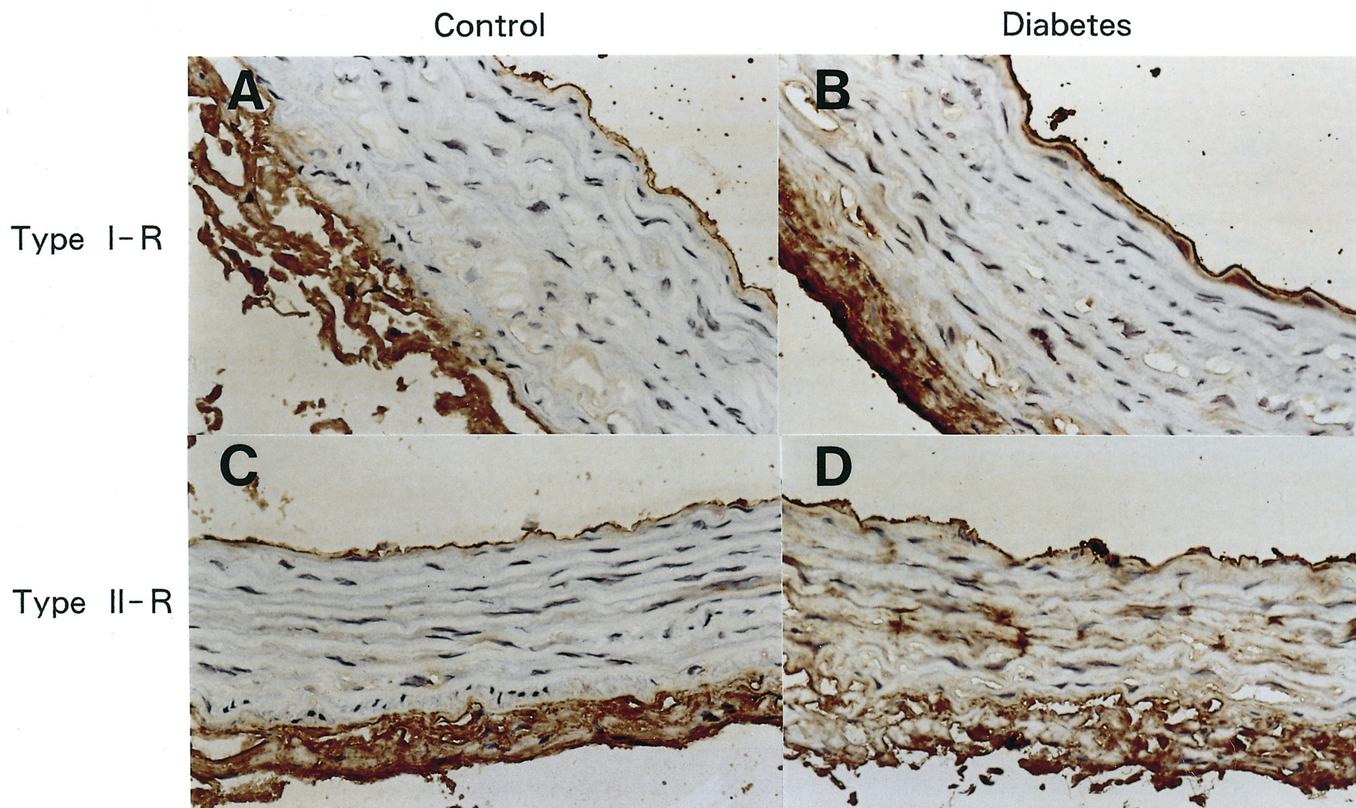

Fig. 6A–D. Immunohistochemical study of TGF-β type I and II receptors in aortas from control and diabetic rats. Immunohistochemistry was carried out in aortas from control (**A**, **C**) and diabetic rats (**B**, **D**). A and B were stained using anti-TGF-β type I receptor antibody and C and D were with anti-TGF-β type II receptor antibody as indicated in Materials and methods

Expressions of TGF-β type I and II receptors in aortas from control and diabetic rats in vivo. To determine whether the high expression of TGF-β type II receptor was observed in diabetic SMC in vivo, we studied the TGF-β receptor expressions in aortas from control and diabetic rats by immunohistochemistry and RT-PCR.

Medial layers in both control and diabetic rats stained very weak with an anti-TGF-β type I receptor antibody (Fig. 6A, B). The media of diabetic rats stained strongly with a TGF-β type II receptor antibody whereas that of control rats was very weak (Fig. 6C, D). Samples using non-immune antibodies at the same concentration (3 μg/ml) had no stainings. These results indicated that diabetic SMC in vivo also expressed more TGF-β type II receptor than control at the protein level. To confirm these results, mRNA expressions of TGF-β receptors were examined.

The expected 503 bp PCR product, confirmed as TGF-β type II receptor by Southern blot analysis, was observed in the aorta of diabetic rats and in cultured SMC, but only a very faint band was present in the aorta of control rats (Fig. 7), indicating that the mRNA of this receptor was more expressed in the aorta of diabetic rats in vivo.

The PCR product of TGF-β type I receptor was observed in cultured SMC but not in aortas from both control and diabetic rats (data not shown), suggesting that there was little, if any, expression of TGF-β type I receptor mRNA in the aortas of the two rats.

Discussion

The present results showed that the expressions of fibronectin and TGF-β receptor were increased in diabetic SMC compared with controls. For quantification, the RT-PCR procedure was designed to measure the level of TGF-β receptors relative to the expression of an endogenous internal standard gene (β-actin). The mRNA expression of TGF-β type II receptor was increased 5.7-fold in diabetic SMC compared with controls, but that of type I receptor hardly differed between control and diabetic SMC, paralleling the results of the cross-linking experiments revealing increased expression of TGF-β type II but not of type I receptor in diabetic SMC.

Very little is known about the regulation of TGF-β receptor gene expression. Hitherto, the responsible metabolic disorder(s) is not known for overexpression of TGF-β receptors in diabetes.

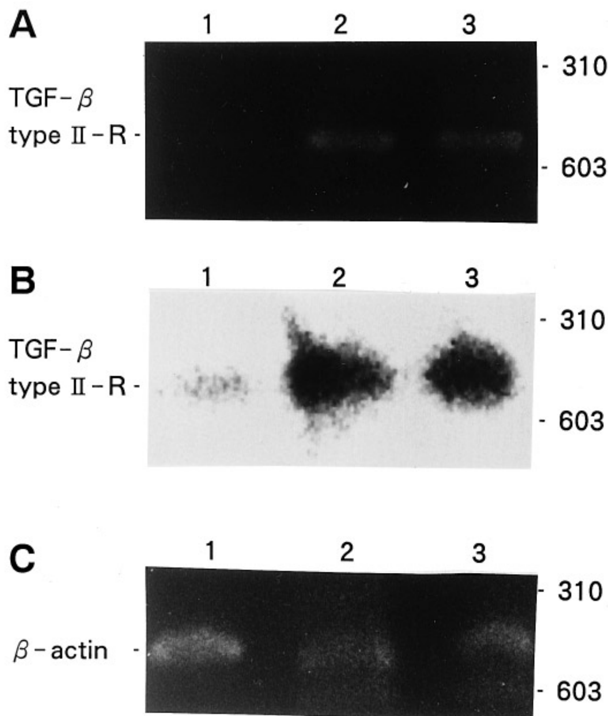

Fig. 7A–C. mRNA expressions of TGF-β type II receptor in aortas of control and diabetic rats. RT-PCR (**A**: TGF-β type II receptor, **C**: β-actin) and Southern blot analysis (**B**: TGF-β type II receptor) were carried out with control arteries (lane 1), diabetic arteries (lane 2) and cultured SMC (lane 3) as described in Materials and methods. The experiments were done with four different pairs of control and diabetic arteries, and typical results are shown. Right: DNA size markers; left: specific PCR products

Insulin and glucose are the most important parameters of the diabetic condition. In this study, streptozotocin-induced diabetes, an insulin-dependent diabetic model, showed low insulin and high glucose levels with the increased expression of TGF-β type II receptor in aortic SMC. We also examined different types of diabetic rats (Otsuka Long-Evans Tokushima Fatty [OLETF] rats) [21], which show high insulin and glucose levels similar to human non-insulin dependent diabetes mellitus. OLETF rat SMC also expressed more PDGF β-receptor and TGF-β type II receptor than control SMC (unpublished data). These data suggest that insulin level is not relevant to TGF-β type II receptor regulation, but glucose or a metabolite, such as advanced glycated end product is closely related to type II receptor up-regulation in diabetes.

Several reports indicate that most pathogenesis related to TGF-β is through TGF-β type II receptor. For example, some tumour cell lines including retinoblastoma, pheochromocytoma, neuroblastoma and breast carcinoma, which are resistant to the growth inhibitory effects of TGF-β, fail to express the type II receptor [22–24]. Here we showed another example of type II receptor-dependent pathological condition, i.e., overexpression of TGF-β type II receptor leads to increased synthesis of fibronectin in diabetic SMC.

Current opinions suggest that TGF-β type III receptor may be indirectly involved in signal transduction as, for example, by presenting ligands to the type I and type II receptors [7]. TGF-β type I and type II receptors have serine/threonine kinase activities and are closely related to signal tranduction probably by a heteromeric complex of both components [8]. It is not clear whether a variety of functions of TGF-β are transduced by different TGF-β receptors or post-receptor systems. Carcamo et al. [25] reported that the TGF-β type I receptor in COS cells or epithelial cells specifies signals sent by TGF-β through binding to TGF-β in the presence of type II receptor. However, Chen et al. [26] reported that TGF-β type II receptor is related to growth inhibition and the inhibition of phosphorylation of RB protein but not to the expression of fibronectin and plasminogen activator inhibitor-1. McCaffrey et al. [27] also reported that TGF-β type II receptor is decreased in cells from atherosclerotic lesions with the increase of collagen synthesis. Centrella et al. [28] reported that bone morphogenetic protein-2 decreases the expression of TGF-β type II receptor in osteoblast cells. These reports suggest that TGF-β type II receptor also has a variety of functions of TGF-β. In our findings from in vitro cultured diabetic SMC, the TGF-β type II receptor showed a possible correlation with increasing fibronectin synthesis but the TGF-β type I receptor did not. Moreover, our data showed that

Table 2. Ratios of intensities in transforming growth factor (TGF)-β type I receptor (I-R)/β-actin and TGF-β type II receptor (II-R)/β-actin at varying amounts of cDNA in control and diabetic smooth muscle cells (SMC) by RT-PCR

Receptor	Control cDNA (μl)				Diabetic cDNA (μl)			
	0.75	1.5	3	6	0.75	1.5	3	6
I-R/β-actin	1.1 ± 0.25	1.3 ± 0.28	1.5 ± 0.29	1.2 ± 0.34	1.6 ± 0.68	1.5 ± 0.54	1.7 ± 0.56	1.5 ± 0.41
II-R/β-actin	0.79 ± 0.24	0.85 ± 0.32	0.94 ± 0.25	0.87 ± 0.24	4.9[a] ± 0.75	4.8[a] ± 0.84	4.9[a] ± 0.81	4.9[a] ± 0.62

Values are mean ± SD
[a] $p < 0.01$ between control and diabetic SMC
Experiments carried out with 10 different pairs of control and diabetic SMC

medial SMCs express very little type I receptor in vivo, suggesting that in vivo fibronectin synthesis would not occur in SMC of both control and diabetic rats by TGF-β1. However, the result of the increased fibronectin in aortas of diabetic rats and the report that fibronectin content was increased in the media of the intact arteries of diabetic patients [5] would indicate that fibronectin synthesis in the artery is actually stimulated by the diabetic state. There are several possibilities to explain these phenomena; 1) most likely, diabetic aortas do express the TGF-β type I receptor necessary for signal transduction together with the type II receptor, but this expression is below the detecting level of our method, and 2) TGF-β may function as a homodimer of type II receptors and different post-receptor signal transduction systems operate in different cell types. The mechanism(s) remain to be elucidated in the fibronectin synthesis of the diabetic arterial wall through TGF-β receptor and post-receptor systems.

SMC in vivo showed different characteristics from cultured SMC in terms of the expression of TGF-β receptors. Type I receptor was little expressed in SMC in vivo, but was expressed in cultured SMC according to RT-PCR. This suggests that SMC will express more type I receptor by a phenotypic change from contractile (in vivo) to synthetic (cultured). However, differences of expression of TGF-β type II receptor between control and diabetic SMC were observed in both in vivo arteries and cultured SMC. We speculate that the specific expression of type II receptor in in vivo diabetic SMC is retained in the cultured diabetic SMC in spite of a phenotypic change.

In summary, the TGF-β-fibronectin system may play a vital role in the characteristic changes of SMC, such as overexpression of PDGF-β receptor in the diabetic artery. Further studies on the TGF-β receptor signalling, the activation mechanism of TGF-β and the actual messenger to cause abnormal TGF-β-fibronectin system in diabetic SMC will be necessary to determine the significance of this system in diabetic SMC.

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