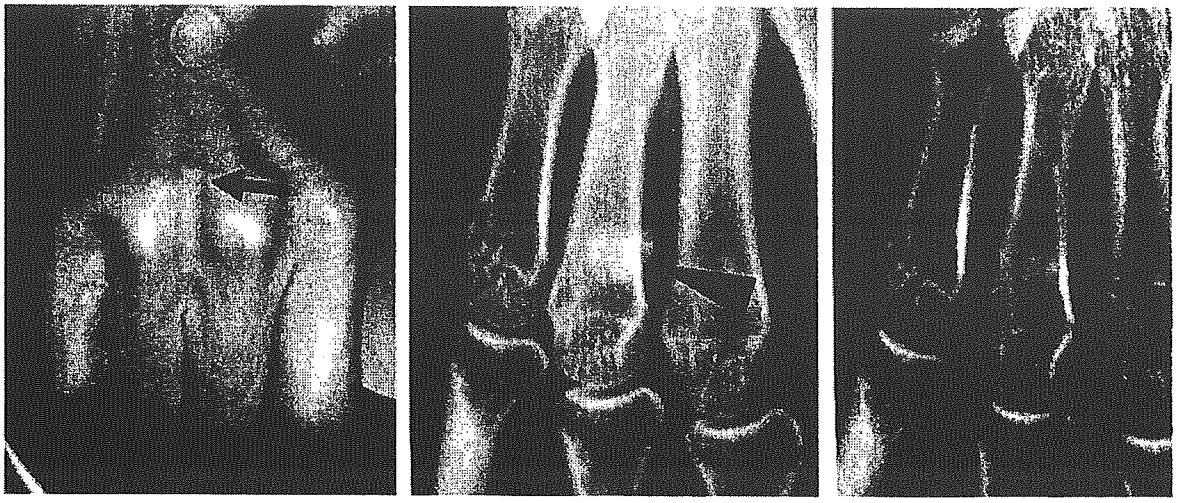

Figure 1. Left panel, subcutaneous nodule with pain (arrow). Radiographic examination before (middle panel) and after 3 months on etidronate (right panel). Arrowheads indicate the positions of nodular calcification.

Our present clinical experience demonstrates a novel therapeutic option for an otherwise incurable complication of Werner syndrome. Moreover, it rediscovers the usefulness of bisphosphonate for ectopic calcification.

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HYPOADIPONECTINEMIA IN BEDRIDDEN FEMALE PATIENTS YOUNGER THAN 75

To the Editor: Older people have several hormonal alterations, but the effect on the endocrine function of adipose tissue in older bedridden patients has not been fully elucidated. Adiponectin is a newly discovered antiinflammatory protein, secreted exclusively by adipocytes, that plays a protective role against atherosclerosis.[1] Hypoadiponectinemia plays a crucial role in atherosclerosis in men, but there have been no studies of plasma adiponectin in bedridden women. The aim of the present study was to estimate plasma adiponectin concentration in bedridden elderly female patients in comparison with age-matched healthy volunteers.

Seventy-four bedridden female patients admitted to geriatric wards and nursing homes in Osaka, Japan, and age-matched volunteers were studied. Clinical diagnoses were defined using detailed physical examination and routine biochemical analyses of blood and urine, as well as clinical tools including computed tomography. Their mean bedridden period ± standard deviation was 49.4 ± 37.4 months. All plasma analyses were performed on samples from fasting subjects. Adiponectin was measured using high-sensitive radioimmunoassay (Linco Research, St. Louis, MO). Bedridden subjects and healthy volunteers were divided into two groups: younger than 75 and aged 75 and older. All statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL). The statistical differences in the variables were compared using the Mann-Whitney *U* test, and the association between any two parameters was assessed using Spearman correlation.

Targeted Disruption of TGF-β–Smad3 Signaling Leads to Enhanced Neointimal Hyperplasia With Diminished Matrix Deposition in Response to Vascular Injury

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Abstract—The role of transforming growth factor (TGF)-β and its signal in atherogenesis is not fully understood. Here, we examined mice lacking Smad3, a major downstream mediator of TGF-β, to clarify the precise role of Smad3-dependent signaling in vascular response to injury. Femoral arteries were injured in wild-type and Smad3-null (null) male mice on C57Bl/6 background. Histopathological evaluation of the arteries 1 to 3 weeks after the injury revealed significant enhancement of neointimal hyperplasia in null compared with wild-type mice. Transplantation of null bone marrow to wild-type mice did not enhance neointimal thickening, suggesting that vascular cells in situ play a major role in the response. Null intima contained more proliferating smooth muscle cells (SMC) with less amount of collagen compared with wild-type intima. TGF-β caused significant inhibition of cellular proliferation in wild-type aortic SMC, whereas the growth of null SMC was only weakly inhibited by TGF-β in vitro, indicating a crucial role of Smad3 in the growth inhibitory function. On the other hand, Smad3-deficiency did not attenuate chemotaxis of SMC toward TGF-β. TGF-β increased transcript level of α2 type I collagen and tissue inhibitor of metalloproteinases-1, and suppressed expression and activity of matrix metalloproteinases in wild-type SMC. However, these effects of TGF-β were diminished in null SMC. Our findings altogether show that the loss of Smad3 pathway causes enhanced neointimal hyperplasia on injury through modulation of growth and matrix regulation in vascular SMC. These results indicate a vasculoprotective role of endogenous Smad3 in response to injury. (*Circ Res.* **2005;96:904-912.**)

Key Words: transforming growth factor-β ■ Smad3 ■ atherosclerosis ■ neointimal hyperplasia ■ smooth muscle cells

Transforming growth factor (TGF)-β is a prototypic member of the TGF-β superfamily that exerts a wide range of biological effects on various cell types.[1] Well described functions of TGF-β including growth inhibition, cell migration, differentiation, extracellular matrix production, and immunomodulation. Abnormality in TGF-β signaling may cause pathological conditions such as tumorigenesis, fibrotic disorders, and vascular diseases.[2] At present, however, the role of TGF-β and its signaling molecules in atherogenesis is not fully understood.

TGF-β is often regarded to have proatherosclerotic effect on arteries. For example, TGF-β expression is increased in human restenotic lesions as well as in neointimal hyperplasia after balloon injury in animals.[3] TGF-β facilitates extracellular matrix deposition by stimulating production of procollagen and fibronectin, downregulating the expression of proteases, and upregulating protease inhibitors, such as plasminogen activator inhibitor type I (PAI-I) and tissue inhibitor of metalloproteinase-1 (TIMP-1).[4–8] TGF-β transgene into vascular wall causes fibroproliferative intimal thickening in animal models in the presence or absence of vascular injury.[9,10] Moreover, TGF-β antagonism by antibody, soluble receptor, or ribozyme reduces constrictive remodeling after balloon injury in animals.[11–13]

On the other hand, considerable evidence implies antiatherosclerotic effects of TGF-β. TGF-β has been shown to inhibit proliferation and migration of vascular smooth muscle cells (SMCs) in vitro.[14,15] Inhibition of TGF-β signal systemically by use of neutralizing antibody and soluble TGF-β receptor type (TβR)-II or in T-cells by expressing a dominant-negative TβR-II results in an unstable plaque phenotype in mouse models of atherosclerosis.[16–18] SMCs

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obtained from human atherosclerotic plaques were shown to be defective in the TGF-β signal pathway and were resistant to TGF-β–mediated growth suppression and apoptosis.[19,20] Furthermore, low blood levels of active TGF-β were associated with severity of vascular disease in a manner consistent with an antiatherosclerotic effect of TGF-β.[21]

TGF-β elicits its effects via signaling through tetramerization of two different receptor serine/threonine kinases, TβR-I and TβR-II.[22,23] Activation of the receptors leads to phosphorylation of cytoplasmic signal transducers Smad2 and Smad3, classified as so-called receptor-activated Smads (R-Smad). The activated R-Smad heteroligomerizes with Smad4, a common mediator Smad, and the complex is transported to the nucleus where it regulates gene expression. In addition, pathways independent of Smads, which involve MAP kinases have also been described.[23] In mice lacking TGF-β signaling molecules, ie, TβR-I and TβR-II, Smad2 and Smad4 turned out to be embryonic lethal.[24–26] However, it was recently found that the mice null for Smad3 survive into adulthood.[27]

We undertook the present study examining Smad3-null mice in vivo and in vitro to elucidate the precise role of Smad3-dependent TGF-β signaling in the vascular response to injury.

Materials and Methods

Reagents

Reagents are described in an expanded Materials and Methods section in the online data supplement available at http://circres.ahajournals.org.

Mice

The generation of $Smad3^{ex8/ex8}$ null mice by homologous recombination was described previously.[27] See expanded Materials and Methods section for details.

Femoral Artery Injury

Mice femoral arteries were injured by use of photochemically-induced thrombosis method.[28] See expanded Materials and Methods section for details.

Histological Evaluation

Fixed femoral artery segments were embedded in paraffin and cut into 5-μm–thick serial sections. Six sections per one irradiated segment at 1-mm intervals were stained with hematoxylin and eosin. Neointima was defined as the region between the lumen and the internal elastic lamina. The media was defined as the region between the internal and external elastic lamina. The cross-sectional areas of intima and media were measured using NIH image version 1.62f (National Institutes of Health, USA). The intima-to-media (I/M) ratio was then calculated, and the mean I/M of all 6 sections per one irradiated segments was determined. The sections with intimal hyperplasia were also subjected to Masson's trichrome staining and immunohistochemistry. Masson's trichrome-positive intimal area was analyzed using Photoshop version 7.0 (Adobe). All the measurements were made in blinded manner.

Immunohistochemistry

Immunohistochemistry is described in the expanded Materials and Methods section.

Bone Marrow Transplantation

Bone marrow transplantation (BMT) was performed principally as described previously.[29] Briefly, bone marrow cell suspensions obtained from either Smad3-null or wild-type mice thigh bone were treated with ACK lysis buffer (0.155 mol/L ammonium chloride, 0.1 mol/L disodium EDTA, and 0.01 mol/L potassium bicarbonate) to lyse erythrocytes. The cells were intravenously injected to recipient Smad3-null or wild-type mice (1×10^6 per body) between the age of 6 and 9 weeks, 3 hours after lethal irradiation (8.5 Gy). Engraftment of the transferred bone marrow was confirmed by polymerase chain reaction (PCR) on peripheral blood DNA according to the protocol by Yang et al.[26] Femoral artery injury was performed 6 weeks after the bone marrow transfer.

Cell Culture

Mouse aortic SMCs were obtained and cultured as described by Ohmi et al[30] (see expanded Materials and Methods section). Experiments were performed on cells after 5 to 10 passages from the primary culture.

Immunocytochemistry

Immunocytochemical staining using anti–α-smooth muscle actin (SMA) and smooth muscle myosin (SMM) antibodies was performed as described by Hasegawa et al[31] with some modification (see expanded Materials and Methods section).

Immunoblotting

Immunoblotting was essentially performed as previously described[32] (see expanded Materials and Methods section).

Growth Inhibition Assay

Growth inhibition assay was performed as described by Datto et al[33] (see expanded Materials and Methods section).

Cell Migration Assay

SMC migration was evaluated by modified Boyden chamber method[34] (see expanded Materials and Methods section).

Real-Time Quantitative PCR

Real-time quantitative PCR is described in expanded Materials and Methods section.

Gelatin Zymography

Gelatin zymography is described in the expanded Materials and Methods section.

Statistical Analysis

Results were presented as mean±SEM. Statistical analyses used two-tailed, unpaired student *t* test.

Results

Mice Lacking Smad3 Show Enhanced Neointimal Hyperplasia in Response to Injury

To evaluate a role of Smad3 in the pathogenesis of neointimal hyperplasia, femoral arteries of wild-type (n=12) and Smad3-null (n=10) male mice were injured by use of the photochemically-induced thrombosis method.[28] Histopathological examination of the arteries 1 to 3 weeks after the injury revealed markedly enhanced neointimal thickening in Smad3-null mice compared with wild-type mice (Figure 1A and 1B). As shown in Figure 1C, mean I/M ratios evaluated at 1 and 3 weeks after the injury were significantly higher in Smad3-null arteries (0.193±0.034 at 1 week and 0.541±0.093 at 3 weeks) than those of wild-type arteries (0.059±0.018 at 1 week and 0.115±0.060 at 3 weeks, $P<0.01$ at each time point).

Immunohistochemical examination showed that both neointimal and medial cells were positive for α-SMA (Figure 2A and 2B) but negative for pan-leukocyte marker CD45 (Figure

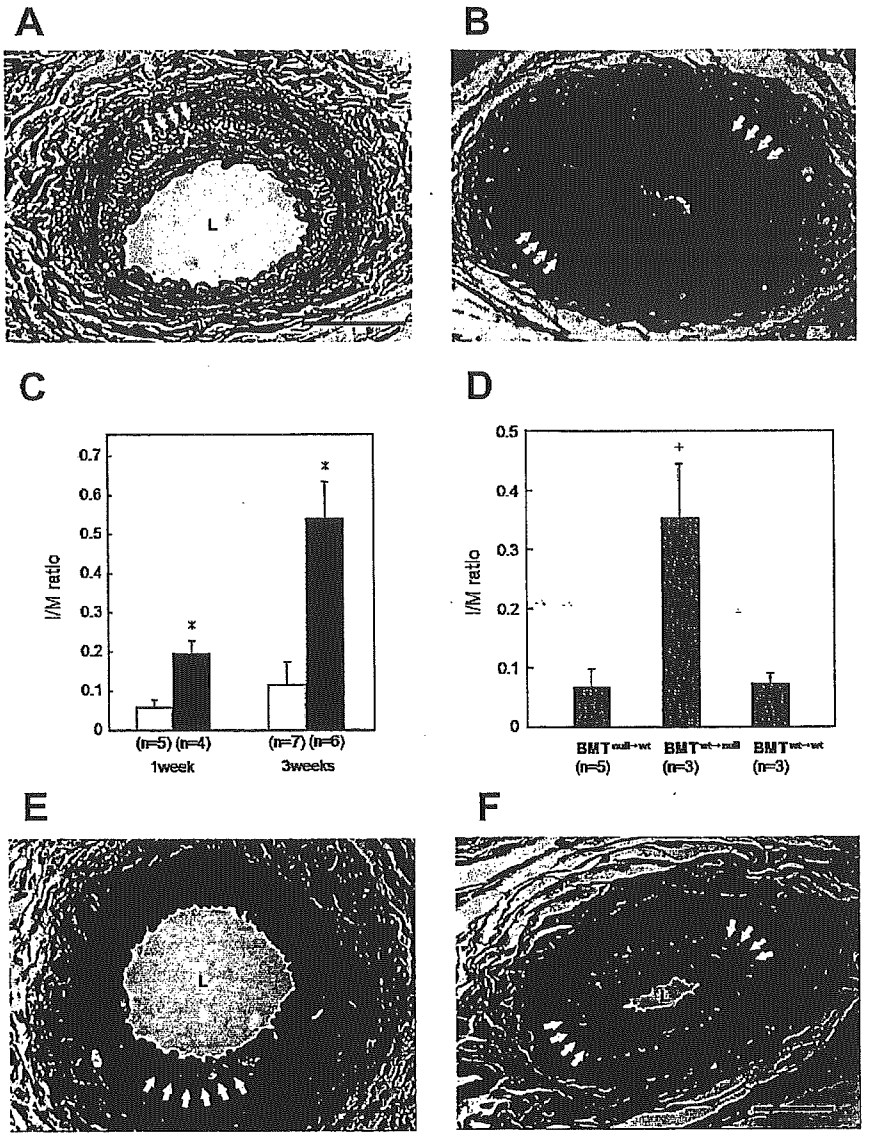

Figure 1. Neointimal thickening in injured femoral arteries of wild-type and Smad3-null mice. Photomicrographs showing representative cross sections of hematoxylin and eosin–stained femoral arteries from wild-type (A) and Smad3-null (B) and $BMT^{null\rightarrow wild}$ (E) and $BMT^{wild\rightarrow null}$ (F) mice 3 weeks after endothelial injury. L indicates vascular lumen. Arrows indicate the positions of the internal elastic lamina. Original magnification ×200; bar=50 μm. Intima-to-media (I/M) ratios at 1 and 3 weeks in wild-type and Smad3-null mice (C) and in $BMT^{null\rightarrow wild}$, $BMT^{wild\rightarrow null}$, and $BMT^{wild\rightarrow wild}$ at 3 weeks (D) were calculated from cross sectional areas morphometrically measured using an image analyzer. Open and closed columns indicate wild-type and Smad3-null mice, respectively. *$P<0.01$ compared with wild type at each time point; †$P<0.05$ compared with $BMT^{null\rightarrow wild}$.

2C and 2D), indicating that the intima was exclusively composed of SMCs. The same anti-CD45 antibody recognized leukocytes in vasa vasorum (Figure 2D) as well as lymphocytes in the mouse spleen (Figure 2E).

TGF-β is well known for its antiinflammatory effect.[1,2] To determine whether systemic inflammation due to Smad3 deficiency contributes to enhanced neointimal formation, we injured femoral artery of wild-type and Smad3-null mice after bone marrow transplantation (BMT). Lethally irradiated Smad3-null mice received 1×10^6 bone marrow cells from a wild-type mouse ($BMT^{wild\rightarrow null}$ mice). At the same time, irradiated wild-type mice were given bone marrow either from Smad3-null or wild-type mice ($BMT^{null\rightarrow wild}$ and $BMT^{wild\rightarrow wild}$ mice). Photochemical injury was performed 6 weeks after the bone marrow transfer, and the arterial cross section was analyzed 3 weeks later. As shown in Figure 1D, mean I/M ratio was significantly higher in $BMT^{wild\rightarrow null}$ arteries (0.353±0.091) than those of $BMT^{null\rightarrow wild}$ (0.067±0.031, $P=0.011$) or $BMT^{wild\rightarrow wild}$ (0.073±0.018, $P=0.039$) arteries. I/M ratios in $BMT^{wild\rightarrow null}$ and $BMT^{null\rightarrow wild}$ mice tended to be lower than those of Smad3-null and wild-type mice, respectively, presumably due to the effect of vascular irradiation.[35,36] Representative cross sections of $BMT^{null\rightarrow wild}$ and $BMT^{wild\rightarrow null}$ femoral arteries are shown in Figure 1E and 1F.

Smad3-Null Intima Is Rich in Proliferating Cells but Contains Low Amounts of Collagen Fibers

Intimal cell proliferation was assessed by immunohistochemical detection of proliferating cell nuclear antigen (PCNA) in the femoral artery sections 1 week after the injury (Figure 3A and 3B). The ratio of the PCNA-positive nuclei to total cell nuclei was higher by 1.8-fold in Smad3-null intima compared with wild-type intima (Figure 3C). The result shows an increased proliferative activity of SMCs in Smad3-null artery at the early stage after injury.

We next evaluated intimal cell density in hematoxylin and eosin–stained arterial sections 3 weeks after the injury. As shown in Figure 4A, the ratio of intimal cell number to total intimal area was 1.6-fold higher in Smad3-null artery

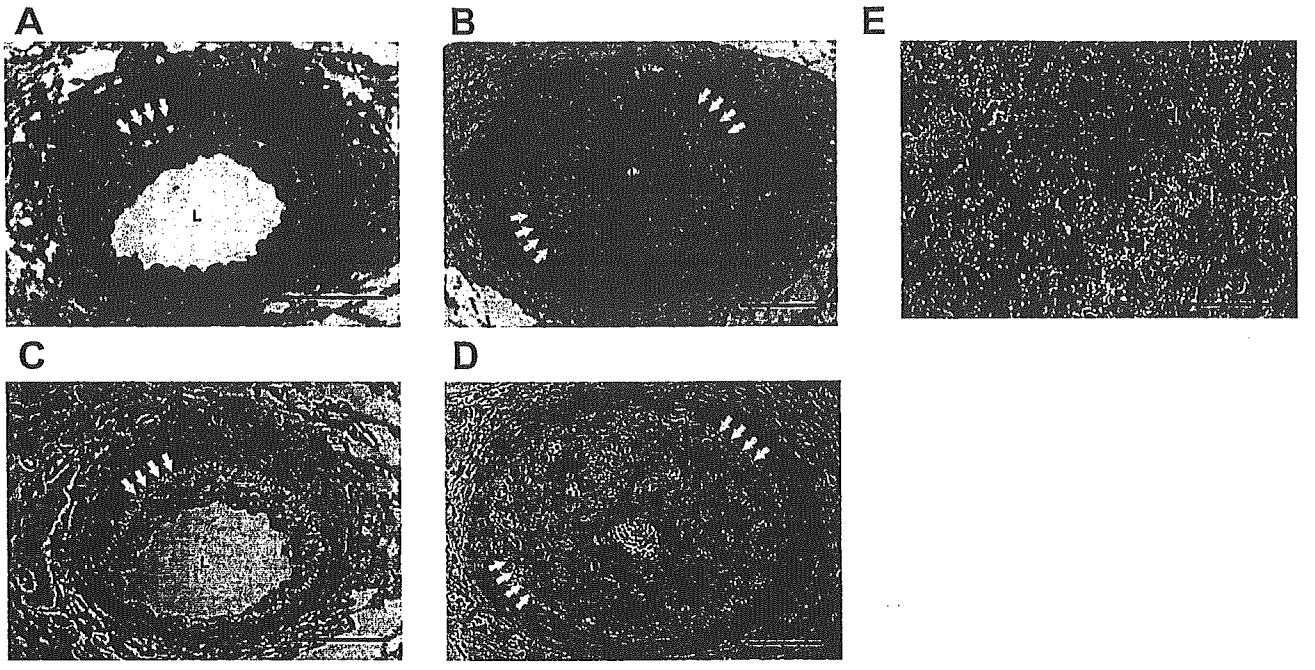

Figure 2. Immunohistochemical analysis of neointimal components. Cross sections of femoral arteries from wild-type (A and C) and Smad3-null (B and D) mice 3 weeks after endothelial injury and of mouse spleen (E). Immunostaining was performed using specific antibodies for α-SMA (A and B) and CD45 (C, D, and E). L indicates vascular lumen. Arrows indicate the positions of the internal elastic lamina. Arrowheads indicate the positions of representative CD45-positive leukocytes. Original magnification ×200; bar=50 μm.

(133±8.6) compared with wild-type artery (85.3±7.7, $P<0.01$), indicating higher cell density relative to extracellular area in Smad3-null intima. Because TGF-β/Smad3 signal is implicated in extracellular matrix (ECM) deposition, Masson's trichrome staining was also performed on a 3-week artery specimen to evaluate the amount of extracellular collagen fibers (Figures 4C and 4D). As summarized in Figure 4B, Smad3-null neointima showed 60% reduction in the ratio of Masson's trichrome-positive area to total intimal area compared with that of wild-type intima. These results suggest that Smad3 deficiency caused increased SMC number with less collagen deposition in neointima.

Growth Inhibition by TGF-β Is Attenuated in SMCs Lacking Smad3

To identify the mechanisms by which Smad3 deficiency caused exaggerated intimal hyperplasia, biological responses of the aortic SMCs obtained from wild-type and Smad3-null mice were examined in vitro. The cells were positive for both α-SMA and SMM (Figure 5A and 5B) as examined by immunocytochemistry. They also exhibited the classic "hills and valley" appearance, a feature characteristic of confluent cultured vascular SMCs. No morphological differences were observed between wild-type and Smad3-null SMCs (data not shown). It was confirmed by immunoblotting that SMCs derived from Smad3-null mice lacked expression of Smad3, whereas Smad2 level was similar in both cells (Figure 5C).

The SMCs were first tested for proliferation. As shown in Figure 6A, TGF-β dose-dependently inhibited FBS-stimulated DNA synthesis in wild-type SMCs with the maximal inhibition of 70% at 1 ng/mL and higher doses. In contrast, growth of Smad3-null SMCs was only weakly (<30%) inhibited by TGF-β. In addition, the basal growth rate of the null cells was ≈1.4-fold higher than that of the wild-type. Similar results were obtained for two additional cell lines of each genotype. The results firmly establish an essential role for Smad3 in TGF-β–mediated inhibition of cellular proliferation in vascular SMCs.

Smad3 Deficiency Does Not Attenuate TGF-β–Mediated Migratory Response in SMCs

The cells were next examined for migration, another function crucial to neointimal formation. Aschcroft et al[37] previously reported that Smad3-null monocytes and neutrophils were unable to migrate toward TGF-β, suggesting Smad3 is required for migration signal downstream of TGF-β. As shown in Figure 6B, Smad3-null SMCs dose-dependently migrated toward TGF-β at least to a similar extent as wild-type SMCs in a modified Boyden chamber assay. Moreover, Smad3-null cells showed a higher migratory capacity ($P<0.05$) than wild-type cells at 10 ng/mL TGF-β. The result suggests that Smad3-dependent signal is not essential for TGF-β–induced chemotaxis in murine vascular SMCs.

SMCs Require Smad3 for the Regulation of Type I Collagen, Matrix Metalloproteinases, and TIMP-1 by TGF-β

Previous studies suggested that migration of medial SMCs to intima involves extracellular matrix degradation.[38,39] Because TGF-β is implicated in extracellular matrix metabolism through transcriptional regulation of collagens, matrix metal-

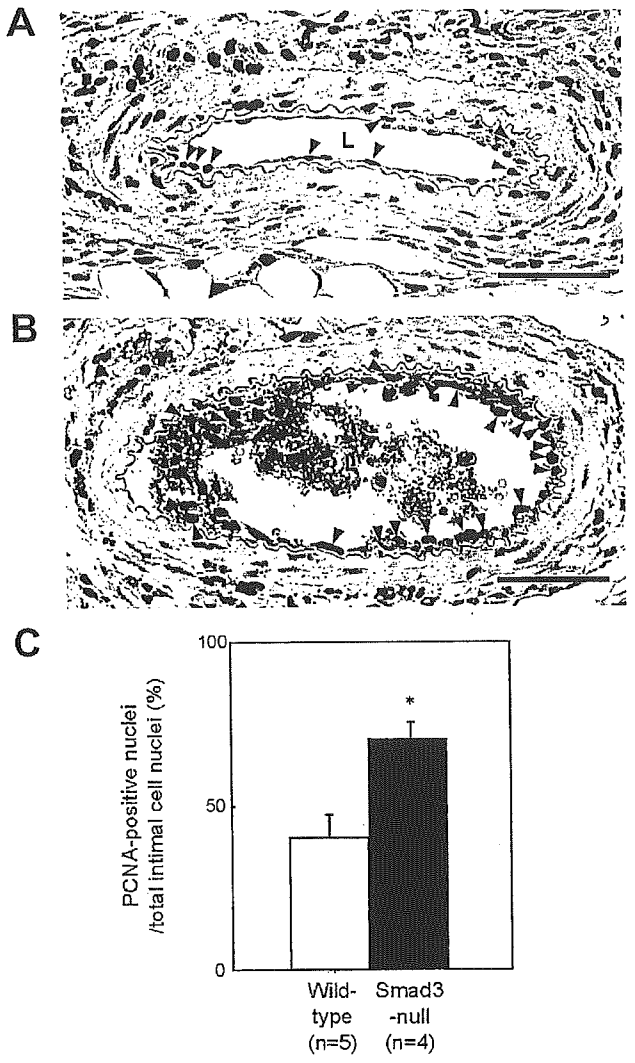

Figure 3. In vivo evaluation of cell proliferation in neointima. Representative anti-PCNA–stained cross sections of femoral arteries from wild-type (A) and Smad3-null (B) mice obtained 1 week after the injury. Arrowheads indicate PCNA-positive cells in intima. C, Ratios of PCNA-positive intimal cell number to total intimal cell number. L indicates vascular lumen. Original magnification ×200; bar=50 μm. *$P<0.05$ compared with the wild type.

loproteinases (MMPs), and TIMP-1,[7,8] we examined the ability of TGF-β to regulate mRNA expression of these components in wild-type and Smad3-null SMC. Transcript levels of COL1A2, membrane-type matrix metalloproteinase 1 (MT1-MMP), and TIMP-1 were evaluated by real-time quantitative PCR. As shown in Figure 7A, TGF-β time-dependently upregulated mRNA level of COL1A2 in wild-type SMCs with a maximal increase of 3-fold. Induction of COL1A2 by TGF-β was significantly less in Smad3-null SMCs compared with wild-type cells at all time points. TGF-β suppressed mRNA expression of MT1-MMP, an activator of pro–MMP-2,[40] to 64% of the basal level in wild-type SMCs (Figure 7B). However, MT1-MMP level was not affected by TGF-β in Smad3-null SMCs. Moreover, TGF-β increased TIMP-1 expression by 5-fold over the basal level in wild-type SMCs (Figure 7C), whereas no significant induction was observed in Smad3-null SMCs. Finally, the effect of TGF-β on MMP activity in SMC culture media was examined by gelatin zymography (Figure 7D). The basal gelatinolytic activity of MMP-2 in a serum-free conditioned media was similar for wild-type and Smad3-null SMCs. TGF-β time-dependently suppressed MMP-2 activity in wild-type cells with the maximal suppression of 29% at 24 hours, but it did not show significant effect in Smad3-null SMCs. These results suggest that Smad3 plays an essential role in TGF-β–mediated regulation of type I collagen, MMPs, and TIMP-1 in vascular SMCs.

Discussion

We report six novel findings in this article. First, mice lacking Smad3 showed a significant enhancement of neointimal hyperplasia on endothelial injury compared with corresponding wild-type mice. Second, neointima of Smad3-null mouse after injury contained a larger number of PCNA-positive cells compared with wild-type, indicating an increased proliferative activity of Smad3-null SMCs in vivo. Third, Smad3-null neointima showed higher cell density with reduced collagen area. Fourth, TGF-β–induced growth inhibition was diminished in Smad3-null SMCs in vitro. Fifth, Smad3-null SMCs retained migratory activity toward TGF-β. And finally, Smad3-null SMCs were impaired in induction of type I collagen and TIMP-1 as well as in suppression of MMPs by TGF-β. These results confirm a regulatory role of endogenous Smad3 in vascular remodeling in response to injury.

Enhanced neointimal hyperplasia in Smad3-null mice (Figure 1) lend support to previous reports describing the association of low TGF-β activity either at the ligand or receptor levels with intimal lesion formation. Grainger et al[41] showed that transgenic expression of apolipoprotein(a) promoted SMC proliferation and subsequent development of early vascular lesions by inhibiting proteolytic activation of TGF-β. Conversely, treatment with the antiestrogen tamoxifen increased serum TGF-β_1 levels and suppressed the formation of aortic lesions in mice[42]; a similar effect was also observed in human subjects.[43] McCaffrey et al[19] reported that reduced TβR-II activity due to genomic mutations led to SMC expansion in human atherosclerosis. Moreover, inhibition of TGF-β by use of a soluble type II receptor or a neutralizing antibody accelerated atherosclerosis and induced an unstable plaque phenotype in apoE-deficient mice.[17,18] And our present findings, for the first time, demonstrate a direct evidence that attenuation of TGF-β signal at the postreceptor level results in enhanced neointimal formation on injury.

Increased PCNA-positive intimal cells in vivo (Figure 3) and defect in TGF-β–induced growth suppression in vitro (Figure 6A) suggest that increased proliferative activity of SMCs contributes to the prominent neointimal formation in Smad3-null mice. Importance of Smad3 in TGF-β–mediated growth inhibition has well been described in other cell types such as αCD-stimulated primary splenocytes and embryonic fibroblasts.[33] Our results verify that Smad3, also in vascular SMCs, plays a major role in growth inhibitory function of

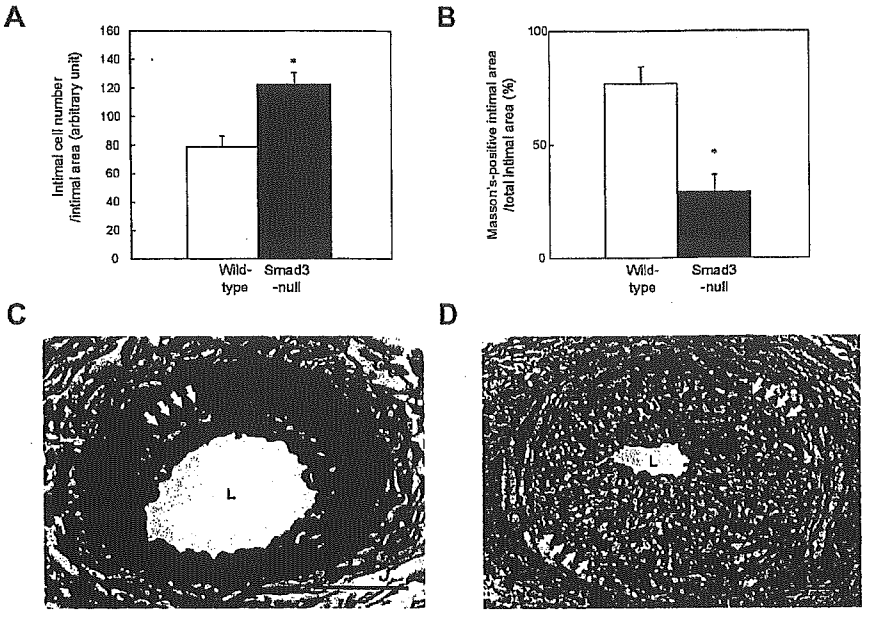

Figure 4. Evaluation of cell density and matrix deposition in neointima. A, Ratios of intimal cell number to total intimal area evaluated on hematoxylin and eosin–stained femoral arterial sections from wild-type (n=7) and Smad3-null (n=6) mice obtained 3 weeks after the injury. B, Ratios of Masson's trichrome–positive intimal area to total intimal area in femoral arterial sections from wild-type (n=7) and Smad3-null (n=6) mice 3 weeks after the injury. C and D, Photomicrographs showing the representative Masson's trichrome–stained sections of wild-type (C) and Smad3-null (D) femoral arteries. Arrows indicate the positions of the internal elastic lamina. L indicates vascular lumen. Original magnification ×200; bar=50 μm. *$P<0.01$ compared with the wild type.

TGF-β. It is to be noted that lack of Smad3 did not eliminate TGF-β–induced growth suppression in SMCs (Figure 6A). The residual growth inhibitory activity is likely to depend on another mediator downstream of TGF-β receptors, possibly Smad2.

Ashcroft et al[37] reported that Smad3 is required for TGF-β–induced migration of monocytes, leukocytes, and keratinocytes. Unexpectedly, Smad3-null SMCs were able to migrate toward TGF-β (Figure 6B). The finding suggests that, in contrast to the growth inhibitory function, Smad3-dependent signal is not essential for chemotaxis by TGF-β in murine vascular SMCs. It is therefore likely that the ability of medial SMCs to migrate into intima is preserved in Smad3-null arteries. The signaling pathway responsible for TGF-β–induced SMC motility remains to be elucidated.

TGF-β is known as a potent inducer of ECM deposition. It has been demonstrated that overexpression and intravenous administration of TGF-β caused arterial intimal thickening largely consisted of increased ECM.[10,44] TGF-β exerts fibrogenic activity through enhancement of ECM synthesis as well as inhibition of ECM degradation by downregulating MMP expression and upregulating MMP inhibitors.[6–8] Previous studies, mainly performed on dermal fibroblasts, showed that TGF-β–mediated regulation of many ECM-related genes, such as type I, III, V, and VI collagens, TIMP-1 and MMP-1 was Smad3-dependent.[45–47] In this study, we reported that Smad3-null neointima was rich in SMCs with relatively less matrix-deposition compared with wild-type intima, as evaluated by intimal cell density and Masson's trichrome staining (Figure 4), confirming a crucial role of Smad3-dependent signals in vascular ECM regulation. Moreover, TGF-β was unable to enhance mRNA expression of COL1A2 and TIMP-1 or suppress MT1-MMP expression in Smad3-null SMCs (Figure 7), establishing Smad3-dependency of these genes in vascular SMCs. Regulation of MMP-2 or gelatinase also seems to depend on Smad3-pathway in SMCs, because TGF-β attenuated MMP-2 activity in the culture media of wild-type but not in Smad3-null SMCs. Because degradation of matrix scaffold by MMPs enables cell movement and general tissue reorganization,[38,39] inability of TGF-β to suppress MMPs in Smad3-null SMCs may facilitate cell migration from media to intima in vivo.[48] Our in vitro finding that Smad3-null SMCs show a higher migration than wild-type at 10 ng/mL TGF-β (Figure 6B) may support this idea. MMP activity uninhibited by TGF-β as well as decreased matrix deposition might also have contributed to enhancement of intimal thickening in Smad3-null mice.

There have been reports on injury models suggesting that TGF-β promotes intimal thickening.[3,9–13,49] The present result that Smad3 deficiency accelerates intimal response to injury appears inconsistent with these results. However, we do not think that our findings contradict other reports on TGF-β transgene or antagonism. Our model differs from any other previous models in the point it specifically lacks Smad3 signal but not other TGF-β signal components, eg, Smad2 and MAP kinases. Smad3 not only transduces signal downstream of TGF-β, but also plays a major role in signaling of activins,[22,23] other members of the TGF-β superfamily. Activin A is expressed in atherosclerotic lesion[50] and promotes the contractile or nonproliferative phenotype of SMCs,[51] playing a role in stabilization of atherosclerotic plaque. Adenovirus-mediated overexpression of activin A suppresses neointimal formation.[51] Although we have not examined the involvement of activin A in the present study, it is assumable that the defect in activin A signal in addition to TGF-β accounts for the drastic neointimal hyperplasia in Smad3-null mice. It is of interest to determine whether specific activation of Smad3 in arterial SMCs in vivo attenuates neointimal hyperplasia. As another possibility, proinflammatory status caused by systemic Smad3 deficiency[27] might have influenced neointimal response. Although our BMT results (Figure 2D through 2F) show that the degree of intimal hyper-

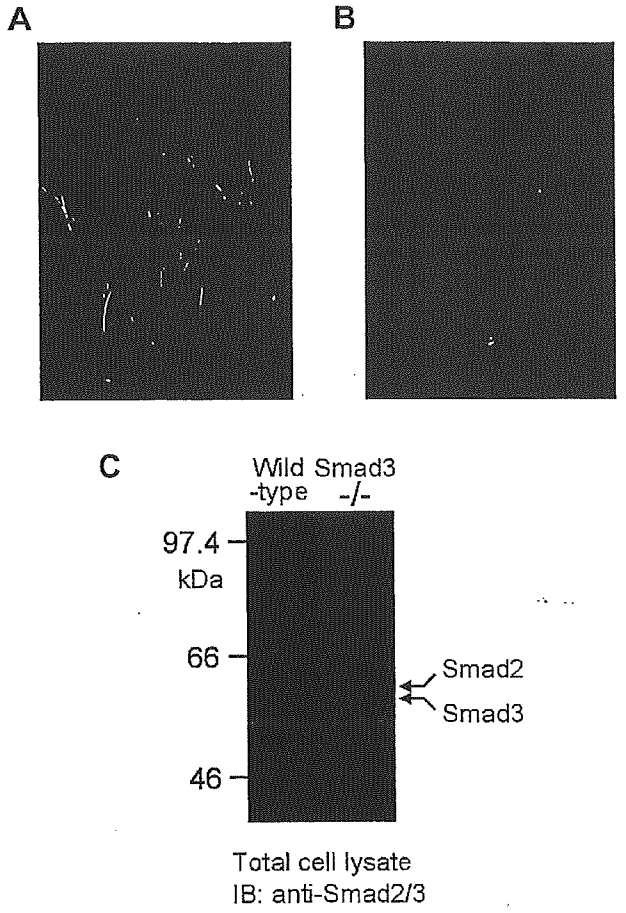

Figure 5. Characterization of cultured mice aortic SMCs. SMCs enzymatically isolated from the aorta of wild-type mice were immunocytochemically stained using anti-SMA (A, green) and anti-SMM (B, red) antibodies, counterstained with DAPI (blue, for nuclei), and subjected to fluorescent microscopy. Original magnification ×200. C, Total cell lysates of wild-type and Smad3-null SMCs were analyzed by SDS-PAGE and subjected to immunoblotting with an anti-Smad2/3 antibody. Migration positions of Smad2 and Smad3 are indicated.

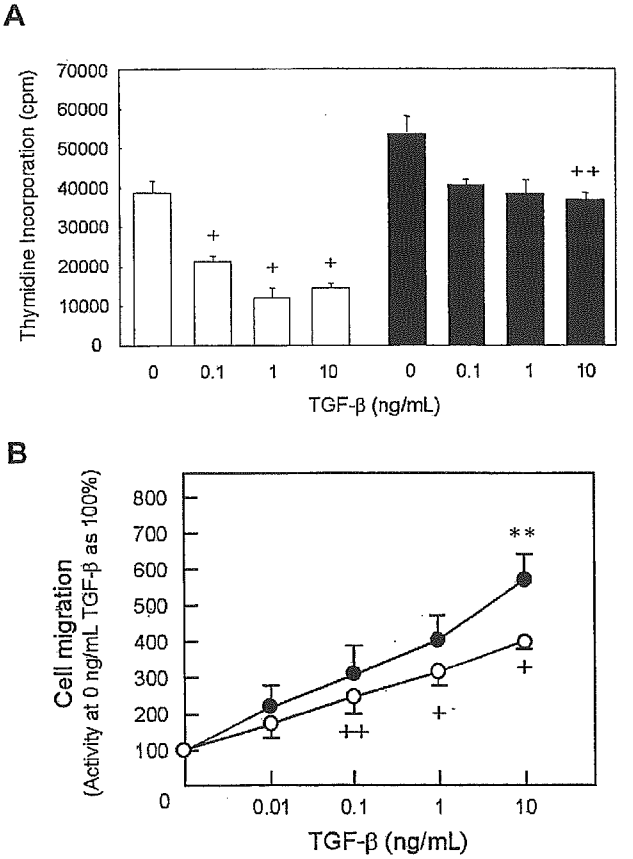

Figure 6. TGF-β–induced growth inhibition and migration of wild-type and Smad3-null SMCs. A, Wild-type (open columns) and Smad3-null (closed columns) SMCs were assayed for TGF-β–induced growth inhibition using ^{3}H-thymidine incorporation. Data are expressed as the means of three separate experiments, each performed in quadruplicate. $+P<0.01$, $++P<0.05$, compared with the value of 0 ng/mL TGF-β. B, Migration of wild-type (open circles) and Smad3-null (closed circles) SMCs toward various doses of TGF-β was measured by use of modified Boyden chamber method. Data represent the percentage of cell numbers relative to those in the absence of TGF-β and are expressed as the means of 5 separate experiments, each performed in triplicate. $+P<0.01$, $++P<0.05$, compared with the value of 0 ng/mL TGF-β. $**P<0.05$, compared with the value of wild-type at 10 ng/mL TGF-β.

plasia mainly depends on the origin of blood vessels and not of bone marrow cells, further investigation is needed to elucidate the entire role of inflammation in Smad3-null vascular response.

Finally, overactivation of TGF-β–Smad3 pathway is implicated in various fibrotic diseases involving organs such as skin, lung, liver, and kidney. Molecular agents that block Smad3-dependent TGF-β signal are anticipated as an ideal therapeutic option for these disorders.[46] However, our present results lead us to surmise that systemic suppression of Smad3 signaling can cause undesirable effects in the arteries by facilitating proliferative intimal lesions. Therefore, selective drug-delivery to the affected organs as well as careful monitoring of possible vascular lesions should be considered on clinical application of Smad3 inhibitors for fibrotic diseases.

In conclusion, mice lacking Smad3 developed marked neointimal hyperplasia on injury accompanying modulation of growth and matrix regulation in vascular SMCs. This study documents direct evidence and novel information on the functional significance: a vasculoprotective role of Smad3-dependent TGF-β signaling in response to injury.

Acknowledgments

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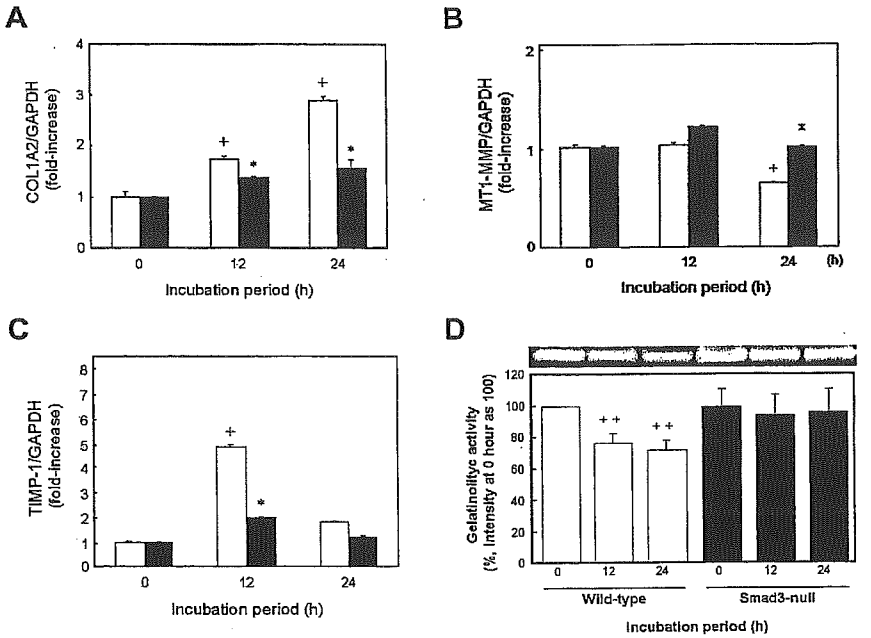

Figure 7. Effect of TGF-β on expression of type I collagen, MMPs, and TIMP-1 in wild-type and Smad3-null SMCs. Transcript levels of COL1A2 (A), MT1-MMP (B), and TIMP-1 (C) in wild-type and Smad3-null SMCs treated with TGF-β. Wild-type (open columns) and Smad3-null (closed columns) SMC were incubated with 10 ng/mL TGF-β for the indicated periods, the total RNA was isolated and used for cDNA synthesis. Quantitative real-time PCR was performed using the SYBR Green PCR Master Mix and analyzed on an ABI PRISM 7000 Sequence Detector System. Data were calculated relative to the value for the cells without TGF-β and are expressed as the means of 3 separate experiments, each performed in triplicate. $+P<0.01$, compared with the value of 0 hour; $*P<0.01$, compared with the wild type at the same time point. D, MMP-2 gelatinolytic activity in the culture media of wild-type and Smad3-null SMCs treated with TGF-β. Culture media of SMCs incubated with 10 ng/mL TGF-β for the indicated periods was analyzed by gelatin zymogram. Proteolytic degradation of gelatin by MMP was visualized as a translucent band on the dark background. Graph shows the gelatinolytic activity, evaluated by densitometrical scanning of the bands, relative to those of wild-type SMCs at 0 hour. Data were expressed as the means of 4 separate experiments. $++P<0.05$, compared with the value of 0 hour.

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増殖因子とその受容体—PDGF，TGF-β—

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I．増殖因子とは

ヒトの身体は，数十兆の細胞が適切に配置して組織を構築し，協調しあいながら特化して生理機能を発揮している．そのためには，多種多様に分化した細胞の数のバランスが適切に制御される必要があり，このプロセスに重要な役割を果たしている物質群の一つが細胞増殖因子(以下，増殖因子)である．増殖因子とは，文字通り細胞の増殖を促進するペプチドであり，単なる栄養物でないものを指す．

代表的な増殖因子として知られる血小板由来増殖因子(platelet-derived growth factor，以下PDGF)は，A鎖(PDGF-A)，B鎖(PDGF-B)と呼ばれる2種類のポリペプチドからなる二量体分子として，1979年に初めて精製された[1]．その後20年を経て，最近，PDGF-C，PDGF-Dという新しい分子が発見された[2]．現在，PDGFには，AA，AB，BB，CC，DDという5種類の二量体アイソフォームが確認されている．これらは，主として間葉系細胞の表面に発現する2種類のPDGF受容体(α受容体とβ受容体)と特異的に結合する(図1)．PDGF受容体はその細胞内にチロシンキナーゼ活性を有し，PDGFとの結合による二量体化を経て，その活性が上昇する．そして種々の細胞内シグナル分子をチロシンリン酸化により活性化させ，主にRas-MAPキナーゼ経路やPI3キナーゼ経路を通じて遺伝子発現や細胞骨格の変化をもたらし，細胞の増殖や遊走を刺激すると考えられている．

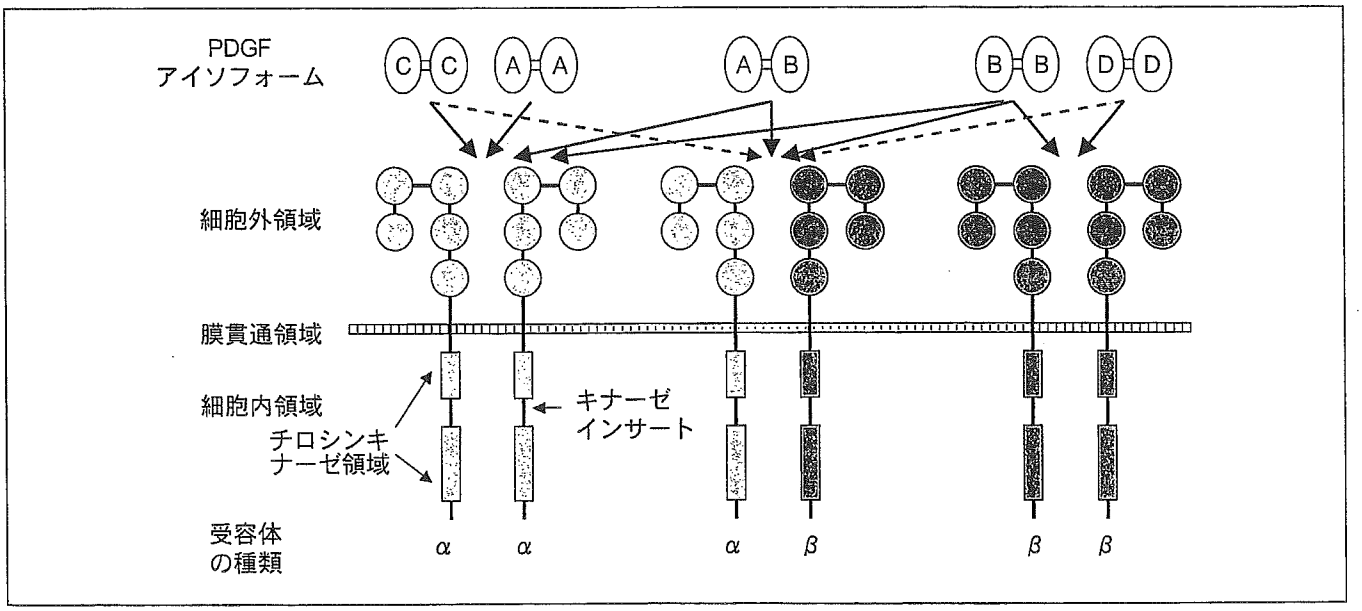

図1　5種類のPDGFアイソフォームとPDGFα，β受容体の構造
PDGFα，β受容体は5つの免疫グロブリンドメインからなる細胞外領域と，細胞内領域にはインサートにより分断されたキナーゼドメインを有し，ホモないしヘテロ二量体を形成する．実線は，それぞれのPDGFアイソフォームと結合し，活性化される受容体二量体の組み合わせを示す．PDGF-CCおよびDDは破線で示すヘテロ二量体をも活性化しうると報告されているが，その生物学的意義は不明である．(文献2)より改変引用)

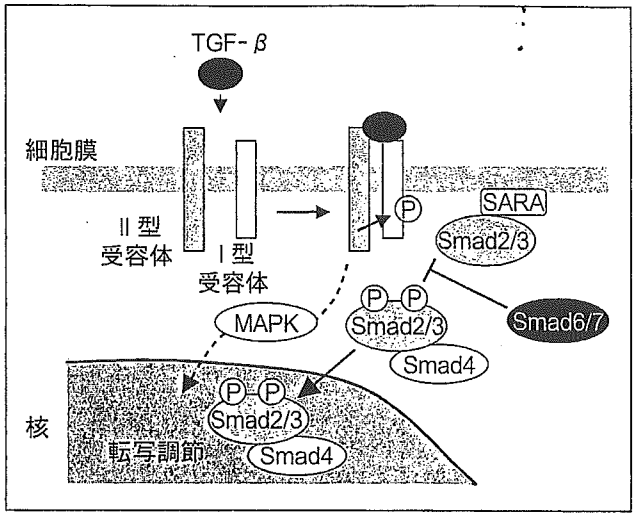

図2　TGF-βによる細胞内シグナル伝達
TGF-βが受容体に結合すると，I型受容体とII型受容体の会合が起こり，II型受容体がI型受容体の細胞膜貫通部直下をリン酸化する．これにより活性化されたI型受容体は特異的SmadであるSmad2およびSmad3をリン酸化し，これらは共有型SmadであるSmad4と複合体を結合する．Smad複合体は細胞質から核へと移行し，核内で他の転写因子と共同して標的遺伝子の発現を誘導する．一方，Smad6，Smad7はこれらのシグナルを抑制することから，抑制型Smadと呼ばれる．また，TGF-βはSmad分子とは別にある種のMAPキナーゼ(MAPK)の活性化をもたらすことも報告されている．

TGF-β(transforming growth factor-β)は，そもそもその名の通り，ある培養条件下で細胞をトランスフォームすなわち形質変換/癌化させる因子として1983年に血小板などから精製された．しかし，その後の研究から，この形質変換はTGF-βによって発現が変化する他の増殖因子などを介した間接作用であること，TGF-βの本質的な作用は逆に強力な細胞増殖抑制と細胞外マトリクス産生であることが明らかとなった[3]．TGF-βには，I型およびII型と名付けられた2種類の受容体があり，いずれも細胞内にセリン・スレオニンキナーゼ活性を有する．図2に示すように，TGF-βとの結合により活性化された受容体は，Smadと呼ばれる細胞内シグナル分子をセリン・スレオニンリン酸化する．自身が転写因子であるSmad分子は核内へと移行し，各種遺伝子の発現を調節し，増殖抑制作用，マトリクス産生作用などをもたらすと考えられている[4]．TGF-βには，骨形成にかかわるBMP(bone morphogenic protein)や細胞の分化に重要なアクチビンなど類似した構造をもつ仲間があり，これらは合わせてTGF-βスーパーファミリーと呼ばれている．

II. PDGF，TGF-βと動脈硬化

1976年，Russel Rossらは血管平滑筋細胞の増殖にPDGFが重要であることを明らかにした．PDGFが精製される3年前のことである．彼らは，何らかの原因により血管内皮細胞が傷害を受けて剝離した箇所に血小板血栓が付着し，ここから放出されたPDGFに反応して血管壁中膜の平滑筋細胞が内膜へと遊走，増殖を重ねて肥厚内膜を形成することが動脈硬化の本質であると提唱した[5]．動脈硬化の古典的な学説となった「傷害反応仮説」である．この考え方は，経皮冠動脈形成術(percutaneous transluminal intervention：PCI)後にみられる再狭窄病変形成機序の説明として現在も受け継がれている．一方，高脂血症に起因する粥状動脈硬化では，内皮細胞が剝離せずとも内膜肥厚をもたらされること，流血中の単球に由来する泡沫化マクロファージが主体であること，さらに不安定プラークとその破綻(unstable plaque and its rupture)の概念などが確立され[6]，PDGFの占める重要性は相対的に小さくなった感がある．ところが最近，高コレステロール血症患者の流血中単球でPDGF-A，Bの発現が増加していることや[7]，PDGFβ受容体に対する抗体がApoEノックアウトマウスの動脈硬化病変形成を軽減すること，LRP1(LDL receptor-related protein 1)ノックアウトマウスの動脈硬化にPDGFシグナルが重要であることなどが報告され[8,9]，新たな注目を集めている．

TGF-βと動脈硬化とのかかわりについても，これまで数多くの研究がなされてきた．表1に示すように，TGF-βが動脈硬化を促進するか抑制的に働くかは過去の報告が相半ばしている．近年，T細胞特異的にTGF-βシグナルを抑制したマウスモデルなどの成績から，粥状動脈硬化病変の形成に対してはTGF-βがこれを抑制するとの考え方が主流となっている[22]．一方，内皮傷害に伴う再狭窄モデルでは，主としてTGF-βの病変促進性が示唆されている．興味深いことに，

表1　動脈硬化におけるTGF-βの役割を論じた主な研究報告

抑制する	促進する
・重症冠疾患では血中TGF-βが低値である[10,11] ・タモキシフェンはTGF-βの活性化を通じて冠動脈病変を抑制する[12,13] ・中和抗体や可溶型II型受容体によるTGF-βの抑制は不安定プラーク様動脈病変を生じる[14,15] ・TGF-βは平滑筋細胞の増殖を抑制する[16~18]	・TGF-βの過剰発現は新生内膜形成を増強する[19,20] ・TGF-βを抑制する新生内膜の形成が抑制される[21,22]

TGF-βの主要シグナル分子であるSmad3のノックアウトマウスでは，内皮傷害に対する新生内膜の形成が著しく増強する(Kobayashiら未発表データ)．すなわち，動脈硬化巣におけるTGF-βの作用は，TGF-βとその受容体，シグナル分子の発現にも関連して，複雑に制御されている可能性がある．

III．糖尿病にみられる動脈硬化と増殖因子の役割

糖尿病患者に心血管障害の発症率やPCI後の再狭窄の発症頻度が高く，心イベントを生じた後の生命予後も悪いことが知られている．しかし，その原因についてはいまだ十分には解明されていない．

PDGFやTGF-βは糖尿病に合併する動脈硬化の形成にどのように関与するのだろうか．培養細胞を用いた検討から，ヒトの血管内皮細胞やマクロファージ，そしてラットやウサギの平滑筋細胞において，高血糖がPDGFβ受容体の発現を増強させることが示されている[23,24]．また，2型糖尿病モデルラットの中膜(平滑筋細胞)では，やはりβ受容体の発現が増加している[25]．さらに，糖尿病血管障害に関連するさまざまな液性因子，例えばアンギオテンシンII[26]，エンドセリン[27]，炎症性サイトカイン[28]の数々，終末糖化産物(advanced glycation end-products：AGE)[29]などが，PDGFの産生を刺激することも知られている．このほか，高血糖下でプロテインキナーゼCや低分子量G蛋白Rhoの活性化を通じて血管壁での発現が増えるオステオポンチンという蛋白は，PDGFによる平滑筋細胞の増殖をさらに増強させる働きをもつ[30]．このように糖尿病状態は，直接的あるいは間接的に血管壁でのPDGFシグナル増強を招き，動脈硬化病変の形成に寄与すると考えられる．

PDGF受容体キナーゼの阻害剤であり慢性骨髄性白血病の治療薬として臨床応用もされているイマチニブ(imatinib，商品名グリベック)が，糖尿病にしたApoEノックアウトマウスの大動脈粥状硬化病変を有意に抑制することが最近報告された[32]．イマチニブの投与は，単球遊走因子であるMCP-1(monocyte chemoattractant protein-1)やマトリクス産生因子CTGF(connective tissue growth factor)の発現も血管壁において低下させることから，糖尿病血管壁ではPDGFが多彩な作用を通じて動脈硬化病変の形成に携わることが示唆される．

一方，TGF-βも糖尿病・高血糖によりその発現を増すことが幾つかの臓器ならびに細胞レベルの検討から明らかとなっている．特に腎糸球体では，高血糖に伴うプロテインキナーゼCの活性化を通じてTGF-β発現が上昇し，マトリクスの沈着を促す結果，腎症の硬化性変化を進展させると考えられている．動脈壁においては，糖尿病がTGFβ受容体の発現上昇をもたらすことが確認されており，その結果，フィブロネクチンなどのマトリクス産生が増加する．これら一連の変化は，糖尿病患者でしばしば認められる“びまん性に硬化した動脈壁”の形成を考えるうえで興味深い．ただし，これまで報告されている1型，2型糖尿病患者の血中TGF-β濃度については必ずしも一定の傾向がみられず，またTGF-βには内皮機能を保護し，プラークを安定化させる働きもあることなどから，糖尿病の動脈硬化におけるTGF-βの役割は病変の進展度に応じて異なる可能性がある．

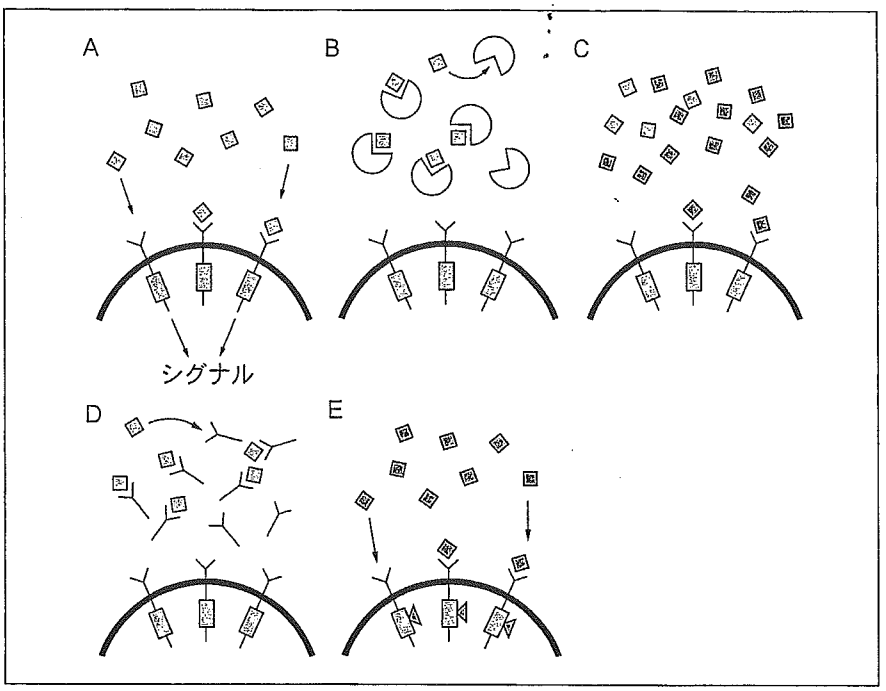

図3　増殖因子に対するさまざまな拮抗剤
A：増殖因子が正常に働く場合に比べ，B：増殖因子に対する中和剤，C：増殖因子受容体への結合に競合する薬剤，D：シグナルを伝達しない可溶性の増殖因子受容体が存在すると，増殖因子は受容体と結合できない．E：受容体の細胞内ドメインに作用してシグナル伝達を阻害する拮抗剤も可能である．(文献32)より改変引用)

IV．増殖因子を標的とした治療の可能性

現在，増殖因子やその受容体の発現を抑制する薬剤，シグナル伝達を抑制する方法などが盛んに研究されている．図3に示すような作用機序の拮抗剤は，各種増殖因子に対して応用が可能であり，糖尿病に伴う動脈硬化の新しい治療薬となる可能性をはらんでいる．図3のAは，増殖因子が受容体に結合する結果，増殖シグナルが伝達される通常の状態を示している．これに対してBでは，増殖因子に何らかの物質が結合した結果，増殖因子が受容体と結合できなくなる．Cでは受容体に結合する物質が増殖因子の受容体への結合と競合し，増殖因子が受容体に結合できない．Dでは細胞内にシグナルを伝えない受容体が大量に存在するため，増殖因子はこちらの方に結合してしまい，細胞膜表面の受容体には結合できなくなる．さらにEでは増殖因子と受容体の結合は正常に起きるが，下流へのシグナル伝達が阻害されている．特に，C，D，Eについては増殖因子拮抗剤の有力な候補がすでに現われており，前述のPDGF受容体キナーゼ阻害剤イマチニブの作用機序はEに該当する．

増殖因子は病変部だけでなく全身の正常細胞に作用しうるため，糖尿病患者の動脈硬化治療を目的にこれら阻害剤薬を臨床応用するためには，全身投与に伴う副作用の懸念を乗り越える必要がある．すでにPCI後の再狭窄予防のために世界的に使用されている薬剤溶出ステント(drug-eluting stent)などは薬物を局所に限定して投与できることから有望なデバイスとなろう．

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Werner症候群

Werner症候群とは

1904年にドイツの医師Otto Wernerにより「強皮症を伴う白内障症例」として初めて報告された常染色体劣性の遺伝性疾患である。思春期以降，さまざまな老化徴候が出現することから，代表的な早老症候群の一つに数えられている。本症は，第8染色体短腕上に存在するRecQ型DNA/RNAヘリカーゼ（WRNヘリカーゼ）のホモ接合体変異により生じる。

頻度

わが国におけるWerner症候群の発症頻度は100万人に1～3名の割合とされ，近親婚の多い地域に高い傾向がある。これまで全世界で少なくとも1,200症例が報告されているが，うち800例以上が日本人であり，とくにわが国に頻度の高い疾患と考えられる。一方，神奈川県の一般成人を対象とした調査では，1,000名中6名が*WRN*遺伝子変異をヘテロ接合体で保有していた。この数値から単純に計算すると，*WRN*変異をヘテロ接合体で保有する人の数は全国で70万人にのぼると考えられ，毎年20名強の赤ん坊が*WRN*変異をホモ接合体に保有して（つまり発症のリスクを抱えて）生まれてくることになる。

症候と病態生理

Werner症候群患者は幼児期，思春期までは健康人と区別することが難しい。思春期以降，白髪や皮膚の萎縮，角化などの症候が出現するが【図1】，実際の診断は30歳前後で白内障の出現を契機になされることが多い。Epsteinらによって記載されたWerner症候群の代表的な症候を【表1】に示す。このほか，高コレステロール血症の合併や腹部内臓脂肪の蓄積を高頻度に認める。40歳以上の症例では，しばしば足部や肘部に難治性皮膚潰瘍を生じ，ADL低下の大きな要因となる。平均寿命は47歳前後とされてきたが，近年，各種合併症治療の進歩により延長傾向にある。本症の原因であるWRNヘリカーゼの欠損はゲノムの不安定性（genomic instability）を招き，その結果として細胞老化や突然変異の発生が促進されると考えられている。しかし，インスリン抵抗性を初めとする各種代謝異常をもたらす機序については十分に解明されていない。

Werner症候群：ウェルナー症候群

Side Memo

早老症候群

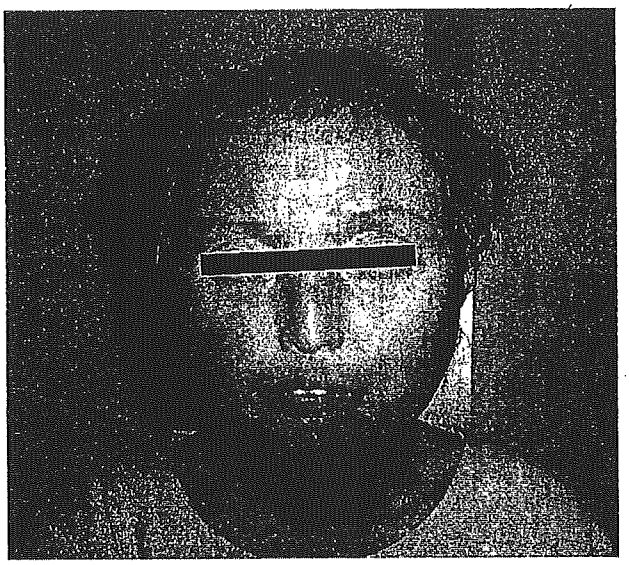
老年者一般に認められる徴候が若いうちに現れるという特徴に基づき分類された雑多な疾患群である。近年，その原因遺伝子の多くが明らかになった。Werner症候群以外では，Huchinson-Gilford（ハッチンソン-ギルフォード）症候群，Cockayne（コケイン）症候群，ataxia teleangiectasia（毛細血管拡張性運動失調），Down（ダウン）症候群などが早老症候群に分類される。

診断・鑑別診断

古くは前述の臨床的特徴と常染色体劣性遺伝形式とをもって診断された。現在ではその原因遺伝子が明らかにされているため，蛋白または，ゲノムDNAレベルでWRNヘリカーゼ変異を特定することが診断の確定に必要である。四肢末梢を主体とする皮膚の硬化と萎縮は強皮性との鑑別を要するが，全般的な臨床症状の相違が診断の決め手となる。

図1　Werner症候群の症候

① 46歳，女性。薄く白い毛髪，薄い皮膚，口唇の皺など，老年者にみられる特徴がこの年代より現れている。
② 52歳，男性。薄い毛髪，曲がった腰，細い四肢など一見して実年齢より老けて見える。



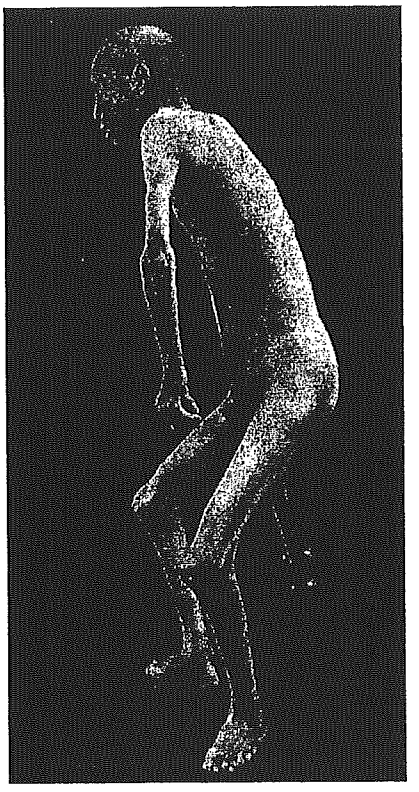


表1　Werner症候群の臨床的特徴

①低身長
②皮膚萎縮・角化・潰瘍
③四肢の筋・脂肪組織の萎縮
④毛髪の変化（白髪・禿頭）
⑤音声の変化（高調性嗄声）
⑥白内障
⑦骨粗鬆症
⑧耐糖能異常
⑨性腺機能低下
⑩軟部組織石灰化
⑪悪性腫瘍合併
⑫高インスリン血症

Basic Point　biochemistry

●老化（aging）とは

成熟期以後，加齢とともに各臓器の機能あるいはそれらを統合する機能が低下し，個体の恒常性を維持することが不可能となり，ついには不可逆的に死に至る過程をさす。この過程で起きるすべての現象を老化現象とよぶ。生理的な老化（physiological aging）が著しく加速され，病的状態を引き起こす場合を病的老化（pathological aging）とよぶことがあり，骨粗鬆症，Alzheimer病，早老症候群などが含まれる。

治療

本症患者の2大死因は間葉系の悪性腫瘍と動脈硬化性心血管障害である。*WRN*遺伝子異変に対する根本的治療法が存在しない現状では，悪性腫瘍の早期発見と動脈硬化性リスクの軽減が患者の予後向上に重要な意味をもつ。本症に合併する糖尿病は強いインスリン抵抗性を示し，しばしばコントロールに苦慮するが，通常はチアゾリジン誘導体の内服が有効である。皮膚潰瘍は通常療法に反応しづらく，状況によっては皮膚移植も考慮すべきである。

Level up View

●Werner症候群とアディポサイトカイン

Werner症候群患者はインスリン抵抗性，耐糖能障害，高脂血症など，肥満者によくみられる代謝異常を呈するが，そのBMI（body mass index：体重(kg)/身長(m²)，25以上が肥満）は22以下と見かけ上は太っていないことが多い。ところがその一方で，腹腔内内臓脂肪の蓄積を伴いやすい。近年，アディポサイトカインと総称され脂肪細胞から分泌される生理活性物質の変動が，生活習慣病の成り立ちに重要と考えられている。Werner症候群では，インスリン抵抗性を惹起するTNF（tumor necrosis factor）-αや抗凝固作用をもつPAI（プラスミノーゲンアクチベーターインヒビター）-Iといったアディポサイトカインの血中濃度が健常者に比べて有意に高いことがわかった。このように，Werner症候群にみられる各種代謝異常の成り立ちには，特異な脂肪分布とその機能変化が関与している可能性がある。

Self Check

Werner症候群について正しいのはどれか。

1. 常染色体優性遺伝形式をとる。
2. DNA/RNAヘリカーゼ遺伝子の変異を原因とする。
3. 毛髪の変化や白内障を示すことが多い。
4. 思春期以降，種々の老化徴候を示す。
5. 1型糖尿病を合併することが多い。

a. (1)(2)(3)　b. (1)(2)(5)　c. (1)(4)(5)　d. (2)(3)(4)　e. (3)(4)(5)　〈横手幸太郎〉

シンポジウム(6)

インスリンシグナルと動脈硬化症の関連性

[3]
インスリンと血管平滑筋細胞

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本稿では，①高インスリン血症が単独で動脈硬化のリスクとなるか，②血管平滑筋細胞のインスリン作用とインスリン抵抗性，③プロインスリンCペプチドと平滑筋細胞，という3項目を柱として，インスリンと平滑筋細胞について論じる．

これまでに行われてきた複数の臨床研究の成績から，高インスリン血症と動脈硬化性疾患との関連が示唆されている．しかし，臨床的に観察される高インスリン血症の多くはその基盤にインスリン抵抗性を有し，代償性機転によって生じると考えられる．このため，高インスリン血症が単独で動脈硬化の発症と進展に寄与するかは不明確である．筆者らは，50～65歳の15年間にわたり著しい高インスリン血症を呈したインスリノーマの男性例を経験したが，脂質や血圧値には異常を認めず，症候性の動脈硬化性疾患はなく，また頸動脈超音波検査によるIMTは左右とも0.7 mm，プラークも観察されなかった．インスリノーマを対象とした過去の報告[1]ならびにラット膵移植モデル[2]の検討結果を考慮すると，高インスリン血症はインスリン抵抗性あるいは他のリスクファクターとの共存下でのみ動脈硬化促進的に働くことが示唆された．

動脈硬化の進みやすい病態として注目されているメタボリックシンドローム（metabolic syndrome）の成り立ちや2型糖尿病の発症にはインスリン抵抗性が重要と考えられている．そこで，血管平滑筋細胞内のインスリンシグナルにおいても“抵抗性”を生じうるのか，またそれが動脈硬化の形成に関与するのか，臨床ならびに基礎的考察を行った．昨今，経皮的冠動脈インターベンション（PCI）後の再狭窄に対し，薬剤溶出ステントの高い有効性が示されている．ラパマイシン（シロリムス：免疫抑制剤）溶出ステントはその先駆けであり，わが国でも用いられているが，インスリン使用糖尿病患者ではその効果が不十分であることが報告されている[3]．これに対して，パクリタキセル（タキソール）という抗癌剤を用いた溶出ステントはインスリン使用糖尿病患者においても有意な抑制効果を示し話題となった[4]．ラパマイシンはp70S6キナーゼの阻害剤でありインスリンシグナルの一部分のみを抑制するが増殖に重要なMAPキナーゼ経路には影響を与えない[5]．これに対してパクリタキセルは，細胞分裂という“最終アウトプット”を阻害する働きを持つことが，外因性インスリンを使用する糖尿病患者の再狭窄予防効果の差異に現れている可能性がある．

一般にインスリン抵抗性は，肝や骨格筋細胞におけるPI3キナーゼ経路の減弱と関連することが知られている．筆者らの成績では，血管平滑筋細胞におけるPI3キナーゼ経路はMAPキナーゼ経路に対して抑制性に働く．つ

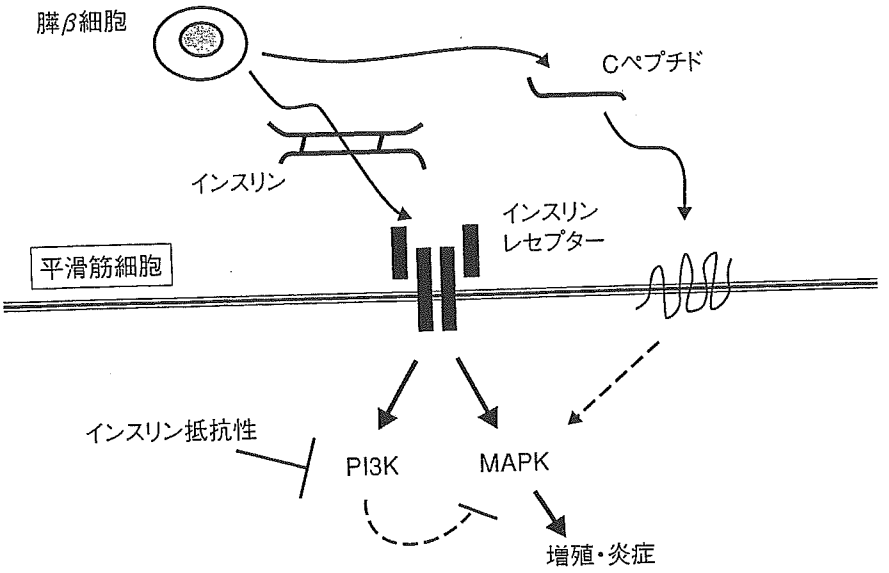

図1　血管平滑筋細胞におけるインスリン抵抗性とシグナル伝達

まり，平滑筋細胞における“インスリン抵抗性＝PI3 キナーゼ活性の低下”は，アポトーシスの抑制や細胞増殖，炎症の増強などをもたらし，それ自体が動脈硬化に対して促進的に働いている可能性がある．

また，これまでインスリン分泌過程の副産物と考えられてきたプロインスリン由来Cペプチドに，近年，種々の生理作用が報告されている[6]が，平滑筋細胞においてはMAPキナーゼ経路を活性化し，炎症惹起性遺伝子の発現を促した．インスリン抵抗性状態や慢性腎不全などの動脈硬化が進みやすい病態において高Cペプチド血症が見られることは示唆的といえよう．

このように，血管平滑筋細胞におけるインスリン抵抗性状態がMAPキナーゼシグナルの増強をもたらして動脈硬化病変の形成に寄与し，Cペプチドがその作用を修飾しうるという仮説は，2型糖尿病患者の血管合併症進展の一側面を説明するものであり，これらを標的とした新しい治療法創出の可能性を示している（図1）．

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