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Vascular Remodeling in Hypertensive Transgenic Mice

Naomi NISHIJO[1, 4], Shoko TAKAMINE[1], Fumihiro SUGIYAMA[1, 2], Koichi KIMOTO[4], Keiko TANIGUCHI[1], Hisashi HORIGUCHI[5], Takesaburo OGATA[5], Kazuo MURAKAMI[2, 3], Akiyoshi FUKAMIZU[2, 3], and Ken-ichi YAGAMI[1]

[1]Laboratory Animal Research Center, [2]Center for Tsukuba Advanced Research Alliance, [3]Institute of Applied Biochemistry, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki 305-8575, [4]Tokyo Kasei University, Kaga, Itabashi, Tokyo 173-8602, and [5]Ibaraki Prefectural University of Health Sciences, Ami, Ibaraki 300-0394, Japan

Abstract: *We physiologically and histopathologically analyzed vascular damage due to hypertension and vascular remodeling in hypertensive transgenic mice (Tsukuba hypertensive mice; THM). Pubertal (6-week-old) THM already had hypertension similar to blood pressure in adult THM due to an enhanced renin angiotensin system (RAS). They progressively developed remarkable vascular hypertrophy composed of dedifferentiation of vascular smooth muscle cells (VSMCs) and extracellular matrix accumulation in the thoracic aorta, and VSMC hyperplasia was predominant in the abdominal aorta. THM are therefore a useful animal model for studying vascular remodeling mediated by enhanced RAS.*

Key words: *hypertension, transgenic mice, vascular remodeling*

Human hypertension is generally recognized as a multifactorial disease associated with etiological factors. Both genetic and environmental factors, either singly or in combination, appear to play important roles in the etiology of hypertension. To study hypertension etiology and pathogenesis, researchers have used animal models such as the spontaneous hypertensive rat (SHR) [10], the stroke-prone spontaneous hypertensive rat (SHR-SP) [11] and the Dahl rat [2], but genetically defined factors related to hypertension in these animal models are poorly understood.

We established a hypertensive transgenic mouse line, the Tsukuba hypertensive mouse (THM) [4], that was produced by cross-mating transgenic mice carrying the human renin gene [3] with mice bearing the human angiotensinogen gene [16]. THM present higher angiotensin II (Ang II) and blood pressure levels than normotensive nontransgenic lines, and are therefore an animal model of human essential hypertension due to genetically defined factors of the renin angiotensin system (RAS) [4]. We recently demonstrated that atherosclerotic damage to the aortic vasculature is accelerated in THM fed an atherogenic diet [15], and that excessive salt ingestion by THM leads to acute aortic aneurysm and rupture [9]. We studied vascular damage due to hypertension and vascular remodeling in THM.

THM females were bred at our facility as described

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Address corresponding: K. Yagami, Laboratory Animal Research Center, University of Tsukuba, 1–1–1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan

elsewhere [4], and those 6 weeks old were used. Age-matched C57BL/6J controls were purchased from CLEA Japan Inc. (Tokyo, Japan). They were housed in an air-ventilated cabinet under specific-pathogen-free conditions. The cabinet was controlled at 23 ± 1°C, 55 ± 5% relative humidity, and artificial lighting from 06:00 to 20:00. The mice had free access to food (NMF, Oriental Yeast Co. Ltd., Tokyo, Japan) and autoclaved water.

We measured the systolic blood pressure of THM and control C57BL/6J mice with a programmable sphygmomanometer (BP-98A; Softron, Tokyo, Japan) in the morning between 09:00 and 11:00. Unanesthetized mice wrapped in a cotton holder were put into a warming tube thermostatically controlled at 37°C. Systolic blood pressure was calculated as the average of 10 measurements for each mouse. The systolic blood pressure of THM was 127.9 ± 3.8 mmHg, and that of controls 97.1 ± 9.1 mmHg, indicating a significant difference between the mouse lines (Fig. 1a). For comparison, the SHR rat has rapidly increased blood pressure from 7 to 10 weeks of age and an additional increase due to aging, with the highest blood pressure roughly 200 mmHg [10]. The present result indicates that pubertal (6-week-old) THM already have hypertension similar to blood pressure in adult THM [4].

To clarify whether hypertension in pubertal THM was accompanied by an enhanced RAS, plasma renin activity (PRA) in both mouse strains was measured. Blood was collected from the inferior vena cava of mice anesthetized with pentobarbital (30 mg/kg). Plasma containing anticoagulant (EDTA 2Na) was stored at −80°C until use. PRA was estimated by measuring the rate of Ang I formation, with subsequently generated Ang I quantitated by radioimmunoassay (RIA), as described elsewhere [4]. PRA in THM was roughly 25-fold higher than that in normotensive C57BL/6J mice (Fig. 1b), suggesting that functional enhancement of RAS in THM begins at an early stage of growth.

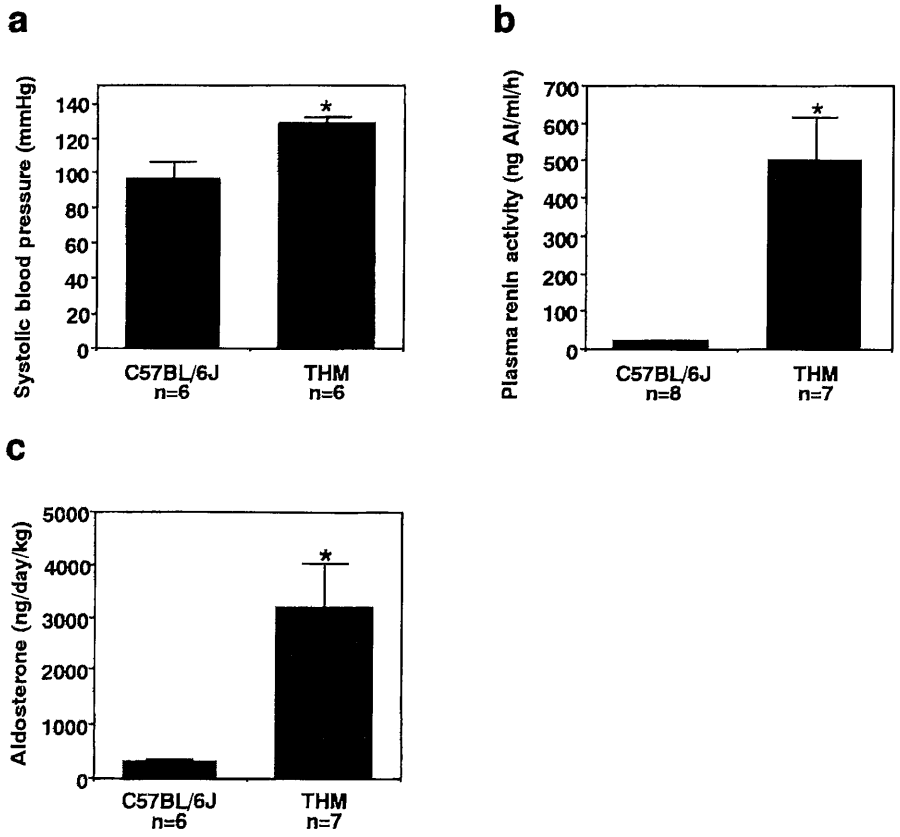

Fig. 1. Systolic blood pressure (a), plasma renin activity (b) and urinary aldosteron concentration (c) of THM and C57BL/6J mice. * $p<0.0001$ vs C57BL/6J.

A major function of RAS is to maintain water and electrolytes mediated with aldosterone whose release from the adrenal gland is induced by Ang II. We measured urine aldosterone concentrations in the THM and controls to determine the reabsorption of water and electrolytes in the renal tubules, which suspected of being related to increasing blood pressure in THM. Urine was collected daily for 4 days with a metabolic cage (CL-0305; CLEA Japan Inc. Tokyo, Japan) and frozen until use. To measure urine aldosterone, an aliquot of collected urine was pretreated with an equal volume of 0.2N HCl and incubated at 30°C for 18 hr. The urine aldosterone concentration was determined by RIA (Aldosterone RIA Kit II, Dinabot, Tokyo, Japan). That in THM was roughly 10 times higher than in controls (Fig. 1c). In pubertal THM, an enhanced aldosterone release appears due to overproduction of Ang II derived from human renin and angiotensinogen, indicating that THM already have hypertension due to activated RAS when young.

We subsequently histopathologically analyzed vascular damage in the THM due to hypertension induced by activated RAS. After euthanasia with pentobarbital administration, phosphate buffered saline (PBS) was introduced into the left ventricle via a 26 gauge needle and allowed to flow out through a cut in the right atrium. When the perfusate became clear, 4% formaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) and 3% glutaraldehyde in PB were perfused for light and electron microscopy. For light microscopy, the formaldehyde-fixed aorta, heart and kidneys were embedded in paraffin. The fixed aorta was separated into the thoracic and abdominal aortae. The thoracic aorta was further divided into ascending thoracic aorta, aortic arch, and descending thoracic aorta, and the abdominal aorta into upper and lower abdominal aortae. Sections were stained with hematoxylin and eosin (HE) to observe the cellular structure in the aortic wall, and with elastica-van Gieson (EVG) staining for elastic lamellas. For electron microscopy, the glutaraldehyde-fixed aortic arch and abdominal aorta were minced into small blocks and postfixed with 1% osmium tetraoxide in PB, dehydrated in graded ethanol, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a JEM 100 CX electron microscope (JEOL, Tokyo, Japan).

Microscopically, the aortic arch of normotensive C57BL/6J mice consisted of a single layer of endothelial cells, five to six layers of media composed of vascular smooth muscle cells (VSMCs) and elastic lamellas, and loose connective adventitial tissue (Fig. 2a and 2c). In contrast, the THM aortic wall, especially in the media of the aortic arch, was significantly thickened due to VSMC hypertrophy, but neither quantitative nor qualitative changes were observed in elastic lamellas (Fig. 2b and 2d). Similar findings were seen in thoracic ascending and descending aortae.

Ultrastructurally, VSMCs in C57BL/6J were spindle-shape and contained intracellular myofilaments and undeveloped organella. These cell junctions appeared tight, and the extracellular matrix was scarce (Fig. 3a). Most VSMCs in THM manifested extreme hypertrophy, containing decreased intracellular myofilaments and prominently developed organella such as rough-surfaced endoplasmic reticulum and mitochondria. Increased reticular extracellular matrix was also noted in THM (Fig. 3b and 3e). These findings indicate that VSMCs of THM involve phenotypic modification from spindle-shaped contractive to synthetic.

In the upper abdominal aorta of THM, VSMC hypertrophy was not significant, although media were dramatically thickened compared to C57BL/6J. Vascular thickening was associated with fibroblastic proliferation, and a roughly 2-fold increase in the number of elastic lamella and increased collagen fiber in inner and outer adventitial tissues (Fig. 2e, 2f, 2g and 2h). Similar findings were observed in the lower abdominal aorta. Ultrastructurally, VSMCs and elastic lamella were dramatically increased in THM. Most VSMCs were contractive, increased intracellular myofilaments, although inmost VSMCs were square and closely placed. Increased reticular extracellular matrix was also noted in THM (Fig. 3c and 3d). Pathological differences between thoracic aorta and abdominal aorta in THM therefore appeared to be VSMC hypertrophy and hyperplasia.

VSMC hypertrophy and hyperplasia are closely related to local RAS in the vascular response to arterial injury [13], and with mechanical stress including shear stress and blood pressure, and the growth factors such as fibroblast growth factor(FGF), transforming growth factor β (TGF-β) and platelet derived growth factor (PDGF), stimulated their synthesis in VSMCs due to Ang II [5, 7, 8]. Autocrine release of Ang II also leads

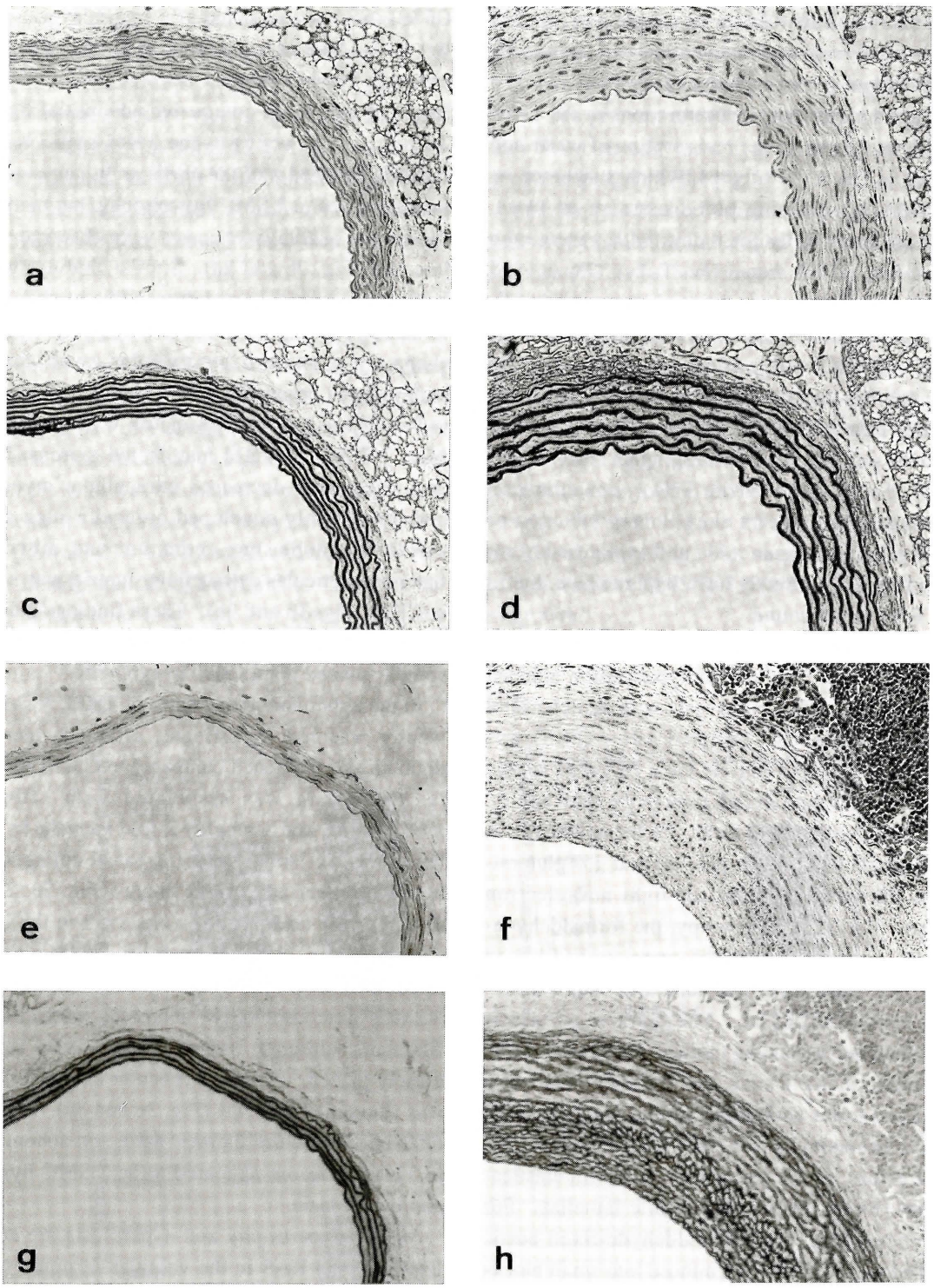

Fig. 2. Coronal sections of the aortic arch in C57BL/6J (a and c) and THM (b and d), and the abdominal aorta in C57BL/6J (e and g) and THM (f and h). In the aortic arch, the media of THM (b) was thickened compared to that of C57BL/6J (a). The thickened media was due to VSMC hypertrophy, but not to quantitative and qualitative changes in elastic lamellae (c and d). In the abdominal aorta, the media of THM (f) was much thicker than that of C57BL/6J (e). A dramatical increase in VSMCs and elastic lamellae was noted in THM (h), compared to C57BL/6J (g). HE staining (a, b, e and f), × 200. EVG staining (c, d, g and h), × 200.

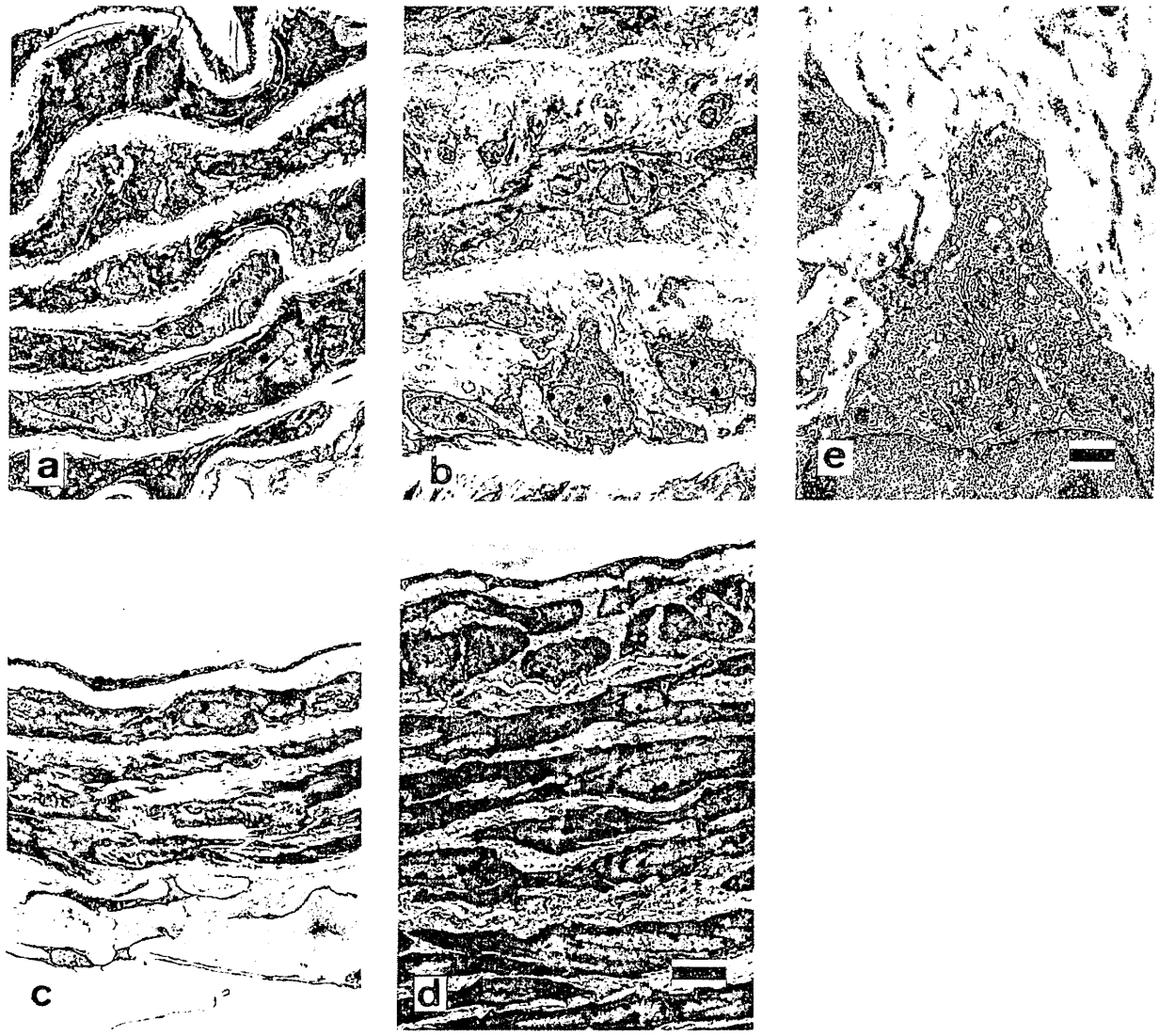

Fig. 3. Ultrastructure of the aortic arch in C57BL/6J (a) and THM (b and e), and the abdominal aorta in C57BL/6J (c) and THM (d). The lumen appears in the upper parts of the panels. In the aortic arch, and most of VSMCs in THM manifested remarked hypertrophy (b), containing prominently developed organelles such as rough-surfaced endoplasmic reticulum and mitochondria (e). In the abdominal aorta, a dramatical increase in VSMCs and elastic lamellae was noted in THM(d), compared to C57BL/6J (c). Most of the VSMCs in THM were spindle-shaped and contained having undeveloped organellae (d). Bar= 5 μm (a, b, c and d). Bar=1 μm (e).

directly to hypertrophy of cultured myocardial cells through the Ang I receptor [14]. Hypertrophy of VSMCs in THM is suggested to be reactivation of protein synthesis in these cells.

Several studies have shown that administering TGF-β to VSMCs induces cellular hypertrophy and inhibits mitogen-stimulated proliferation [1, 12]. Itoh *et al.* [7] showed that VSMC proliferation was induced by FGF mediated by Ang II. Since mechanical stress enhanced TGF-β expression in VSMCs via Ang II [5], VSMC hypertrophy demonstrated in THM could be prominent in the thoracic aorta, which is influenced strongly by mechanical stress. Ang II may lead to predominant FGF expression and result in VSMC hyperplasia in the abdominal aorta. Square VSMCs in the inmost site of the THM abdominal aorta are considered to be cells immediately after proliferation.

We studied kidney and heart sections stained with

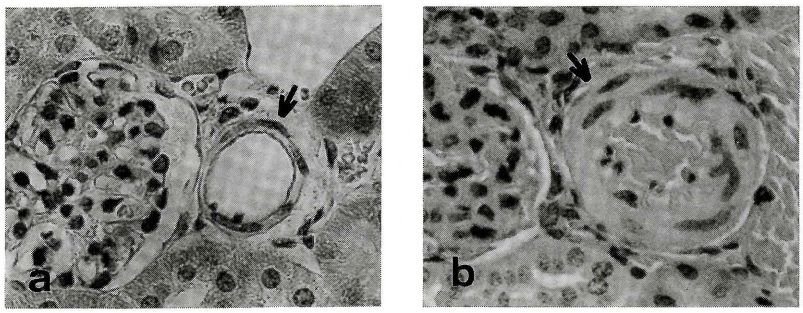

Fig. 4. Cross-section of the small intrarenal artery in C57BL/6J (a) and THM (b). Vascular thickening was notable in small arteries neighbouring the glomeruli of the kidneys. The arteries are indicated by arrows. HE staining, × 400.

HE, to compare the vascular structures of the small arteries in THM and C57BL/6J. Compared to controls, vascular thickening was notable in small renal arteries near glomeruli (Fig. 4a and 4b). Similar findings were also demonstrated in small cardiac arteries.

In conclusion, we demonstrated that pubertal THM progressively develop vascular remodeling involving dedifferentiation of VSMCs and extracellular matrix accumulation, and that qualitative differences, hypertrophy and hyperplasia exist in vascular remodeling in the thoracic and abdominal aortae. Possible reasons for qualitative differences in vascular remodeling are the degree of mechanical stress, the balance of growth factor synthesis, and the vascular structure at the respective aortic sites. THM are therefore a useful animal model for studying vascular remodeling mediated by enhanced RAS.

Acknowledgment

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