development of atherosclerotic lesions in *ApoE*$^{-/-}$ mice fed a high cholesterol diet for 6 weeks (Figure 6G and H; Supplementary Figure S6F).

Recently, it has been revealed that an SNP in the promoter region of the human MRTF-A gene (−184C>T), which results in a high transcriptional activity in HeLa and K562 cells, is associated with susceptibility to CAD (Hinohara *et al*, 2009). We found that the −930 bp of MRTF-A promoter containing −184T, which is associated with high CAD susceptibility, showed significantly stronger transcriptional activity than the wild-type promoter in cultured RAVSMCs (Figure 6I). These results further support our notion that inhibition of MRTF-A could be an effective novel approach to the treatment and prevention of vascular disorders.

Discussion

In the present study, we used three vascular injury models (femoral artery wire injury, carotid artery ligation and diet-induced atherosclerosis in *APOE*$^{-/-}$ mice) in *Mkl1*$^{-/-}$ mice to elucidate the roles played by MRTF-A in pathological vascular remodelling. We initially found that expression of MRTF-A mRNA and protein was significantly increased in injured arteries and aortic tissues containing atherosclerotic lesions in *ApoE*$^{-/-}$ mice, while expression of myocardin was reciprocally decreased. In each model, neointima formation or atherosclerotic lesions were significantly smaller in *Mkl1*$^{-/-}$ mice than in the respective controls. The expression of vinculin, MMP-9 and integrin β1 genes, which are targets of SRF and key regulators of cellular migration, was significantly diminished in the injured arteries of *Mkl1*$^{-/-}$ mice. Knocking down MRTF-A in RAVSMCs reduced expression of these genes in response to extracellular stimuli, which significantly impaired cell migration. These results demonstrate that induced expression of MRTF-A is crucial for acquisition of the capacity to migrate in response to environmental stress in dedifferentiated VSMCs (Liu *et al*, 2005). We also found that MRTF-A gene expression in VSMCs is, at least in part, regulated by miR-1, which is in turn regulated by myocardin and SRF (Jiang *et al*, 2010; Chen *et al*, 2011). Expression of miR-1 was reduced in dedifferentiated VSMCs, along with that of myocardin. This apparently led to an increase in MRTF-A expression, though it is possible that another as yet unidentified mechanism, such as transcriptional regulation through sites located in the distal 5′-FR or within introns, also contribute to the reciprocal regulation of myocardin and MRTF-A expression. Finally, we showed that a small molecule inhibitor of MRTF-A, CCG-1423, significantly reduced neointima formation following wire injury to mouse femoral arteries. Collectively, these results demonstrate that induction of MRTF-A plays a key role in vascular remodelling by maintaining SRF activity, thereby conferring a capacity for migration in response to extracellular stimuli on dedifferentiated VSMCs. MRTF-A is thus a potentially useful therapeutic target that may be more specific and efficient than the upstream Rho family GTPases, which can affect diverse intracellular signalling events.

In differentiated VSMCs, myocardin strongly activates SRF and the expression of VSMC-specific contractile proteins, thereby contributing to the maintenance of the contractile phenotype (Wang *et al*, 2003). Myocardin is constitutively located in the nucleus, where it suppresses MRTF-A expression via activation of miR-1. The ability of MRTF-B to transduce Rho signalling into the nucleus is much weaker than that of MRTF-A (Kuwahara *et al*, 2005; Nakamura *et al*, 2010),

Table II Luminal and neointimal area of femoral arteries 3weeks after vascular injury

	n	Lumen ($\times 10^3/\mu m^2$)	Intima ($\times 10^3/\mu m^2$)	Media ($\times 10^3/\mu m^2$)	IEL ($\times 10^3/\mu m^2$)	EEL ($\times 10^3/\mu m^2$)	Intima/Media ratio
Control injury	6	15.0 ± 3.0	39.9 ± 4.1	22.9 ± 2.1	55.0 ± 2.4	77.8 ± 3.7	1.85 ± 0.17
CCG1423 injury	8	22.3 ± 5.6	20.2 ± 4.8*	24.8 ± 1.6	43.0 ± 7.2	67.8 ± 2.8	0.81 ± 0.19*

The ratio of intima to media was calculated as the intimal area/medial area. Values are means ± s.e.m. IEL, internal elastic lamina; EEL, external elastic lamina. *$P<0.01$ versus control injured arteries.

Figure 6 CCG-1423, an MRTF-A inhibitor, attenuated neointima formation induced by wire injury in mouse femoral arteries. (**A**) CCG-1423 diminished the nuclear accumulation of endogenous MRTF-A induced by 20% FCS in RAVSMCs. Cells were stained with anti-MRTF-A antibody (green) and DAPI (blue). (**B**) CCG-1423 significantly inhibited MRTF-A-induced SRF activity in RAVSMCs. Graphs show the relative luciferase activities of 3 × CArG-luc. STARS: expression plasmid encoding striated muscle activator of Rho signalling. Data were obtained from two experiments performed in quintuplicate. (**C**) PDGF-BB-induced migration was assessed in RAVSMCs treated without or with 0.1 μm (+) or 1 μm (++) of CCG-1423. Data were obtained from two experiments performed in sextuplicate. (**D**) FCS-induced proliferation was assessed in RAVSMCs treated without or with 0.1 μm (+) or 1 μm (++) of CCG-1423. Data were obtained from two experiments performed in quadruplicate. (**E, F**) Effect of CCG-1423 on neointima formation in wire-injured femoral arteries in mice. Graph showing the neointima (NI)-to-media (M) ratio in wire-injured arteries from mice treated without (control) or with CCG-1423 ($n=3$ in control group and 4 in CCG1423 group) (**E**). Representative images of neointima are shown (**F**). (**G**) Representative images of atherosclerotic lesions in cross-sections of proximal aorta from *ApoE*$^{-/-}$ mice fed a high-cholesterol diet with or without CCG-1423 for 6 weeks. Oil-red O: Oil-red O staining. Bar indicates 100 μm. (**H**) Graphs showing the relative (%) area of atherosclerotic lesions in cross-sections of proximal aorta from *ApoE*$^{-/-}$ mice fed a high-cholesterol diet and treated with or without CCG-1423 for 6 weeks ($n=3$ in control group and 4 in CCG-1423 group). **$P<0.01$. (**I**) Effect of an SNP in the promoter region of MRTF-A gene (−184C>T) on the promoter activity in RAVSMCs. Relative activities of −930 bp MRTF-A(−184C)-luc and −930 bp MRTF-A(−184T)-luc in two different experiments performed in quadruplicate are shown. All graphs are shown as means ± s.e.m. **$P<0.01$. (**J**) A proposed model of the role of MRTF-A in vascular remodelling. In differentiated, contractile VSMCs, constitutively nuclear myocardin strongly activates SRF, leading to expression of VSMC-specific contractile proteins, and suppresses MRTF-A expression through activation of miR-1. Under these conditions, cytosolic Rho signalling is confined almost exclusively to regulation of contraction. In dedifferentiated VSMCs, MRTF-A expression is induced by reductions in miR-1 expression and basal SRF activity, thereby maintaining the lower basal SRF activity necessary for cellular migration and proliferation. Because MRTF-A is shuttled between the cytosol and nucleus and because it activates SRF downstream of Rho signalling, in dedifferentiated VSMCs, extracellular stimuli activating Rho signalling can substantively affect cellular proliferation and migration by modulating SRF activity. MYOCD: myocardin.

so that Rho family signalling is almost exclusively confined to regulating contraction through modifying Ca^{2+} sensitivity in the cytosol. By contrast, in dedifferentiated synthetic VSMCs, the reduction in myocardin expression leads to a reduction in basal SRF activity and then to a loss of VSMC-specific contractile components. Under these conditions, MRTF-A expression is induced, at least in part, by the reduction in miR-1 also caused by diminished myocardin, and is sufficient to maintain the SRF activity necessary for cellular migration and proliferation. Because MRTF-A is shuttled between the cytosol and nucleus, where it activates SRF downstream of Rho family GTPase-actin signalling, in dedifferentiated VSMCs extracellular stimuli activating Rho GTPase signalling can substantively affect cellular proliferation and migration by modulating SRF activity (Medjkane *et al*, 2009; Olson and Nordheim, 2010). Loss or inhibition of MRTF-A reduced stimulus-induced cell migration and proliferation, making cells static (Figure 6J). This suggests that the reciprocal expression of MRTF-A and myocardin mediated by miR-1 regulates the plasticity of effectors downstream of Rho family signalling, thereby contributing to phenotypic modulation of VSMC during vascular remodelling.

In addition to the classical concept that dedifferentiated intimal VSMCs are derived from medial VSMCs, recent evidence raises the possibility that VSMC progenitor cells in the circulation or adventitia also contribute to intimal VSMCs (Sata *et al*, 2002; Hoglund *et al*, 2010). We have not addressed the role of MRTF-A in the process of intimal VSMC differentiation from such progenitor cells in this study. In that context, however, MRTF-A has been shown to be involved in the differentiation of mesenchymal stem cells into VSMCs (Jeon *et al*, 2008). Thus, MRTF-A may also play an important role in the molecular processes underlying migration, proliferation and differentiation of VSMC progenitor cells into intimal VSMCs during vascular remodelling.

Recently, human genetic screening to identify novel susceptibility loci for CAD using microsatellite markers and SNP analysis revealed that an SNP in the promoter region of the MRTF-A gene ($-184C>T$) is associated with susceptibility to CAD (Hinohara *et al*, 2009). Moreover, functional analysis suggested that heightened MRTF-A expression is associated with increased susceptibility to CAD. We observed that the MRTF-A promoter containing $-184T$, which is associated with high CAD susceptibility, showed significantly stronger transcriptional activity than the wild-type promoter in cultured VSMCs (Figure 6I). These observations further support the conclusion that MRTF-A is crucially involved in pathological vascular remodelling underlying the development of vascular diseases, and imply that MRTF-A is a potentially useful therapeutic target for prevention of the progression of vascular diseases.

Materials and methods

Plasmids

-930 bp MRTF-A($-184C$)-luc (MRTF-A-luc), vinculin-luc, vinculin CArG-mut-luc and $3\times$CArG-luc were described previously (Kuwahara *et al*, 2007; Morita *et al*, 2007; Hinohara *et al*, 2009). Expression vectors used in the experiments were described previously (Kuwahara *et al*, 2007). MRTF-A 3′UTR-luc and mutMRTF-A 3′UTR-luc were respectively generated by inserting the MRTF-A 3′UTR containing wild type or mutated miR-1 target sequences downstream of the luciferase gene in a pMIR-REPORTER kit miRNA reporter expression vector (Ambion). -5500 bp MRTF-A-luc was generated by inserting 5500 bp of the 5′-FR of MRTF-A gene upstream of the luciferase gene in pGL4 vector (Promega).

Animal experiments

MRTF-A$^{-/-}$ mice were kindly provided from Dr EN Olson (The University of Texas, Southwestern Medical Center at Dallas) (Li *et al*, 2006). *ApoE*$^{-/-}$ and *MRTF-A*$^{-/-}$ mice (C57BL/6 background) were cross-bred (Kobayashi *et al*, 2004; Li *et al*, 2006). The animal care and all experimental protocols were reviewed and approved by the Animal Research Committee at Kyoto University Graduate School of Medicine.

Cell culture and transfection

RAVSMCs (Cell Applications. Inc.), A7r5 (DS Pharma Biomedical), NIH3T3 and COS7 cells were maintained in DMEM supplemented with 10% FCS. Co-transfection of RAVSMCs with $3\times$CArG-luc plus expression plasmids encoding MRTF-A (1 ng) and STARS (100 ng), or with MRTF-A-luc plus expression plasmids encoding myocardin and MRTF-A (0, 1 or 10 ng each) was accomplished using FuGene6 (Roche). pRL-TK (Roche) was included in all transfections as an internal control. MiRIDIAN microRNA mimic for miR-1, miRIDIAN microRNA hairpin inhibitor for miR-1 or a negative control for each (Thermo Scientific) was transfected into RAVSMCs grown in 6-cm dishes using Dharmafect2. MAVSMCs were obtained as previously reported (Nakamura *et al*, 2010).

RNA interference

RAVSMCs grown in 6-cm dishes were transfected with 200 pmol of ON-TARGET plus® siRNA reagent targeting rat MRTF-A or myocardin, or control scrambled siRNA (Thermo Scientific) using Dharmafect 2. For luciferase assays, RAVSMCs grown in 24-well dishes were transfected with 100 pmol of siRNA and 500 ng of luciferase reporter plasmid using Fugene 6.

Mouse vascular injury

Vascular wire injury was induced in femoral arteries of male C57BL/6 wild-type or *Mkl1*$^{-/-}$ mice at 8–10 weeks of age, as described previously (Sata *et al*, 2000; Takaoka *et al*, 2009). LNA oligonucleotide anti-miR-1 microRNA inhibitor or LNA microRNA inhibitor negative control (20 mg/kg) (5′-FAM prelabelled, Exiqon) was injected into sham-operated or injured femoral arteries from the muscular branch using a syringe with 29 gauge needle (TERUMO).

Quantification of neointimal hyperplasia

We harvested the femoral and carotid arteries 4 weeks after wire injury, unless otherwise indicated. Digitalized images were analysed using image analysis software (Image J, NIH), and the intimal and medial areas were recorded. The average of the neointima/media ratios in FIVE serial sections was designated as the value to represent each individual.

Analysis of atherosclerotic lesion area in ApoE$^{-/-}$ *mice*

Mkl1$^{+/+}$;*ApoE*$^{-/-}$ and *Mkl1A*$^{-/-}$;*ApoE*$^{-/-}$ mice were fed normal chow for 4 weeks beginning when the mice were 4 weeks old. Then beginning when they were 8 weeks old, they were fed a high-cholesterol diet (F2HFD1, Oriental Biotechnology) for 8 weeks. Atherosclerotic lesions were analysed by en-face analysis of the whole aorta and quantified by cross-sectional analysis of the proximal aorta, as described previously (Paigen *et al*, 1987; Palinski *et al*, 1994; Kobayashi *et al*, 2004).

Immunohistochemical analysis

Paraffin-embedded sections (4 μm thick) of femoral arteries harvested 4 weeks after wire injury were stained with anti-Mac3, anti-CD31, mouse monoclonal anti-αSMA (Sigma-Aldrich), rabbit polyclonal anti-SM-MHC (BT-562, Biomedical Technologies Inc.) or anti-BSAC antibodies (Sasazuki *et al*, 2002). Ratios of total numbers of Mac-3-positive cells in the intima and the media and CD31-positive endothelial cells in *Mkl1*$^{+/+}$ and *Mkl1*$^{-/-}$ mice were quantified ($n=4$ and 5 in each group, respectively). Sections of proximal aortas from *ApoE*$^{-/-}$ mice were stained with anti-α-SMA, anti-SM-MHC or anti-BSAC antibody.

Real-time RT–PCR

Real-time one-step RT–PCR was performed using One-step RT-PCR master mix reagent (Applied Biosystems). MiR-1 expression was determined using a Taqman MicroRNA RT kit and Taqman Universal PCR Master Mix II (Applied Biosystems). All taqman primers and probes were purchased from Applied Biosystems.

Western blot analysis

Western blot analysis was performed using rabbit polyclonal anti-myocardin, anti-MRTF-A and anti-MRTF-B antibodies as described previously (Kuwahara *et al*, 2005; Nakamura *et al*, 2010).

Statistical analysis

Data are presented as means ± s.e.m. Unpaired *t*-tests were used for comparison between two groups, and ANOVA with *post hoc* Fisher's test was used for comparison among groups. Values of $P<0.05$ were considered as significant. Data obtained from the two-way factorial design were analysed with the two-way ANOVA.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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Author Contributions: TM conducted most of the experiments and contributed to data analysis. KK and NK conceived of and directed the project. MT, HK, YK, MS, TS and RN provided technical help on animal experiments. YN, HN, TN, KS and AK performed some experiments using luciferase reporter assays, immunohistochemical analysis and western blot analysis with TM. KN, YY, CY, JS, SU, TN and YK contributed to data analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Bentzon JF, Weile C, Sondergaard CS, Hindkjaer J, Kassem M, Falk E (2006) Smooth muscle cells in atherosclerosis originate from the local vessel wall and not circulating progenitor cells in ApoE knockout mice. *Arterioscler Thromb Vasc Biol* **26:** 2696–2702

Chen J, Yin H, Jiang Y, Radhakrishnan SK, Huang ZP, Li J, Shi Z, Kilsdonk EP, Gui Y, Wang DZ, Zheng XL (2011) Induction of microRNA-1 by myocardin in smooth muscle cells inhibits cell proliferation. *Arterioscler Thromb Vasc Biol* **31:** 368–375

Daniel JM, Bielenberg W, Stieger P, Weinert S, Tillmanns H, Sedding DG (2010) Time-course analysis on the differentiation of bone marrow-derived progenitor cells into smooth muscle cells during neointima formation. *Arterioscler Thromb Vasc Biol* **30:** 1890–1896

Evelyn CR, Wade SM, Wang Q, Wu M, Iniguez-Lluhi JA, Merajver SD, Neubig RR (2007) CCG-1423: a small-molecule inhibitor of RhoA transcriptional signaling. *Mol Cancer Ther* **6:** 2249–2260

Glass CK, Witztum JL (2001) Atherosclerosis. The road ahead. *Cell* **104:** 503–516

Hinohara K, Nakajima T, Yasunami M, Houda S, Sasaoka T, Yamamoto K, Lee BS, Shibata H, Tanaka-Takahashi Y, Takahashi M, Arimura T, Sato A, Naruse T, Ban J, Inoko H, Yamada Y, Sawabe M, Park JE, Izumi T, Kimura A (2009) Megakaryoblastic leukemia factor-1 gene in the susceptibility to coronary artery disease. *Hum Genet* **126:** 539–547

Hinson JS, Medlin MD, Lockman K, Taylor JM, Mack CP (2007) Smooth muscle cell-specific transcription is regulated by nuclear localization of the myocardin-related transcription factors. *Am J Physiol Heart Circ Physiol* **292:** H1170–H1180

Hoglund VJ, Dong XR, Majesky MW (2010) Neointima formation: a local affair. *Arterioscler Thromb Vasc Biol* **30:** 1877–1879

Jeon ES, Park WS, Lee MJ, Kim YM, Han J, Kim JH (2008) A Rho kinase/myocardin-related transcription factor-A-dependent mechanism underlies the sphingosylphosphorylcholine-induced differentiation of mesenchymal stem cells into contractile smooth muscle cells. *Circ Res* **103:** 635–642

Jiang Y, Yin H, Zheng XL (2010) MicroRNA-1 inhibits myocardin-induced contractility of human vascular smooth muscle cells. *J Cell Physiol* **225:** 506–511

Jin W, Goldfine AB, Boes T, Henry RR, Ciaraldi TP, Kim EY, Emecan M, Fitzpatrick C, Sen A, Shah A, Mun E, Vokes V, Schroeder J, Tatro E, Jimenez-Chillaron J, Patti ME (2011) Increased SRF transcriptional activity in human and mouse skeletal muscle is a signature of insulin resistance. *J Clin Invest* **121:** 918–929

Kenagy RD, Hart CE, Stetler-Stevenson WG, Clowes AW (1997) Primate smooth muscle cell migration from aortic explants is mediated by endogenous platelet-derived growth factor and basic fibroblast growth factor acting through matrix metalloproteinases 2 and 9. *Circulation* **96:** 3555–3560

Kobayashi T, Tahara Y, Matsumoto M, Iguchi M, Sano H, Murayama T, Arai H, Oida H, Yurugi-Kobayashi T, Yamashita JK, Katagiri H, Majima M, Yokode M, Kita T, Narumiya S (2004) Roles of thromboxane A(2) and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J Clin Invest* **114:** 784–794

Kuwahara K, Barrientos T, Pipes GC, Li S, Olson EN (2005) Muscle-specific signaling mechanism that links actin dynamics to serum response factor. *Mol Cell Biol* **25:** 3173–3181

Kuwahara K, Teg Pipes GC, McAnally J, Richardson JA, Hill JA, Bassel-Duby R, Olson EN (2007) Modulation of adverse cardiac remodeling by STARS, a mediator of MEF2 signaling and SRF activity. *J Clin Invest* **117:** 1324–1334

Li S, Chang S, Qi X, Richardson JA, Olson EN (2006) Requirement of a myocardin-related transcription factor for development of mammary myoepithelial cells. *Mol Cell Biol* **26:** 5797–5808

Li S, Wang DZ, Wang Z, Richardson JA, Olson EN (2003) The serum response factor coactivator myocardin is required for vascular smooth muscle development. *Proc Natl Acad Sci USA* **100:** 9366–9370

Liu Y, Sinha S, McDonald OG, Shang Y, Hoofnagle MH, Owens GK (2005) Kruppel-like factor 4 abrogates myocardin-induced activation of smooth muscle gene expression. *J Biol Chem* **280:** 9719–9727

Medjkane S, Perez-Sanchez C, Gaggioli C, Sahai E, Treisman R (2009) Myocardin-related transcription factors and SRF are required for cytoskeletal dynamics and experimental metastasis. *Nat Cell Biol* **11:** 257–268

Miano JM (2003) Serum response factor: toggling between disparate programs of gene expression. *J Mol Cell Cardiol* **35:** 577–593

Miralles F, Posern G, Zaromytidou AI, Treisman R (2003) Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113:** 329–342

Morita T, Mayanagi T, Sobue K (2007) Reorganization of the actin cytoskeleton via transcriptional regulation of cytoskeletal/focal adhesion genes by myocardin-related transcription factors (MRTFs/MAL/MKLs). *Exp Cell Res* **313:** 3432–3445

Nakamura S, Hayashi K, Iwasaki K, Fujioka T, Egusa H, Yatani H, Sobue K (2010) Nuclear import mechanism for myocardin family

members and their correlation with vascular smooth muscle cell phenotype. *J Biol Chem* **285:** 37314–37323

Nishimura G, Manabe I, Tsushima K, Fujiu K, Oishi Y, Imai Y, Maemura K, Miyagishi M, Higashi Y, Kondoh H, Nagai R (2006) DeltaEF1 mediates TGF-beta signaling in vascular smooth muscle cell differentiation. *Dev Cell* **11:** 93–104

Olson EN, Nordheim A (2010) Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat Rev Mol Cell Biol* **11:** 353–365

Owens GK, Kumar MS, Wamhoff BR (2004) Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* **84:** 767–801

Paigen B, Morrow A, Holmes PA, Mitchell D, Williams RA (1987) Quantitative assessment of atherosclerotic lesions in mice. *Atherosclerosis* **68:** 231–240

Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL (1994) ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb* **14:** 605–616

Sasazuki T, Sawada T, Sakon S, Kitamura T, Kishi T, Okazaki T, Katano M, Tanaka M, Watanabe M, Yagita H, Okumura K, Nakano H (2002) Identification of a novel transcriptional activator, BSAC, by a functional cloning to inhibit tumor necrosis factor-induced cell death. *J Biol Chem* **277:** 28853–28860

Sata M, Maejima Y, Adachi F, Fukino K, Saiura A, Sugiura S, Aoyagi T, Imai Y, Kurihara H, Kimura K, Omata M, Makuuchi M, Hirata Y, Nagai R (2000) A mouse model of vascular injury that induces rapid onset of medial cell apoptosis followed by reproducible neointimal hyperplasia. *J Mol Cell Cardiol* **32:** 2097–2104

Sata M, Saiura A, Kunisato A, Tojo A, Okada S, Tokuhisa T, Hirai H, Makuuchi M, Hirata Y, Nagai R (2002) Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. *Nat Med* **8:** 403–409

Schwartz SM, deBlois D, O'Brien ER (1995) The intima. Soil for atherosclerosis and restenosis. *Circ Res* **77:** 445–465

Shoji M, Sata M, Fukuda D, Tanaka K, Sato T, Iso Y, Shibata M, Suzuki H, Koba S, Geshi E, Katagiri T (2004) Temporal and spatial characterization of cellular constituents during neointimal hyperplasia after vascular injury: Potential contribution of bone-marrow-derived progenitors to arterial remodeling. *Cardiovasc Pathol* **13:** 306–312

Sun Y, Boyd K, Xu W, Ma J, Jackson CW, Fu A, Shillingford JM, Robinson GW, Hennighausen L, Hitzler JK, Ma Z, Morris SW (2006) Acute myeloid leukemia-associated Mkl1 (Mrtf-a) is a key regulator of mammary gland function. *Mol Cell Biol* **26:** 5809–5826

Takaoka M, Nagata D, Kihara S, Shimomura I, Kimura Y, Tabata Y, Saito Y, Nagai R, Sata M (2009) Periadventitial adipose tissue plays a critical role in vascular remodeling. *Circ Res* **105:** 906–911

Wang D, Chang PS, Wang Z, Sutherland L, Richardson JA, Small E, Krieg PA, Olson EN (2001) Activation of cardiac gene expression by myocardin, a transcriptional cofactor for serum response factor. *Cell* **105:** 851–862

Wang DZ, Li S, Hockemeyer D, Sutherland L, Wang Z, Schratt G, Richardson JA, Nordheim A, Olson EN (2002) Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc Natl Acad Sci USA* **99:** 14855–14860

Wang Z, Wang DZ, Pipes GC, Olson EN (2003) Myocardin is a master regulator of smooth muscle gene expression. *Proc Natl Acad Sci USA* **100:** 7129–7134

Watanabe N, Kurabayashi M, Shimomura Y, Kawai-Kowase K, Hoshino Y, Manabe I, Watanabe M, Aikawa M, Kuro-o M, Suzuki T, Yazaki Y, Nagai R (1999) BTEB2, a Kruppel-like transcription factor, regulates expression of the SMemb/Nonmuscle myosin heavy chain B (SMemb/NMHC-B) gene. *Circ Res* **85:** 182–191

Xu W, Baribault H, Adamson ED (1998) Vinculin knockout results in heart and brain defects during embryonic development. *Development* **125:** 327–337

Zhao Y, Samal E, Srivastava D (2005) Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature* **436:** 214–220

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新規創薬を目指した生活習慣病・難治性疾患モデル遺伝子変異ラットの開発と解析
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