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Sirt7 Deficiency Attenuates Neointimal Formation Following Vascular Injury by Modulating Vascular Smooth Muscle Cell Proliferation

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Background: Sirt7 is a recently identified sirtuin and has important roles in various pathological conditions, including cancer progression and metabolic disorders. It has previously been reported that Sirt7 is a key molecule in acute myocardial wound healing and pressure overload-induced cardiac hypertrophy. In this study, the role of Sirt7 in neointimal formation after vascular injury is investigated.

Methods and Results: Systemic ($Sirt7^{-/-}$) and smooth muscle cell-specific Sirt7-deficient mice were subjected to femoral artery wire injury. Primary vascular smooth muscle cells (VSMCs) were isolated from the aorta of wild type (WT) and $Sirt7^{-/-}$ mice and their capacity for cell proliferation and migration was compared. Sirt7 expression was increased in vascular tissue at the sites of injury. $Sirt7^{-/-}$ mice demonstrated significant reduction in neointimal formation compared to WT mice. In vitro, Sirt7 deficiency attenuated the proliferation of serum-induced VSMCs. Serum stimulation-induced upregulation of cyclins and cyclin-dependent-kinase 2 (CDK2) was significantly attenuated in VSMCs of $Sirt7^{-/-}$ compared with WT mice. These changes were accompanied by enhanced expression of the microRNA 290-295 cluster, the translational negative regulator of CDK2, in VSMCs of $Sirt7^{-/-}$ mice. It was confirmed that smooth muscle cell-specific Sirt7-deficient mice showed significant reduction in neointima compared with control mice.

Conclusions: Sirt7 deficiency attenuates neointimal formation after vascular injury. Given the predominant role in vascular neointimal formation, Sirt7 is a potentially suitable target for treatment of vascular diseases.

Key Words: Neointimal formation; Sirtuin; Smooth muscle cell

Silent information regulator 2 (Sir2) proteins and sirtuin families have been identified as NAD-dependent histones and protein deacetylases that regulate a wide range of biological processes, such as aging, metabolism and cancer progression.[1–3] In mammals, 7 homologues of Sir2 have been identified,[4] and Sirt7 is the most recently identified sirtuin, which was originally considered a nucleolus-localized sirtuin that regulates RNA polymerase I transcription.[5] However, subsequent studies found it was also localized in the nucleus and cytoplasm.[6] In addition, a recent study showed that Sirt7 acts as not only as a NAD-

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dependent histone and protein deacetylase, but also as a histone desuccinylase.[7] Sirt7 is highly expressed in the liver, spleen and testis in mice,[8] but previous studies using homozygous Sirt7-deficient ($Sirt7^{-/-}$) mice showed multiple organ phenotypes,[9–11] suggesting that the exact functional role of Sirt7 is not limited to these tissues.

In cardiovascular tissues, the expression level of cardiac Sirt7 diminishes with aging, and the life span of $Sirt7^{-/-}$ mice is shorter than that of wild-type (WT) mice due to increased cardiomyocyte apoptosis and interstitial fibrosis under physiological conditions.[12] In contrast, we reported recently that acute cardiovascular insult induces high levels of Sirt7 in the border zone of acute myocardial infarction and contributes to appropriate wound healing by stabilizing transforming growth factor-β receptor I (TβRI), enabling efficient TGF-β signaling.[13] In addition, we also showed in a model of myocardial infarction and hindlimb ischemia that mice deficient in Sirt7 exhibited low levels of tissue inflammation.[13]

Vascular smooth muscle cells (VSMCs) play a key role in maintaining vascular structure and homeostasis.[14] Under pathological conditions, VSMC proliferation is regarded as a central feature of lesion formation, such as neointimal formation after vascular injury.[15] It has been reported that some of the members of sirtuin regulate VSMCs functions. For example, Sirt1, the most well-known mammalian sirtuin, has a vascular protective effect mediated by the inhibition of VSMC proliferation and increase in DNA resistance to chronic vascular inflammation.[16,17] In contrast, Sirt6 promotes VSMC differentiation in response to cyclic strain.[18] These reports indicate that each sirtuin has a different role in VSMCs depending on the pathophysiological conditions.

Cell cycle regulation is a fundamental mechanism of VSMC function. In general, progression of cell cycle promotes cell function in VSMCs, resulting in accelerated vascular remodeling.[19] Cyclin-dependent-kinase 2 (CDK2) is a crucial regulator of the cell division cycle and is upregulated at sites of vascular lesions.[20,21] Repression of CDK2 activity has been shown to attenuate vascular remodeling.[22,23] It has been shown that Sirt1 directly regulates the expression of cell cycle-related proteins.[16] However, little is known about the effect of other sirtuin members on the expression of cell cycle-related proteins.

The present study was designed to determine the role of Sirt7 in vascular neointimal formation using systemic and smooth muscle cell-specific Sirt7-deficient mice, and to clarify the molecular mechanism by which Sirt7 modulates disease process, especially focusing on VSMC proliferation.

Methods

Animals

All procedures were performed in accordance with Kumamoto University animal care guidelines and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH, Publication No. 85-23, revised 1996). The study was approved by the animal research ethics committee of Kumamoto University (#A29-057). The generation of homozygous $Sirt7^{-/-}$ mice was described in detail previously.[12] Smooth muscle cell-specific Sirt7-deficient mice were generated by the intercrossing between $Sirt7^{flox/flox}$ mice, which harbored LoxP sites within introns 5 and 9, and SM22-Cre transgenic mice. Generation of $Sirt7^{flox/flox}$ mice was described in detail previously.[9] The SM22-Cre transgenic mice were obtained from Jackson Laboratory.

Wire Injury Model of the Femoral Artery

Male, 8- to 12-week-old mice underwent femoral artery wire injury, as described in detail previously.[15] Briefly, under isoflurane anesthesia, the left femoral artery and femoral vein were exposed and looped together proximally and distally with a 6-0 silk suture. The muscular branch artery was isolated and ligated with a 6-0 silk suture. Femoral veins and connective tissues around the femoral artery were carefully removed. A straight spring wire (C-SF-15-15, COOK, Bloomington NV) was carefully inserted from the muscular branch into the main femoral artery. After removal of the guidewire, the proximal portion of the muscular branch artery was tied off.

Mice were euthanized at 7, 14 or 28 days after operation, and the femoral vessels were harvested for morphometric analysis. The femoral artery was fixed in 4% paraformaldehyde (PFA), dehydrated, and embedded in paraffin. Hematoxylin and eosin (HE) staining was performed and the cross-sectional areas of the intima, media and adventitia were analyzed and measured by ImageJ software, as described previously.[24] The density of VSMCs was assessed by α-smooth muscle actin (α-SMA) staining. Bromodeoxyuridine (BrdU) labeling was performed to identify proliferating cells in the wire-injured vessel by detection of DNA synthesis. BrdU solution (BD Pharmingen, 1 mg/100 μL) was injected intraperitoneally 24 h before sacrifice. A standard avidin-biotin procedure for BrdU staining (BrdU; rabbit polyclonal antibody, Abbiotec, No. 250563) was performed, as described previously.[25] BrdU-labeled and -unlabeled cells in the intimal area were counted by an investigator blinded to the experimental procedure.

Immunostaining

Paraformaldehyde-fixed tissue samples were immunostained for VSMCs (α-SMA; mouse monoclonal, M0851, 1A4, DAKO) and macrophages (Iba-1; rabbit polyclonal, 019-19741, Wako Pure Chemicals), as described previously.[26,27] Histofine® Simple Stain Mouse MAX PO and Histofine® Simple Stain MAX PO(M) were used as secondary antibodies. Morphometric analysis was performed using ImageJ software.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was prepared using a Qiagen RNeasy fibrous minikit according to the protocol supplied by the manufacturer, and cDNA was prepared using the T-PCR System 2700 (Applied Biosystems). Quantitative real-time polymerase chain reaction (PCR) was performed, as described previously.[13] Transcript expression levels were determined as the number of transcripts relative to those of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) normalized to the mean value of the control.

Cell Culture

Primary human aortic smooth muscle cells (HAoSMCs) were obtained from the American Type Culture Collection (ATCC® PCS-100-012™). Primary VSMCs were isolated from the aorta of 8-week-old male WT mice and $Sirt7^{-/-}$ mice, as described in detail previously.[28] Passage 3 to 5 VSMCs were used in all experiments. VSMCs were cultured under standard conditions at 37°C in 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) containing

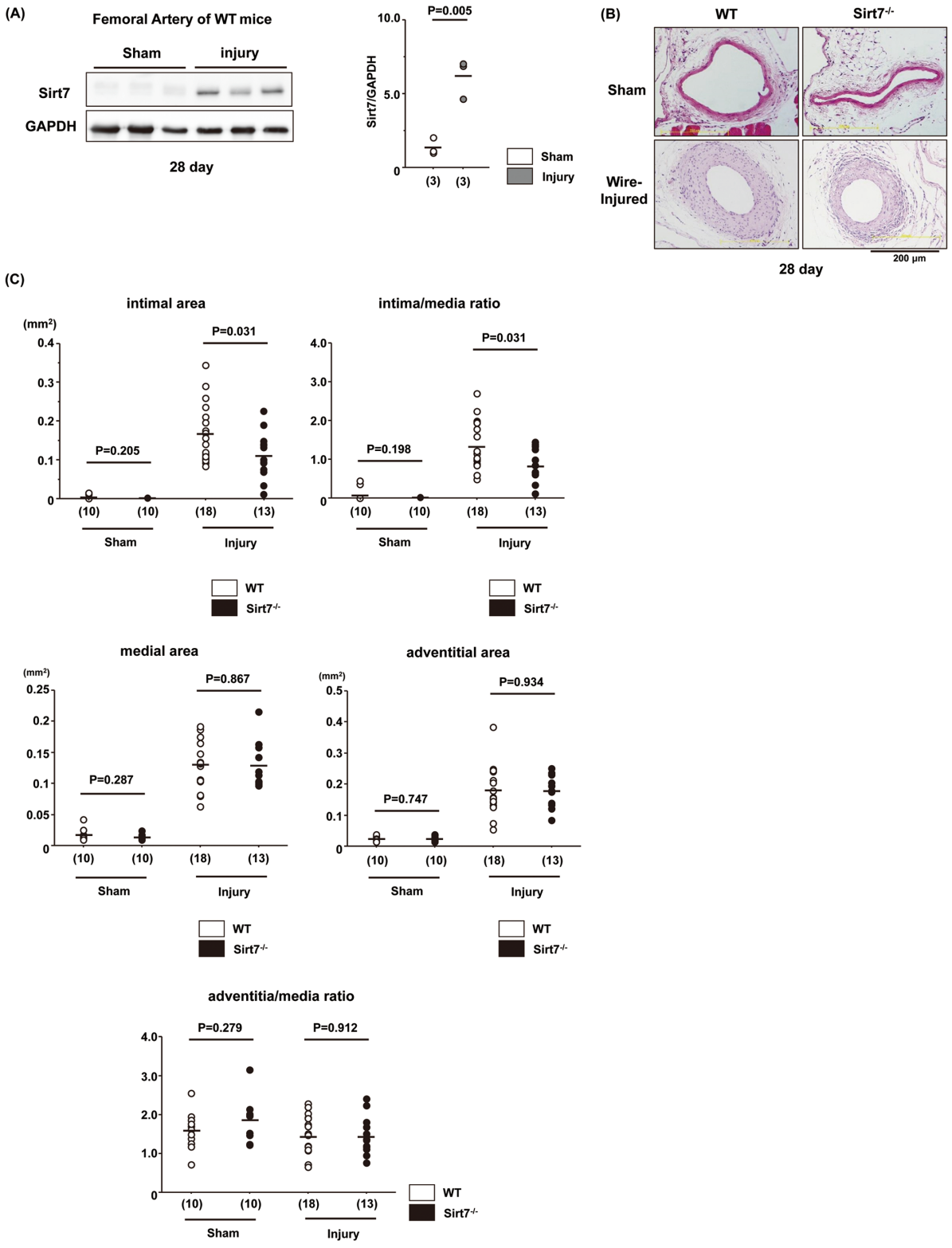

Figure 1. Sirt7 deficiency lessened neointimal formation following vascular injury. (**A**) Sirt7 protein expression in femoral arteries of wild-type (WT) mice at 28 days after sham operation and wire injury. (**B**) Representative images of hematoxylin and eosin (HE)-stained femoral arteries of WT mice and Sirt7 deficient ($Sirt7^{-/-}$) mice at 28 days after wire injury. (**C**) Quantitative analysis of the intimal area, intima/media ratio, medial area, adventitial area and adventitia/media ratio. Data represent individual samples and mean values of the indicated sample sizes.

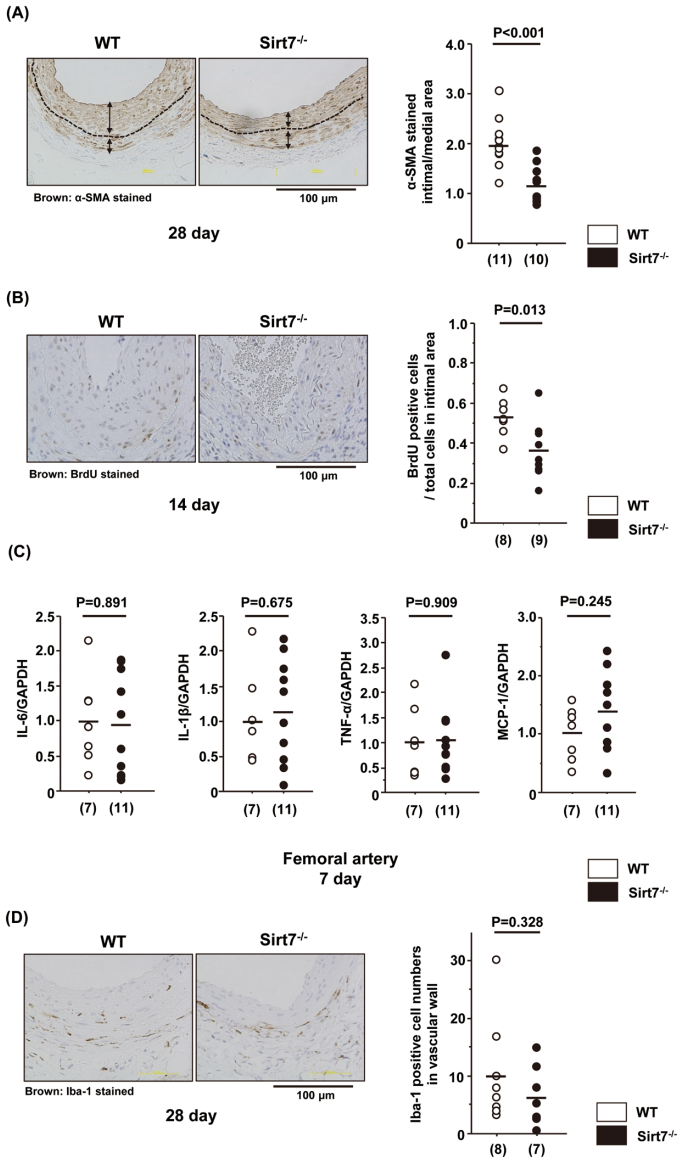

Figure 2. Sirt7 is involved in the neointimal formation after vascular injury via regulation of the vascular smooth muscle cell (VSMC) proliferation. (**A**) **Left**: Representative images of α-smooth muscle actin (SMA)-stained femoral arteries of WT mice and Sirt7$^{-/-}$ mice at 28 days after wire injury. **Right**: Quantitative analysis of the ratio of α-SMA stained intimal/medial area (**B**) **Left**: Representative images of bromodeoxyuridine (BrdU)-stained femoral arteries of wild-type (WT) mice and Sirt7$^{-/-}$ mice at 14 days after wire injury. **Right**: The ratio of BrdU positive cells to total cells in the neointima. (**C**) IL-6, IL-1β, TNF-α and MCP-1 mRNA expression levels in the femoral arteries of WT mice and Sirt7$^{-/-}$ mice at 7 days after wire injury. (**D**) **Left**: Representative images of ionized calcium-binding adapter molecule 1 (Iba-1)-stained femoral arteries of WT mice and Sirt7$^{-/-}$ mice at 28 days after wire injury. **Right**: Quantitative analysis of Iba-1-positive cell numbers in the vascular wall. Data represent individual samples and mean values of the indicated sample sizes.

1 g/L glucose and 2 mmol/L L-glutamine, supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 mg/mL streptomycin.

RNA Interference

The small interference RNAs (siRNAs) that target human Sirt7 were purchased from Invitrogen (Carlsbad, CA). Control cultures were transfected with unrelated siRNAs (Invitrogen). Cells were transfected for 24 h with siRNAs by using Lipofectamine RNAi MAX (Invitrogen) according to the instructions provided by the manufacturer.

Cell Proliferation Assay

VSMCs were seeded at a concentration of 10,000 cells per well in 96-well plates, and incubated with 10% FBS for 24 h. After 24-h serum starvation (DMEM containing 0.1% FBS), VSMCs were cultured for 24 h with or without 10% FBS stimulation. VSMC proliferation was evaluated by the BrdU cell proliferation assay kit (Roche Diagnostics, Indianapolis, IN, USA) and a MTS Celltiter 96 aqueous non-radioactive cell-proliferation assay (Promega, Madison, WI, USA). Absorbance was measured at A370 nm–A492 nm and at A490 nm in a plate reader.

Cell Migration Assay

VSMC migration was assessed by using a 24-well Boyden chamber with polycarbonate membranes with 8-μm pores (the CytoSelect™ Cell Haptotaxis Assay Kit; CellBiolabs, CA), as described previously.[29] After overnight serum starvation, VSMCs (1.0×10^6 cells/well) were added to the upper chamber, and DMEM, supplemented with 10% FBS, was added to the lower chamber. After 24-h incubation at 37°C, VSMCs on the upper surface of the membrane were removed by gentle scraping. The inserts were transferred to clean wells containing 300 μL of Lysis Buffer/CyQuant® GR dye solution. After 20-min incubation at

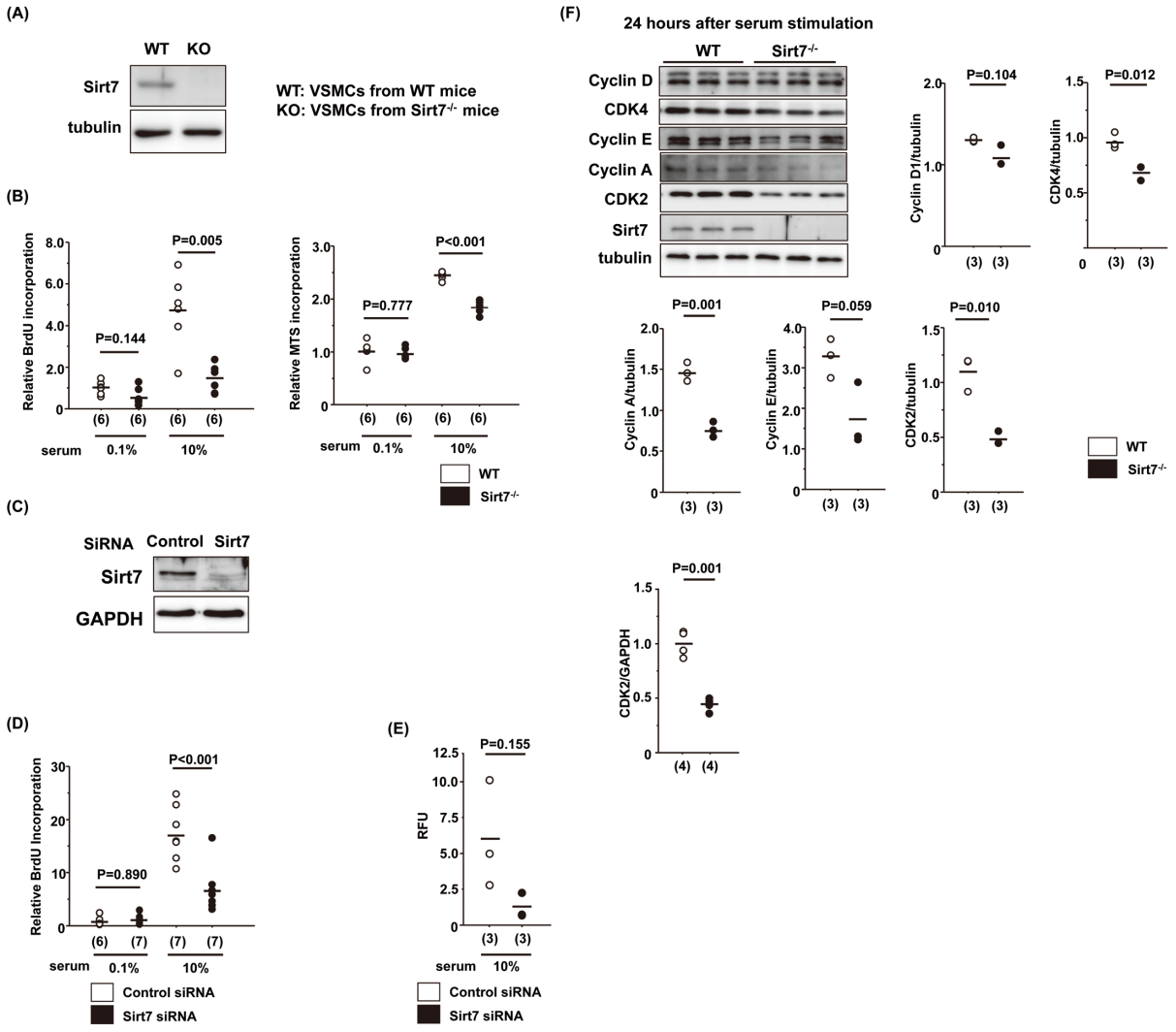

Figure 3. Sirt7-deficient (Sirt7$^{-/-}$) vascular smooth muscle cells (VSMCs) show reduced proliferative capacity. (**A**) Sirt7 protein expression in VSMCs from wild-type (WT) mice and VSMCs from Sirt7$^{-/-}$ mice. (**B**) Serum-induced VSMC cell proliferation of WT and Sirt7$^{-/-}$ assessed by bromodeoxyuridine (BrdU) assay (left) and MTS assay (right). (**C**) Sirt7 protein level in primary human aortic smooth muscle cells (HAOSMCs) treated with control and Sirt7 siRNA. (**D**) Cell proliferation analysis of HAoSMCs treated with control and Sirt7 siRNA assessed by using a BrdU assay. (**E**) Cell migration analysis of HAoSMCs treated with control and Sirt7 siRNA assessed by using the Boyden-Chamber assay. (**F**) The protein expressions of cell-cycle-related proteins, including cyclin D1, E, A, CDK2 and CDK4 in WT VSMCs and Sirt7$^{-/-}$ VSMCs. Data represent individual samples and mean values of the indicated sample sizes.

room temperature, the florescence of the CyQuant® GR dye solution was measured at 480 nm/520 nm in a fluorescence plate reader.

Quantitative miRNA Expression

The miRNAs were isolated from cultured VSMCs or aortas by using a Qiagen miRNeasy Mini kit. The miRNAs were reverse transcribed into first-strand cDNA using a RT 2 miRNA First-Strand Kit (Qiagen). The cDNA was mixed with QuantiTast SYBR Green PCR Master Mix. For real-time PCR, primers and templates were mixed with SYBR Advantage Quantitative PCR Premix (BD Clontech). The DNA was amplified for 40 cycles of denaturation for 5 s at 95°C and annealed for 20 s at 60°C on the C1000 Thermal Cycler (CFX384, BioRad). Transcript levels were normalized to those of U6 in the same sample.

Western Blot Analysis

Western blotting was performed with a SDS-PAGE Electrophoresis System, as described previously.[13] Primary antibodies against cyclin E, CDK2, Sirt7, GAPDH (Cell Signaling Technology, Danvers, MA, USA), cyclin D1, cyclin A, cyclin-dependent-kinase 4 (CDK4) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and tubulin (Calbiochem) were used.

Statistical Analysis

All data are presented as individual samples and mean

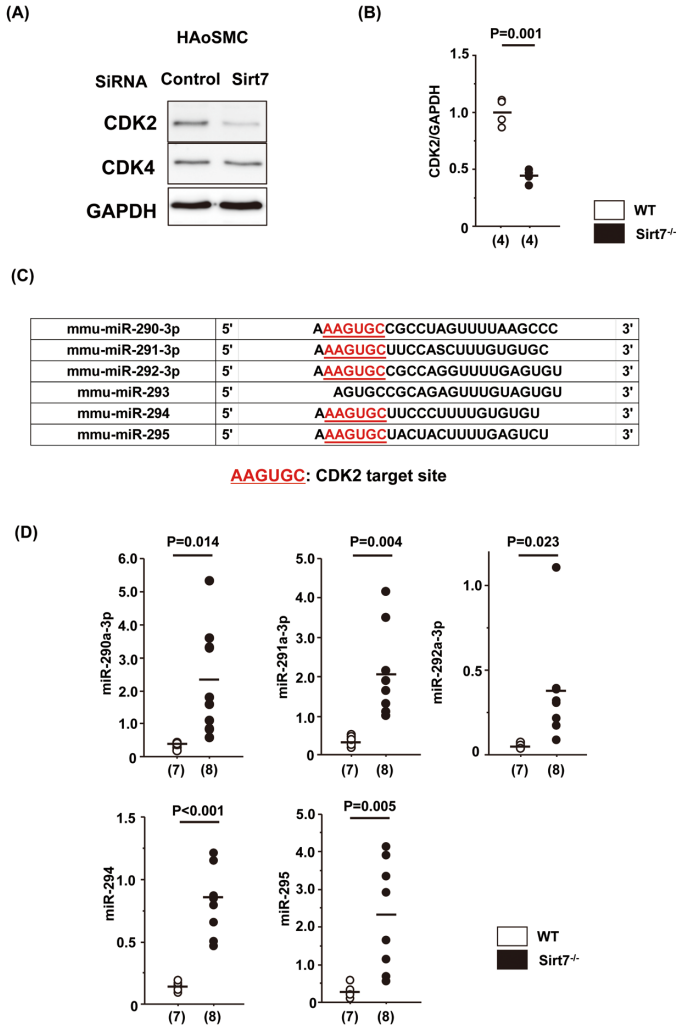

Figure 4. Sirt7 deficiency is associated with CDK2 downregulation and upregulation of the miRNA 290-295 family. (**A**) CDK2 and CDK4 protein expressions in primary human aortic smooth muscle cells (HAOSMCs) treated with control and Sirt7 siRNA. (**B**) CDK2 mRNA expression level in vascular smooth muscle cells (VSMCs) of wild-type (WT) and Sirt7$^{-/-}$ mice. (**C**) miR-290-295 cluster target site in 3'-UTR of CDK2 predicted by miRbase. (**D**) Expression levels of miR-290-295 in VSMCs of WT and Sirt7$^{-/-}$ mice. Data represent individual samples and mean values of the indicated sample sizes.

values or mean±SEM. Differences between groups were analyzed by using the unpaired Welch t-test. In multiple-comparison tests, the P value was adjusted by using the Bonferroni method.

The significance level of a statistical hypothesis test was set at 0.05.

Results

Sirt7$^{-/-}$ Mice Show Attenuated Neointimal Formation Following Vascular Injury

To investigate the role of Sirt7 in neointimal formation, we first examined vascular Sirt7 expression profiles in the femoral artery wire injury model. As shown in **Figure 1A**, Sirt7 protein expression was significantly increased at the sites of injury at 28 days after operation compared with the non-operated vessels.

To investigate the functional role of Sirt7 in the development of neointimal formation following vascular injury, WT and Sirt7$^{-/-}$ mice were subjected to femoral wire injury. Morphometric analysis at 28 days after wire injury showed a significant decrease in the intimal area, with a lower intima/media ratio in Sirt7$^{-/-}$ mice than WT mice (**Figure 1B**,**1C**). In contrast, the medial and adventitial areas and the adventitia/media ratio were not significantly different between WT mice and Sirt7$^{-/-}$ mice (**Figure 1B**,**1C**). Thus, Sirt7 deficiency is associated with reduced neointimal hyperplasia, compared with WT mice, in a mice model of wire injury.

Because VSMCs contribute to the neointimal formation following vascular injury,[15] we analyzed VSMC composition in wire-injured vessels by α-SMA staining. The ratio of intimal α-SMA positive area to medial α-SMA positive area was significantly decreased in Sirt7$^{-/-}$ mice compared with WT mice (**Figure 2A**). We also performed BrdU staining to investigate the vascular cell proliferation in the intimal area at 14 days after wire injury. The ratio of intimal BrdU-positive cells to total cells was significantly decreased in Sirt7$^{-/-}$ mice compared with WT mice (**Figure 2B**). In contrast, vascular inflammation, assessed by the measurements of inflammatory cytokines such as IL-6, IL-1β, TNF-α and MCP-1 and macrophage infiltration by ionized calcium-binding adapter molecule 1 (Iba-1) staining in vascular walls, was not different between WT and Sirt7$^{-/-}$ mice (**Figure 2C**,**2D**). These findings indicate that Sirt7 is involved in the neointimal formation after vascular injury

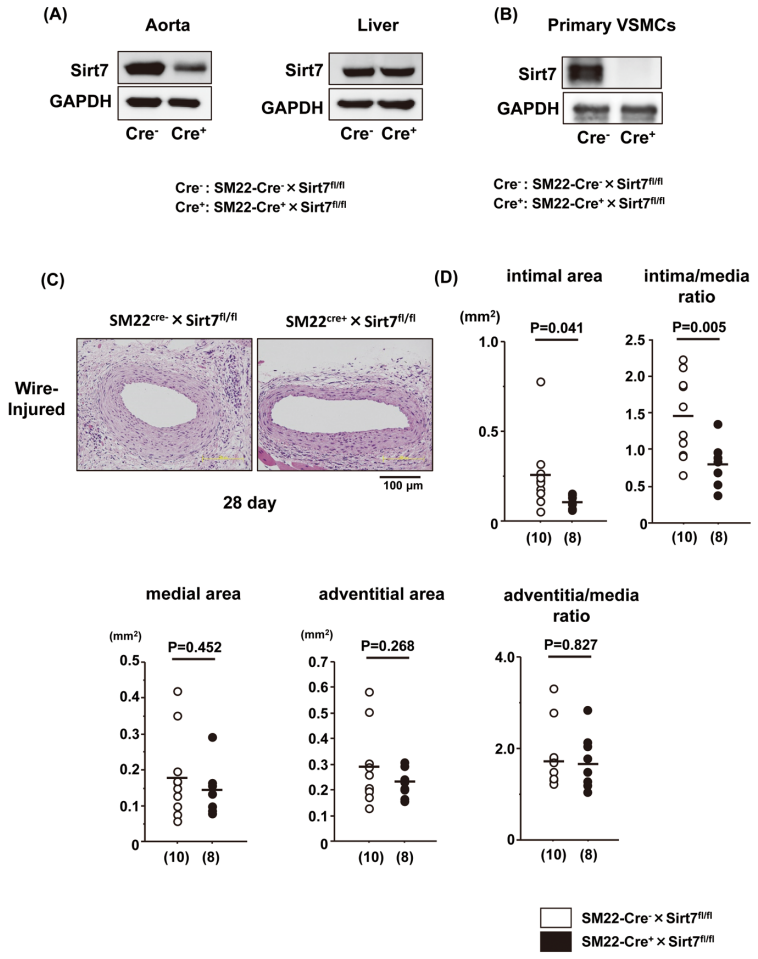

Figure 5. Smooth muscle cell-specific Sirt7 deficiency reduced neointimal formation following vascular injury. (**A**) Sirt7 protein expression in the aorta and liver of control mice and smooth muscle cell (SMC)-specific Sirt7-deficient mice. (**B**) Sirt7 protein expression in vascular smooth muscle cells (VSMCs) from control mice and SMC-specific Sirt7-deficient mice. (**C**) Representative images of hematoxylin and eosin (HE)-stained femoral arteries of control mice and SMC-specific Sirt7-deficient mice at 28 days after wire injury. (**D**) Quantitative analysis of intima, intima/media ratio, media, adventitia, and adventitia/media ratio. Data represent individual samples and mean values of the indicated sample sizes.

via regulation of the VSMC proliferation.

Sirt7-Deficient VSMCs Show Reduced Proliferative Capacity

To examine the functional role of Sirt7 on VSMCs, we isolated primary VSMCs from the aorta of WT and Sirt7$^{-/-}$ mice and compared their capacity for cell proliferation and migration (**Figure 3A**). Stimulation of WT VSMCs with serum significantly increased the cell proliferation, evaluated by the BrdU assay and MTS assay, whereas these changes were attenuated in Sirt7$^{-/-}$ VSMCs (**Figure 3B**). We confirmed these results by RNAi-mediated Sirt7 knockdown experiments in HAoSMCs. Serum stimulation of control siRNA-treated HAoSMCs significantly increased cell proliferation; however, these changes were attenuated in Sirt7 siRNA-treated HAoSMCs (**Figure 3C**,**3D**). Furthermore, knockdown of Sirt7 tended to reduce cell migration in response to serum stimulation; however, the decrease did not reach statistical significance (**Figure 3E**).

As Sirt1 in VSMCs is reported to regulate the expression of cyclin D1,[16] we analyzed the cell cycle-related proteins and mRNA in VSMCs from the aortas of WT and Sirt7$^{-/-}$ mice. As shown in **Figure 3F**, the protein levels of cell cycle-positive regulators, such as cyclin-D1, -E, -A, CDK2 and CDK4, were significantly or tended to be lower in Sirt7$^{-/-}$ VSMCs compared with WT VSMCs following serum stimulation. These results suggest the involvement of Sirt7 in the cell responses to serum stimulation through the modulation of cell cycle-related molecules in VSMCs.

Sirt7 Deficiency Is Associated With Upregulation of the miRNA 290-295 Family and CDK2 Transcriptional Downregulation

CDK2 is generally considered to be critical for the progression of the cell cycle.[30] Consistent with the results of the primary VSMCs from Sirt7$^{-/-}$ mice, the CDK2 protein level was decreased in Sirt7 siRNA-treated HAoSMCs compared with control siRNA-treated HAoSMCs, whereas the CDK4 protein level was not different (**Figure 4A**). Furthermore, Sirt7 deficiency in VSMCs showed a significantly lower level of CDK2 mRNA (**Figure 4B**), and which suggests that the decrease of CDK2 expression in Sirt7-deficient VSMCs possibly was regulated at the transcriptional level or post-transcriptional level by miRNA.

In a recent study, miRNAs were reported to regulate cell cycle proliferation by directly suppressing the expression of cell cycle-related proteins.[31] CDK2 protein was found to be negatively regulated by a miR-371-373 cluster, and the expression of these miRNAs were negatively regulated by Cullin-4B (CUL4B),[32] which is reported to form a complex with Sirt7.[9,33,34] The miR-290-295 are a mice homolog of

the human miR-371-373 cluster.[35] As shown in **Figure 4C**, the miR-290-295 cluster, except for miR-293, share the same seed sequence and are predicted to target CDK2 by miRbase (http://www.mirbase.org/). Therefore, we examined the expression level of miRNA 290-295, excluding miR-293, in VSMCs isolated from WT and Sirt7$^{-/-}$ mice. We found that most of these miRNAs were increased in VSMCs of Sirt7$^{-/-}$ mice compared with WT mice (**Figure 4D**). These results suggest that Sirt7 negatively regulates these miRNAs through interaction with CUL4B, and that deletion of Sirt7 increases their expressions and inhibits CDK2 expression.

Smooth Muscle Cell-Specific Sirt7 Deficiency Reduces Neointimal Formation Following Vascular Injury

To elucidate the functional role of Sirt7 in VSMCs in vivo, we compared the neointimal formation between smooth muscle cell (SMC)-specific Sirt7 deficient mice and its control littermates. SMC-restricted depletion of Sirt7 was confirmed by Western blot analysis (**Figure 5A**,**5B**). As shown in **Figure 5C** and **5D**, the neointimal hyperplasia following vascular injury was also significantly decreased in SMC-specific Sirt7-deficient mice than in control mice. This finding confirmed that Sirt7 in VSMCs is involved in the development of neointimal lesions in the femoral artery wire injury model.

Discussion

Sirt7, the latest identified sirtuin, has emerged as a critical factor in various diseased conditions.[9,10] Previously, we demonstrated that Sirt7 contributed to proper wound healing through stabilization of TβRI, enabling efficient TGF-β signaling.[13] Recently, we have also shown that cardiomyocyte Sirt7 ameliorates stress-induced cardiac hypertrophy by interacting with and deacetylating GATA4.[36] However, little is known about the role of Sirt7 in vascular diseases. In the present study, we found that: (1) Sirt7 expression was increased in vascular tissue at the sites of injury; (2) systemic and VSMC-specific Sirt7-deficient mice showed attenuated neointimal formation in response to vascular injury; (3) Sirt7-deficient VSMCs showed lower levels of cell proliferation capacity, which was possibly due to miRNA-dependent reduced cell cycle-related protein expression. To our knowledge, this is the first study to describe the functional roles of Sirt7 in vascular disease in response to vascular insults.

Accumulating evidence suggests the involvement of sirtuins in various vascular diseases through the regulation of several cellular processes. Among the different sirtuins, Sirt1 protects against stress-induced vascular remodeling,[4] atherosclerosis[17] and abdominal aortic aneurysm[37] in mice. Sirt6 also has a vascular protective effect,[38] whereas Sirt3 depletion has no impact on atherogenesis.[39] These results highlight the distinctive roles of different sirtuins in the development of vascular diseases.

VSMCs are the main components of the vascular wall, and their proliferation is a key process in the progression of vascular remodeling. According to previous in vitro experiments, Sirt7 enhances cancer cell survival and tumor progression through the regulation of epigenesist,[40] ribosomal biogenesis[41] and certain cancer-regulatory transcriptional factors, such as C-myc,[42] HIF-1a and HIF-2a.[43] In this study, Sirt7 deficiency in VSMCs inhibited serum-induced cell proliferation (**Figure 3A**–**3D**), which was associated with direct regulation of cell cycle-related proteins, including CDK2 (**Figure 3F**). CDK2 plays a central role in the cell cycle through G1 phase transition to the S phase,[22] and is upregulated in neointimal hyperplasia.[20]

The miR-371-373 cluster is reported to downregulate CDK2 expression and is transcriptionally repressed by CUL4B.[32] CUL4B binds to the DDB1 (DNA damage-binding protein 1) adaptor protein that interacts with DCAF1 (DDB1-CUL4-associated factor 1) and acts as E3 ubiquitin ligase, contributing to various cellular functions including cell cycle progression.[44] Recent studies showed that Sirt7 regulates the activity of the CUL4B-DDB1-DCAF1 E3 ubiquitin ligase complex through the deacetylation of the DDB1 protein.[33,34] These results indicate that Sirt7 modulates VSMCs proliferation through the regulation of the miRNA-CDK2 pathway, which leads to vascular remodeling. Consistent with our results, Zheng et al reported that Sirt7 inhibited VSMCs proliferation and migration by Wnt/β-catenin-dependent mechanisms.[45] Sirt7 was also reported to mediate oxidation-dependent cell cycle arrest in human aortic smooth muscle cells.[46] These mechanisms might be involved in the vascular phenotype that was observed in the present study.

This study has several limitations. First, the time course of inflammatory response after vascular injury has not been evaluated. Because Sirt7 has been shown to regulate inflammatory response, future study will be required to clarify this point. Second, we could not evaluate the effect of Sirt7 deficiency in re-endothelialization after vascular injury and adhesion molecule expression. However, according to the results of VSMCs staining by α-SMA (**Figure 2A**), we believe that Sirt7 might play a more important role in regulating VSMCs proliferation than other factors in this vascular injury model.

In conclusion, we have demonstrated that Sirt7 deficiency in mice attenuates neointimal formation in response to vascular injury, accompanied by regulation of VSMCs functions. Sirt7 is a potentially suitable target in the design of new treatments for vascular diseases.

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Disclosures

The authors declare no conflicts of interest.

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