Chronic HDAC6 Activation Induces Atrial Fibrillation Through Atrial Electrical and Structural Remodeling in Transgenic Mice

Yohei Sawa,1,2 MD, Naoko Matsushita,2 MD, Sachiko Sato,3 BS, Nanae Ishida,1 BS, Maki Saito,4 PhD, Atsushi Sanbe,5 PhD, Yoshihiro Morino,2 MD, Eiichi Taira,1 MD, Mami Obara,1 BS and Masamichi Hirose,1 MD

Summary
Atrial fibrillation (AF) is a relatively common complication of hypertension. Chronic hypertension induces cardiac HDAC6 catalytic activity. However, whether HDAC6 activation contributes to hypertension-induced AF is still uncertain. We examined whether chronic cardiac HDAC6 activation-induced atrial remodeling, leading to AF induction.

The HDAC6 constitutively active transgenic (TG) (HDAC6 active TG) mouse overexpressing the active HDAC6 protein, specifically in cardiomyocytes, was created to examine the effects of chronic HDAC6 activation on atrial electrical and structural remodeling and AF induction in HDAC6 active TG and non-transgenic (NTG) mice. Left atrial burst pacing (S1S1 = 30 msec) for 15-30 sec significantly increased the frequency of sustained AF in HDAC6 active-TG mice compared with NTG mice. Left steady-state atrial pacing (S1S1 = 80 msec) decreased the atrial conduction velocity in isolated HDAC6 active TG compared with NTG mouse atria. The atrial size was similar between HDAC6 active TG and NTG mice. In contrast, atrial interstitial fibrosis increased in HDAC6 active TG compared with that of NTG mouse atria. While protein expression levels of both CX40 and CX43 were similar between HDAC6 active TG and NTG mouse atria, a heterogeneous distribution of CX40 and CX43 occurred in HDAC6 active-TG mouse atria but not in NTG mouse atria. Gene expression of interleukin 6 increased in HDAC6 active TG compared with NTG mouse atria.

Chronic cardiac HDAC6 activation induced atrial electrical and structural remodeling, and sustained AF. Hypertension-induced cardiac HDAC6 catalytic activity may play important roles in the development of AF.

Key words: Connexin, Interleukin 6, Hypertension

Atrial fibrillation (AF) is a very common sustained cardiac arrhythmia and a major clinical challenge because it increases morbidity and mortality due to complications such as stroke and heart failure.1-3 AF is a relatively common complication of hypertension. It is an important predisposing factor for AF.4 Therefore, identification of the critical mechanisms involved in hypertension-induced AF is important for the treatment of AF. Previous studies showed that hypertension causes chronic cardiac pressure overload, leading to atrial structural remodeling, such as atrial fibrosis, dilatation, and ischemia, that promote AF.5-7 Histone deacetylases (HDACs) are part of a large family of enzymes with crucial roles in numerous biological processes, primarily through their inhibitory effect on transcription.5-7 Recently, it was demonstrated that HDACs participate in the development of cardiac hypertrophy and fibrosis8-10 and HDAC inhibition improves chronic cardiac pressure overload-induced cardiac hypertrophy, which might be induced by hypertension.11 Moreover, HDAC inhibition attenuated cardiac hypertrophy and fibrosis in spontaneously hypertensive rats,12 suggesting that HDACs are important to develop hypertension-induced cardiac remodeling. HDAC6 is the main cytoplasmic deacetylase in mammalian cells. Cytoskeletal proteins such as α-tubulin and cortactin were directly deacetylated by HDAC6.9 Interestingly, a recent study demonstrated that cardiac HDAC6 catalytic activity was induced in response to chronic hypertension.10 Moreover, HDAC6 activation initiates deacetylation of α-tubulin, derailing α-tubulin proteostasis and disrupting the cardiomyocyte microtubule structure, inducing contractile function loss and AF progression.11 Therefore, HDAC6...
activation may participate in the development of hypertension-induced atrial remodelings, such as atrial fibrosis, dilatation, and ischemia, to promote AF. However, whether chronic HDAC6 activation can induce AF through atrial remodeling is still uncertain. This study’s objective was to examine the effects of HDAC6 activation on atrial remodeling, leading to AF.

**Methods**

**Ethics:** The study was conducted in strict accordance with the recommendations in the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health. The Animal Care Committee of Iwate Medical University approved this study. The Committee on the Ethics of Animal Experiments of Iwate Medical University approved the protocol (Permit Number: 28-040). All surgeries were performed under sodium pentobarbital and/or isoflurane anesthesia, and all efforts were made to minimize suffering.

**Experimental animals:** We created the experimental mice by a transgenesis technique and bred them with C57BL/6J female mice (10 weeks old, 25-30 g body weight), which were purchased from Japan SLC. The cDNAs of human HDAC6 were isolated using reverse transcription-PCR. We generated H216A, H611A missense mutations of human HDAC6 (HDAC6H216A, H611A), which are dominant-negative mutations, and all serine or threonine residues of human HDAC6 were replaced with aspartic acid in 43 amino acids of the N-terminal (HDAC6NT-allD). These HDAC6 cDNAs were used to generate transgenic mice, as described previously.13,14 Male mice with cardiac-specific overexpression of human wildtype HDAC6, HDAC6 H216A, H611A, and HDAC6 NT-all D, driven by the modified α-myosin heavy chain promoter, were described previously.13,14 The TG mice were identified by PCR analysis of genomic DNA isolated from tail tips using KAPA Express Extract (NIPPON Genetics, Tokyo, Japan). The isolated heart chambers in positions corresponding to ECG limb lead II.

**Optical mapping:** The optical mapping experiments were performed as previously described.15 The isolated heart was allowed to stabilize for approximately 20 minutes before being stained with the potentiometric fluorophore, RH237 (Promo Kine, Heidelberg, Germany: final concentration of 8 μM). Cardiac contractions were stopped by treating the heart with the electromechanical uncoupler blebbistatin (Toronto Research Chemicals, Toronto, ON, Canada; final concentration of 15 μM) to prevent motion artifacts. In all experiments, the mapping field was positioned at the atrium. The hearts were illuminated with two 530-nm LEDs (LEX2-G, Brain Vision Inc., Tokyo, Japan). Fluorescence was acquired for 2 sec through a 715-nm long-pass filter using a CMOS camera (MiCAMAO2-CMOS, Brain Vision Inc., Tokyo, Japan) with a sampling rate of 1 kHz and 9 × 4.5-mm fields of view (spatial resolution: 50 μm). The isolated hearts were paced from the pulmonary vein atria’s epicardial surface at a basic cycle length of 80 msec. In all experiments, automated algorithms were used to determine the depolarization time relative to a single fiducial point (i.e., the stimulus). The depolarization time was defined as the maximum positive derivative action in the action potential upstroke (dV/dt(max)). Depolarization contour maps were computed for the entire mapping field. The repolarization time was defined as the time when repolarization reached a level of 90%. The optical action potential duration (APD) was calculated from the average of optical APD from more than three consecutive beats. The mean APD was calculated from the average of local APD at more than 150 sites. The method of Bayly18 was modified for optically recorded action potential maps for accurate quantification of the direction and magnitude of conduction velocity (CV) at each recording site. The mean CV was calculated from the average of local conduction velocities at more than 100 sites.

**Histology:** Hearts were quickly excised after a midline sternal incision. After perfusion with 4°C physiological saline, atrial and ventricular tissues were fixed with a 10% solution of formalin in phosphate-buffered saline at 24°C.
for more than 24 hours. They were embedded in paraffin and sliced into 3-μm-thick long-axis sections. Each section was stained with Masson’s Trichrome staining or immunofluorescence for histopathological analysis. Masson’s Trichrome staining was conducted to assess the degree of interstitial fibrosis. To assess the degree of fibrosis, we took digital microscopic images from the sections using light microscopy with BZ-X Analyzer software. Measurements were performed of each section in each preparation. The fibrosis fraction was obtained by calculating the ratio of the total connective area to total myocardial area from four images in each preparation.

Immunohistochemical analyses were performed as described previously.21 Immunofluorescence was conducted to assess the distribution of acetyl alpha-tubulin (α-tubulin), connexin 40 (Cx40), connexin 43 (Cx43), and N-cadherin. Quantitative analysis of acetyl α-tubulin was conducted by calculating the ratio of the acetyl α-tubulin stained area to total myocardial area in each preparation. The spatial distribution of Cx40 and Cx43 was conducted by calculating the ratio of the Cx40 and Cx43 area with N-cadherin to the total Cx40 and Cx43 area in each preparation, respectively. Primary antibodies used were anti-acetylated α-tubulin antibody (Abcam Cambridge, UK), anti-connexin 40 antibody (Invitrogen, California, USA), anti-connexin 43 antibody (Invitrogen California, USA), and anti-N-cadherin antibody (Abcam Cambridge, UK). Secondary antibodies were Alexa546-conjugated anti-rabbit antibody (Santa Cruz Biotechnology Inc.) and Alexa488-conjugated anti-mouse antibody (Cell Signaling Technology, Danvers, USA).

Western blotting: Sample preparation for Western blotting, gel preparation, and electrophoresis was carried out as described previously.12,22,23 Western blot analyses were performed using anti-GAPDH antibody (Chemicon International, Temecula, CA, USA), anti-acetylated α-tubulin antibody (Abcam Cambridge, UK), anti-connexin 40 antibody (Invitrogen, California, USA), and anti-connexin 43 antibody (Invitrogen, California, USA). Secondary antibodies were Alexa546-conjugated anti-rabbit antibody (Santa Cruz Biotechnology Inc.) and Alexa488-conjugated anti-mouse antibody (Cell Signaling Technology, Danvers, USA). The signals from immunoreactive bands were visualized by an Amersham ECL system (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, UK) and quantified using the image analyzing software “Image J (ver. 1.51s).”

Quantification of mRNA by real-time PCR: Total RNA was prepared from the atrial myocardium of anesthetized NTG and HDAC6 active-TG mouse atria (n = 6 for each) with ReliaPrep™ RNA Tissue Miniprep System (Promega, Madison, WI, USA) according to the manufacturer’s instructions. Five hundred nanograms of total RNA were used as a template for reverse transcription with the SuperScript™ III First-Strand synthesis system (Invitrogen, Carlsbad, CA, USA). Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis was performed with an ABI Step One Real-Time PCR System using Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) to detect Nav1.5 (SCN5A), connecetive tissue growth factor (CTGF), collagen type I (collagen 1), transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), monocyte chemotactic protein 1 (MCP-1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The expression of each gene was normalized to that of GAPDH mRNA because the expression of GAPDH mRNA was constant between groups.

Data analysis: All data are shown as the mean ± SE. An analysis of variance with Bonferroni’s test was used to analyze multiple comparisons of data. Fisher’s exact test was used to compare the incidence of sustained AF between different conditions. P < 0.05 was considered significant.

Results

Acetylated α-tubulin expression levels in HDAC6 active-TG atria: The protein expression levels of acetylated α-tubulin were significantly decreased in HDAC6 active TG compared with NTG mouse atria (Figure 1A and B). In contrast, the expression levels of α-tubulin were similar between HDAC6 active TG and NTG mouse atria (Figure 1A and C). Moreover, representative images stained with acetylated α-tubulin antibody (Red) demonstrated that the area of acetylated α-tubulin was decreased in HDAC6 active TG compared with NTG mouse atria (Figure 1D). Quantitative analysis demonstrated that the area of acetylated α-tubulin was significantly decreased in HDAC6 active TG compared with NTG mouse atria (Figure 1E). These results indicate that HDAC6 activity was increased in atrial myocytes of HDAC6 active TG compared with NTG mice.

HDAC6 activation increased the frequency of sustained atrial fibrillation (AF): To assess the effects of atrial chronic HDAC6 activation on AF induction, we performed electrical burst pacing from the esophagus. Before burst pacing, all electrocardiographical parameters were similar between HDAC6 active TG and NTG mice (Table). Electrical burst pacing at a basic cycle length of 30 msec for 15-30 sec induced AF in a HDAC6 active-TG mouse (Figure 2A). The frequency of induction of sustained AF persisting for longer than 5 minutes was significantly increased in HDAC6 active TG (14 of total 24 mice) compared with NTG (2 of total 19 mice) mice (Figure 2B).

HDAC6 activation decreased conduction velocity but not action potential duration and SCN5A gene expression in HDAC6 active-TG mouse atria: Figure 3A shows representative examples of activation isochrone maps recorded from the epicardial surface of the right atrium during steady-state pacing at cycle lengths of 80 msec in two different heart groups. Relative crowding of isochrone lines in a HDAC6 active-TG right atrium (Figure 3A, lower) indicates conduction slowing when compared with that in an NTG right atrium (Figure 3A, upper). When considering all experiments (Figure 3B), the mean atrial CV was significantly prolonged in HDAC6 active TG compared with that in NTG right atria. Gene expression of SCN5A was similar between HDAC6 active TG and NTG mouse atria (Figure 3C). Figure 3D shows representative examples of the optical action potential recorded from the right atrium’s epicardial surface during...
steady-state pacing at cycle lengths of 100 msec in NTG and HDAC6 active-TG mice. The mean optical APD was similar between HDAC6 active TG and NTG mouse right atria (Figure 3E).

**Left atrial size and heart weight/body weight (HW/BW) ratio were similar between HDAC6 active TG and NTG mice:** To assess the effects of HDAC6 activation on left atrial enlargement, we measured the left atrium. The left atria’s mean size was similar between HDAC6 active TG and NTG mice (Figure 4A and B). Moreover, the HW/BW ratio was also similar between HDAC6 active TG and NTG mice (Figure 4C).

**HDAC6 activation increased left atrial fibrosis but not atrial fibrotic gene expression and ventricular fibrosis:** To assess the influence of HDAC6 activation on left atrial fibrosis, we examined the degree of interstitial fibrosis by
Masson’s Trichrome staining in HDAC6 active-TG mice. Extensive interstitial fibrosis in the left atrium was observed in an HDAC6 active-TG mouse compared with an NTG mouse (Figure 4D). The degree of myocardial fibrosis in the left atrium was significantly greater in HDAC6 active TG than NTG mice (Figure 4E). Next, we accessed the gene expression of CTGF, collagen 1, and TGF-β to investigate whether alterations accompanied these morphological observations in gene expression relevant to fibrotic changes. The expression of all three profibrotic genes was similar between HDAC6 active TG and NTG mouse atria (Figure 4F-H). There were no differences in the degree of ventricular fibrosis between NTG and HDAC6 active-TG mice (Figure 4I).

Connexin 40 (CX40) and 43 (CX43) expressions and their spatial distribution pattern: We examined the CX40 and CX43 protein expression levels and spatial distri-

Table. Electrophysiological Parameters in Non-Transgenic (NTG) and HDAC6-Constitutive Active-Transgenic (HDAC6 active TG) Mice

| Parameters | NTG      | HDAC6 active TG |
|------------|----------|-----------------|
| P (msec)   | 12 ± 1   | 12 ± 1          |
| RR (msec)  | 126 ± 4  | 151 ± 9         |
| PR (msec)  | 44 ± 2   | 42 ± 2          |
| QRS (msec) | 14 ± 2   | 13 ± 2          |
| QT (msec)  | 47 ± 5   | 45 ± 5          |

Data are the mean ± SE obtained from 8 mice for each group.

Figure 2. Atrial fibrillation (AF) induction and frequency of sustained AF incidence in HDAC6 active TG and NTG mice. A: A representative example of burst pacing induced AF in a HDAC6 active-TG mouse. B: Frequency of sustained AF was observed. Atrial burst pacing significantly increased the frequency of sustained AF in HDAC6 active-TG mice (14 of total 24 mice) than NTG mice (2 of total 19 mice).
Figure 3. Atrial impulse conduction velocity, action potential duration (APD), and atrial SCN5A gene expression in HDAC6 active TG and NTG mice. A: Representative examples of activation maps during steady-state pacing at a cycle length of 80 msec on the right atrial free wall in HDAC6 active TG and NTG mice. B: Mean conduction velocity in HDAC6 active TG and NTG mouse right atria. C: Mean right atrial APD in HDAC6 active TG and NTG mice. D: Quantitative analyses of SCN5A gene expression by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in HDAC6 active TG and NTG mouse atria. Data are the mean ± SE obtained from 6 to 8 mice for each group.
Figure 4. Left atrial size, HW/BW ratio, and atrial and ventricular fibrosis in HDAC6 active TG and NTG mice. A: Representative images in HDAC6 active TG and NTG mouse hearts. The arrows show the long-axis atrial diameter. B, C: Mean left atrial diameters and mean heart weight/body weight (HW/BW) ratio in HDAC6 active TG and NTG mice. Data are the mean ± SE obtained from 4 to 6 mice for each group. D: Representative examples of staining with Masson’s Trichrome in HDAC6 active TG and NTG mouse left atria. Scale bar = 50 μm. E: Mean % fibrosis in HDAC6 active TG and a NTG mouse left atria. Data are the mean ± SE obtained from 4 to 6 mouse for each group. F-H: Quantitative analyses of connective tissue growth factor (CTGF) (F), collagen 1 (G), and, transforming growth factor-β (TGF-β) (H) gene expression by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) in HDAC6 active TG and NTG mouse atria. Data for CTGF, collagen 1, and TGF-β were normalized to those of GAPDH. Data are the mean ± SE obtained from 6 mice for each group. I: Representative examples of staining with Masson’s Trichrome in HDAC6 active TG and NTG mouse ventricles. Scale bar = 50 μm.
Connexin 40 (CX40) and connexin 43 (CX43) protein expressions, and their spatial distribution in HDAC6 active TG and NTG mouse atria. A, D: Relative expression levels of CX40 (A) and CX43 (D) protein in HDAC6 active TG and NTG mouse atria. B, E: Representative images of left atrial immunofluorescence staining for N-cadherin (orange) + CX40 (green) (B) and N-cadherin (orange) + CX43 (green) (E) in HDAC6 active TG and NTG mouse left atria. Scale bar = 10 μm. C, F: Ratio of CX 40 area (C) and Cx43 area (F) located next to intercalated disc (N-cadherin stained area) to total CX40 and Cx43 area, respectively, in HDAC6 active TG and NTG mouse left atria. Arrows indicate laterality of CX40 and CX43 expression, respectively, in HDAC6 active-TG mouse left atria (C, F). Data are the mean ± SE obtained from 6 mice for each group.

Figure 5. Connexin 40 (CX40) and connexin 43 (CX43) protein expressions, and their spatial distribution in HDAC6 active TG and NTG mouse atria. A, D: Relative expression levels of CX40 (A) and CX43 (D) protein in HDAC6 active TG and NTG mouse atria. B, E: Representative images of left atrial immunofluorescence staining for N-cadherin (orange) + CX40 (green) (B) and N-cadherin (orange) + CX43 (green) (E) in HDAC6 active TG and NTG mouse left atria. Scale bar = 10 μm. C, F: Ratio of CX 40 area (C) and Cx43 area (F) located next to intercalated disc (N-cadherin stained area) to total CX40 and Cx43 area, respectively, in HDAC6 active TG and NTG mouse left atria. Arrows indicate laterality of CX40 and CX43 expression, respectively, in HDAC6 active-TG mouse left atria (C, F). Data are the mean ± SE obtained from 6 mice for each group.

HDAC6 activation increased atrial gene expression of IL-6: We examined the effects of HDAC6 activation on atrial gene expressions of IL-1β, IL-6, TNF-α, and MCP-1 in HDAC6 active-TG mouse atria. IL-6 gene expression increased significantly in HDAC6 active TG compared with NTG mouse atria (Figure 6). In contrast, regarding gene expressions of IL-1β, TNF-α, and MCP-1, there were no differences between HDAC6 active TG and NTG mouse atria (Figure 6).

Discussion

We revealed that chronic HDAC6 activation participated in the development of sustained AF in transgenic mice (Figure 2). Moreover, it slowed atrial impulse propagation and increased interstitial fibrosis and changes in the spatial distribution of CX40 and CX43 in HDAC6 active-
TG mouse atria (Figures 3-5). HDAC inhibition attenuated cardiac hypertrophy and fibrosis in spontaneously hypertensive rats. Cardiac HDAC6 catalytic activity was induced in response to chronic hypertension. Wang, et al. demonstrated that chronic cardiac pressure overload-induced atrial remodeling, such as the distribution of CX 43 from the intercalated disk to lateral membranes, was similar between HDAC6 active TG and NTG mouse atria. These results suggest that HDAC6 plays an important role in the development of hypertension-related AF.

It is well-known that electrophysiological remodeling such as shortening of the APD and slowing of impulse propagation can promote re-entry, leading to AF. In 1914, Mines first defined re-entry as a persisting electrical impulse that reactivates an area of previously activated myocardial tissue that is no longer refractory, which results in a circle of activation. The length of such a cycle depends on its wavelength, defined by the refractory period’s mathematical product (i.e., APD) and the CV. Chronic HDAC6 activation induced atrial impulse slowing with no changes in atrial APD. Therefore, although our results did not show the occurrence of re-entry, the shorter wavelength calculated from APD and CV might induce re-entry, leading to AF in HDAC6 active-TG mouse atria.

It is generally known that CX43 plays key roles in cardiac impulse conduction, and impulse conduction slowing is associated with decreased protein expression levels of CX40 and CX43 in atria. However, our results demonstrated that CX40 and CX43 protein expressions were similar between HDAC6 active TG and NTG mouse atria. Several studies demonstrated that changes in the distribution pattern of CX40 and CX43, such as CX40 and CX43 lateralization, play important roles in impulse conduction slowing, even if the expression levels of both proteins are not decreased. Our previous study also showed that decreases in relative CX43 expression on the intercalated disk compared with the lateral membranes were associated with atrial conduction slowing. Moreover, HDAC inhibition by trichostatin A, an HDAC inhibitor, normalized the expression and size distribution of CX 40 gap junctions and reduced atrial arrhythmias in mice overexpressing homeodomain-only protein, which recruits HDAC activity to induce cardiac hypertrophy. Therefore, chronic HDAC6 activation might cause atrial impulse slowing through changes in the spatial distribution of CX 40 and CX43. Nevertheless, the mechanism for chronic HDAC6 activation-induced redistribution of CX40 and CX 43 from the intercalated disk to lateral membranes is still uncertain. HDAC6 activation initiates deacetylation of α-tubulin, leading to derailment of α-tubulin proteostasis and disruption of the cardiomyocyte microtubule structure. Lauf, et al., demonstrated that microtubules have an important role in the trafficking of CX43 to the gap junction. We demonstrated that the protein expression levels of acetylated α-tubulin decreased significantly in HDAC6 active TG compared with NTG mouse atria (Figure 1A and B). Moreover, acetylated α-tubulin decreased significantly in HDAC6 active TG compared with NTG mouse atria (Figure 1E), which may disrupt the cardiomyocyte microtubule structure and become an obstacle to trafficking CX43 to the gap junction in this study. Furthermore, although the mechanism for chronic HDAC6 activation-induced redistribution of CX40 from the intercalated disk to lateral membranes is still uncertain, the same mechanism may contribute to the redistribution of CX40 in HDAC6 active-TG mouse atria. We demonstrated that chronic HDAC6 activation increased atrial IL-6 gene expression (Figure 6). Various inflammatory markers such as CRP, TNF-α, and IL-6 are known to be associated with AF. Tubastatin, an HDAC6 inhibitor, inhibited IL-6 expression in LPS-stimulated human THP-1 macrophages. Wang, et al. demonstrated that chronic cardiac pressure overload increased atrial gene expression of IL-6. Lazzaroni, et al. showed that increased IL-6 expression was associated with atrial electrical remodeling by down-regulating cardiac connexins. In contrast, IL-6 stimulation increased CX43 expression in rat H9c2 cells. Therefore, although chronic HDAC6 activation-induced increases in IL-6 expression did not change atrial connexin expression levels, it may contribute to CX40 and CX43 remodeling in HDAC6 active-TG mouse atria. Our previous study demonstrated that increased IL-1β gene expression was associated with the development of cardiac pressure overload-induced AF. In contrast, the present study showed that AF was induced and IL-1β gene expression levels did not increase in HDAC6 active TG compared
with NTG mouse atria. In a mouse model of systemic lupus erythematosus, the HDAC6-specific inhibitor CKD-506 did not affect serum IL-1β, suggesting that HDAC6 activation induces AF independent of IL-1β.

Gap junction remodeling can cause impulse conduction slowing, whereas the decreased tissue excitability also causes its conduction slowing. The cardiac Na+ channel (Nav1.5, SCN5A) plays key roles in cardiac conduction through tissue excitability, and impulse conduction slowing is associated with decreased protein expression levels of Nav1.5 in hearts. Xu, et al. demonstrated that trichostatin A decreased the peak I\textsubscript{Na} density in mouse ventricular myocytes. It also reduced Na\textsubscript{v}1.5 protein levels along with the increased Nav1.5 acetylation but did not change SCN5A mRNA levels in mouse ventricular myocytes. Therefore, HDAC6 activation decreases Nav1.5 acetylation, increasing Na\textsubscript{v}1.5 protein in cardiac tissue, which may potentiate cardiac tissue excitability even though SCN5A gene expression did not increase. However, Jang S and Jeong HS\textsuperscript{10} demonstrated that trichostatin A increased SCN5A gene expression in neurogenic induced-human adipose tissue-derived mesenchymal stem cells. Moreover, chronic HDAC6 activation tended to decrease SCN5A gene expression in HDAC6 active-TG mouse atria. Therefore, the decreased SCN5A gene expression level may participate in atrial conduction slowing through decreases in tissue excitability.

Structural remodeling, such as atrial interstitial fibrosis and dilatation, also induce re-entry and AF.\textsuperscript{10,14} HDAC6 activation did not dilate the left atrium but increased atrial interstitial fibrosis with decreased protein expression of acetylated-α tubulin (Figures 1, 4). However, it did not increase fibrogenic gene expressions such as CTGF, collagen I, or TGF-β in HDAC6 active-TG mouse atria. Previous studies showed that HDAC6 participated in the development of cardiac hypertrophy and fibrosis.\textsuperscript{7} Mice overexpressing homeodomain-only protein showed induced atrial fibrosis but decreased TGF-β expression. After treatment with trichostatin A, the mice showed reduced atrial fibrosis and arrhythmias.\textsuperscript{80} Moreover, cardiac mRNA and protein expressions of HDAC6 increased and acetylated-α tubulin expression decreased in an isoproterenol-treated rat cardiac fibrosis model.\textsuperscript{42} Therefore, HDAC6 activation may induce extensive atrial interstitial fibrosis, leading to AF induction through decreases in acetylated-α tubulin even if fibrogenic gene expressions do not increase.

Conclusion

We first found that chronic cardiac-specific HDAC6 activation increased the frequency of sustained AF induction. It induced atrial conduction slowing and increased atrial interstitial fibrosis and the heterogeneous distribution of CX40 and CX43 in atria. Gene expression of IL-6 increased in HDAC6 active TG compared with NTG mouse atria. Therefore, chronic cardiac-specific HDAC6 activation induces atrial electrical and structural remodeling, leading to AF.

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Disclosure

Conflicts of interest: None.

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