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RESEARCH PAPER

MG53/CAV1 regulates transforming growth factor-β1 signaling-induced atrial fibrosis in atrial fibrillation

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ABSTRACT

Atrial fibrosis plays a significant role in the development of atrial fibrillation (AF). Previously, we showed that mitsugumin 53 (MG53) regulates TGF-β1 signaling pathway-induced atrial fibrosis. Recent studies have shown that caveolin-1 (CAV1) is an important anti-fibrosis signaling mediator that inhibits the TGF-β1 signaling pathway. Here, we further study the mechanism underlying the related action of MG53 and CAV1. We demonstrate that CAV1 expression was decreased while MG53 expression was increased in atrial tissue from AF patients. In cultured atrial fibroblasts, MG53 depletion by siRNA caused CAV1 upregulation and TGF-β1/SMAD2 signaling pathway downregulation, while MG53 overexpression via adenovirus had the opposite effect. CAV1 inactivated the TGF-β1/SMAD2 signaling pathway. In addition, using an Ang II–induced fibrosis model, we show that MG53 regulates TGF-β1 signaling via CAV1. Therefore, CAV1 is critical for the MG53 regulation of TGF-β1 signaling pathway-induced atrial fibrosis in AF. These findings reveal the related underlying mechanism of action of MG53 and CAV1 and provide a potential therapeutic target for fibrosis and AF.

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mitsugumin 53; caveolin-1; TGF-β1 signaling pathway; atrial fibrosis; atrial fibrillation

Introduction

Atrial fibrillation (AF) is a common clinical arrhythmia that increases the incidences of stroke and heart failure and, thus, is a serious threat to human health. Atrial fibrosis and atrial enlargement are important characteristic changes in the histopathology of AF, and simultaneously, AF can aggravate fibrosis [1,2]. Noncardiomyocytes in the heart are composed mainly of fibroblasts and play a vital role in cardiac fibrosis [3]. In atrial fibrosis, the transformation of fibroblasts into myofibroblasts produces a large amount of extracellular matrix (ECM) and deposits outside the cell [4,5]. Studies have shown that atrial fibroblasts are more sensitive to fibrosis than ventricular fibroblasts [6,7]. Therefore, inhibiting the fibrotic phenotype of atrial fibroblasts is critical for the treatment of AF.

Mitsugumin 53 (MG53), also named tripartite motif protein 72 (TRIM72), is expressed predominantly in the heart and muscles and has E3 ubiquitin ligase properties [8]. MG53 is a double-edged sword and participates in various physiological and pathological processes. Mice overexpressing MG53 show diabetic cardiomyopathy and myocardial fibrosis symptoms because MG53 acts as an E3 ligase to mediate insulin receptor and insulin receptor substrate 1 degradation, resulting in insulin resistance [9]. However, some studies have shown that MG53 has a protective effect against acute cardiac injury [10]. In addition, MG53 functions to promote membrane repair and wound healing [11]. Our previous studies have shown that MG53 is expressed in human tissues and promotes atrial fibrosis by activating the TGF-β1 signal pathway, thereby increasing susceptibility to AF [12].

Transformation growth factor-β1 (TGF-β1) plays a key role as the strongest extracellular matrix (ECM) deposition promoter driving fibrosis and increasing susceptibility to AF [13–15]. The SMAD protein-dependent pathway is a classical signal transduction pathway through which TGF-β1 plays an important role in the heart [16]. In our early observations of congenital heart disease (CHD) with sinus rhythm (SR), rheumatic heart disease (RHD) with SR, and RHD with AF in the right atrial

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appendage (RRA), AF patients with the most severe atrial fibrosis had high TGF-β1 protein expression [17]. In cultured fibroblasts stimulated by angiotensin II (Ang II), TGF-β1/SMAD2 signaling pathway activation results in the excessive deposition of ECM, such as α-smooth muscle actin (α-SMA, also named ACTA2) and collagen I [18,19].

Caveolins (CAVs) is the main structure that constitutes the caveolae [20]. Currently, there are three subtypes, i.e., CAV1, CAV2 and CAV3 [21]. Among these subtypes, CAV1, which is the main caveolin protein, plays an important role in maintaining the formation, structure and function of the caveolae [22]. Recent studies have found that CAV1 is closely related to myocardial fibrosis. CAV1 knockout mice showed an increased ventricular lumen, a thickened wall, decreased systolic function, and associated interstitial fibrosis [23]. In a ventricular cryoinjury model, compared to wild type-mice conditions, the CAV1 knockout enhanced the TGF-β1 signaling pathway, and more extensive collagen deposition was found in the myocardium [24]. In addition, liver and lung tissue fibrosis showed the same results as follows: supplementation with CAV1 scaffolding protein (CSD) peptides inhibited the TGF-β1 signaling pathway and extracellular matrix deposition [25,26]. However, few studies have investigated atrial fibrosis, and the mechanism of action remains unclear.

Material and methods

Patients and specimens

Fifty-five patients were recruited for this study, and all patients consecutively underwent corrective heart surgery between April 2018 and April 2019 at the First Affiliated Hospital of Chongqing Medical University. The patients were divided into the following three groups: CHD patients with SR (CHD +SR, n = 16) (controls), RHD patients with SR (RHD +SR, n = 18), and RHD patients with AF (RHD+AF, n = 21). All patient's heart function was classified as I–III according to the NYHA classification, and patients with hypertension, coronary heart disease, diabetes mellitus, cardiomyopathy, hyperthyroidism, chronic pulmonary heart disease, or malignant tumors and those aged >70 years were excluded. The patients did not take angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for at least six months before the surgery. RAA tissue (>100 mg) was collected from the cannulation site before initiating extracorporeal circulation.

The Ethics Committee of the First Affiliated Hospital of Chongqing Medical University approved the study protocol, and all patients or family members provided written consent prior to enrollment. The study complied strictly with the principles of the Declaration of Helsinki.

Histological analysis

Atrial fibrosis was assessed by staining sections with Masson's trichrome. Images were captured under an Olympus BX51 microscope (Tokyo, Japan) at × 200 magnification and analyzed by an ImagePro Plus 6.0 image analysis system. The collage volume fraction (CVF) was quantified by measuring the ratio of the blue staining area to the total atrium area.

Immunohistochemistry

The sections (4 μm) were deparaffinized and boiled in a microwave at full power for the antigen retrieval. Then, the sections were blocked at room temperature for 30 min with 1% goat serum (Zsbio Commerce Store, Beijing, China). Next, the sections were incubated separately at 4°C overnight with primary antibodies. Subsequently, the sections were incubated with horseradish peroxidase-conjugated goat and anti-rabbit/mouse secondary antibodies (Zsbio Commerce Store, Beijing, China). The image acquisition was performed after chromogenic reaction with DAB and counterstaining with hematoxylin, and the images were analyzed using the ImagePro Plus 6.0 image analysis system. The levels of MG53 and CAV1 were determined by the integrated option density (IOD). The primary antibodies were as follows: MG53 antibody (1:100; Boiss, Beijing, China) and CAV1 antibody (1:200; Cell Signaling Technology, MA, USA).

Rat atrial fibroblast culture

Atrial fibroblasts were isolated from 2-week-old Sprague–Dawley rats as previously described. The

rats were anesthetized with pentobarbital, and the hearts were quickly excised and washed with pre-cooled PBS. The atrium was separated and cut into 1–2 cm segments in DMEM (Nutrient Mixture F-12, Gibco, CA, USA) supplemented with 10% fetal bovine serum (Wisent, MTL, CA) and 1% penicillin and streptomycin. The tissue blocks were spread evenly in 6-well plates and incubated with 5% CO_2 at 37°C. After 20 min, mixed medium was added to the 6-well plates, and the culture continued. The second and third passage atrial fibroblasts were used for the experiment. All animal experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Animal Ethics Committee of Chongqing Medical University.

Cellular model of atrial fibrosis

Atrial fibroblasts were treated with Ang II (MedChemExpress, NJ, USA) to stimulate atrial fibrogenesis. The atrial fibroblasts were seeded in six-well plates and treated with Ang II (8 umol) for 24 h after confluence reached 70–80%.

RNA interference

To further explore the effects of MG53 and CAV1 in atrial fibroblasts, specific small interfering RNAs (siRNAs) targeting rat MG53 (Hanbio, Shanghai, China) or CAV1 (JTS Scientific, Beijing, China) were constructed. Atrial fibroblasts were seeded in six-well plates, and at 30–50% confluence, they were transfected with MG53-siRNA or NC-siRNA oligomers with Lipofectamine™ 2000 reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The CAV1-siRNA transfection was the same as previously described. The sequences used in the transient transfection process were as follows:

Trim72 – si – 1 RNA, 5′-GCAGCUGUUCGAUGCACCA-3′

Trim72 – si – 2 RNA, 5′- GACAGAAUUCCUCAUGAAA–3′

Trim72 – si – 3 RNA, 5′-GCUGCAGAAGAUUCUGUCA–3′

The following negative control (NC)-siRNA target sequences were provided by Hanbio:

CAV1-si'-1 RNA, 5′-CGAGGUGAAUGAGAAGCAATT-3′

CAV1-si'-2 RNA, 5′-GACGUGGUCAAGAUUGACUTT-3′

CAV1-si'-3 RNA, 5′-CUGUGAUCCACUCUUUGAATT-3′

The negative control (NC)-si'RNA target sequence was provided by JTS Scientific.

Adenovirus vector overexpression

Recombinant adenovirus vectors for MG53 (Ad-MG53) and green fluorescent protein (Ad-GFP) were purchased from Genechem (Shanghai, China). Atrial fibroblasts were seeded in six-well plates and transfected with Ad-MG53 or Ad-GFP at 30-50% confluence. After 24 hours, the cell culture medium was changed.

RNA extraction and real time quantitative PCR

Total RNA was extracted from frozen tissue specimens or cultured atrial fibroblasts using RNAiso Plus reagent (Takara, Japan) according to the manufacturer's instructions. The total RNA was reverse transcribed into cDNA using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara). Real time quantitative PCR (RTQ-PCR) was performed using a SYBR Premix ExTaq II kit (Takara), and GAPDH was used as an internal control. The relative mRNA expression levels were analyzed using the $2^{-\Delta\Delta}$Ct method after normalizing the expression of each gene to that of GAPDH. The primer pairs used are shown in Table 1.

Western blot analysis

The total protein was extracted from RRAs and atrial fibroblasts in different groups using RIPA lysis buffer supplemented with proteinase and phosphatase inhibitors (MedChemExpress, NJ, USA). Equal amounts of protein were loaded and separated by SDS-PAGE and transferred to PVDF membranes (Bio-Rad, Hercules, CA,

Table 1. Primer sets for PCR amplification.

Gene	Oligonucleotide primer sequences (5′–3′)	
Human MG53	Forward:	CCCGTGTACCCCTTCTTCGAC
	Reverse:	CACCCTTCTCCCGTCAGACC
Human CAV1	Forward:	TCAGCAACTCGCTTTAGGTCA
	Reverse:	AACCATACCCACTACGGAT
Human TGF-β1	Forward:	TACCTGAACCCGTGTTGCTCT
	Reverse:	CTGCCGCACAACTCCGGTGA
Human ACTA2	Forward:	CCCTGAAGTACCCGATAGAACA
	Reverse:	TTCAGGGGCAACACGAAGC
Human COL1A1	Forward:	ACGACAAAGCAGAAACATCGG
	Reverse:	TCTTTCAGCAACACAGTTACACA
Human COL1A2	Forward:	AACAGCAGGTTCACTTACACT
	Reverse:	GTCAGCACCACCGATGTCC
Human GAPDH	Forward:	CGCTTCGCTCTCTGCTCCTCCTGTT
	Reverse:	TCCGTTGACTCCGACCTTCACCTTCC
Rat MG53	Forward:	CGTCCTGACTTTCTATGATGC
	Reverse:	AATTCTTGCCCTTGTCGTG
Rat CAV1	Forward:	CGACCCAAGCATCTCAACGA
	Reverse:	CCTTCCAGATGCCGTCGAA
Rat TGF-β1	Forward:	ATTCAAGTCAACTGTGGAGCAAC
	Reverse:	CGAAAGCCCTGTATTCCGTCT
Rat ACTA2	Forward:	TGACTCACAACGTGCCTATC
	Reverse:	CGCTCAGCAGTAGTCACGAA
Rat SMAD2	Forward:	CATCAGTTAAAGCACCTTGTGGA
	Reverse:	CACTGTCTTATGCCCAACGAG
Rat COL1A1	Forward:	TGGTACATCAGCCCAAACCC
	Reverse:	GAACCTTCGCTTCCATACTCG
Rat COL1A2	Forward:	CAGCCTTCACTCAGACCCAA
	Reverse:	TCAATCCAGTAGTAATCGCTCT
Rat GAPDH	Forward:	ACGGCAAGTTCAACGGCACAGTCA
	Reverse:	CCACGACATACTCAGCACCAGCATCA

USA). Subsequently, the membranes were blocked in 5% skim milk for 90 min. Then, each membrane was incubated overnight with the following antibodies: CAV1 (1:1000; Cell Signaling Technology, MA, USA); collagen I (1:1000; Wanleibio, China); TGF-β1 (1:1000; Abcam, UK); P-SMAD2 (1:1000; ABclonal, USA); MG53 (1:1000; Boiss, Beijing, China), α-smooth muscle actin (α-SMA) (1:1000; Wanleibio, China), and GAPDH (1:5000; Cell Signaling Technologies, MA, USA). Finally, enhanced chemiluminescence (Millipore, MA, USA) was used to develop each membrane. The image collection and quantitative analysis were performed using ImageLab 6.0 software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

All data are presented as the mean±SD. The statistical analysis and group comparisons were performed using Student's t-test or one-way analysis of variance. A two-tailed p-value≤0.05 was considered statistically significant. The data were analyzed using GraphPad Prism 6.0 or SPSS 20.0.

Results

Clinical findings

We expanded the data of the clinical specimens. The clinical characteristics of the expanded study participants are shown in Table 2. The echocardiography showed a relative increase in the mean left atrial diameter in the RHD+AF and RHD+SR groups compared to that in the CHD+SR group, which is consistent with the results of previous studies [12]. The left atrial diameter in the RHD +AF group was significantly increased compared with that in the CHD+SR group. There were no differences among the groups in sex, age, left ventricular ejection fraction or right atrial dimension.

Increased MG53 expression and decreased CAV1 expression with increased collagen in the human atrium

The blue-stained collagen fibers, which were mainly distributed in the extramyocardial stroma, were gradually increased in the CHD+SR, RHD +SR and RHD+AF groups. The CVF in the AF group was significantly increased compared with that in the SR group (Figure 1d). The CVF in the RHD+ SR group was higher than that in the CHD +SR group (Figure 1d). Immunohistochemistry was used to assess the amount and location of MG53 and CAV1 expression in atrial tissue (Figure 1(b,c)). MG53, which was mainly distributed in the cytoplasm, was positively correlated with fibrosis (Figure 1(b,e). CAV1 was mainly located in the cell membrane, and its expression level was decreased in the RHD+SR and RHD+AF groups compared with that in the CHD+SR group (figure 1f). The CAV1 level was decreased as fibrosis and MG53 expression increased. To further investigate the relationship between MG53 and CAV1, RTQ-PCR (Figure 1(g,h)) and western blotting (Figure 1 I) were used to detect the atrial

Table 2. Clinical data of the patients.

	CHD + SR	RHD + SR	RHD + AF
Number of cases	16	18	21
Sex (M/F)	7/9	4/14	10/11
Age (years ± SD)	42.1 ± 15.5	50.2 ± 9.6	49.2 ± 10.0
LVEF (% ±SD)	61.4 ± 6.6	59.9 ± 7.1	62.4 ± 6.0
LVEDD (mm±SD)	44.1 ± 7.5	48.8 ± 8.1	48.8 ± 7.9
LAD (mm±SD)	31.4 ± 8.1	39.8 ± 8.2*	47.9 ± 11.2**,#
RAD (mm±SD)	40.4 ± 9.3	35.6 ± 4.1	39.3 ± 7.6

The values are presented as the mean±SD or the number of patients. M = males; F = females; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end-diastolic dimension; LAD = left atrial dimension; RAD = right atrial dimension. The symbol * indicates comparisons with the CHD+SR group, *$p < 0.05$, **$p < 0.01$; and the symbol # indicates comparisons with the RHD+SR group, #$p < 0.05$.

tissue expression levels. MG53 expression was significantly upregulated in the RHD+AF group (Figure 1g and I). CAV1 expression was downregulated in the RHD+SR and RHD+AF groups compared with that in the CHD+SR group, and the downregulation in the AF group was particularly obvious (Figure 1(h,i)). There was a negative correlation between MG53 and CAV1 at the mRNA and protein levels, which was consistent with the results of the histochemical examination. In addition, we verified that the expression levels of TGF-β1, P-SMAD2, collagen I and α-SMA were upregulated in atrial tissue (Attachment 1).

Attachment 1. (a) The relative abundance of TGF-β1, COL1A1, COL1A2 and ACTA2 mRNA levels in the atrium in different groups was analyzed by RTQ-PCR (n = 6 per group). (b) The expression levels of TGF-β1, P-SMAD2, collagen I and α-SMA in the atrium were analyzed by western blotting (n = 8 per group). The symbol * indicates comparisons with the CHD+SR group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; and the symbol # indicates comparisons with the RHD+SR group, #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$.

MG53 overexpression and its effects in atrial fibroblasts

As previously described, MG53 expression is upregulated in AF patients with severe fibrosis. To further investigate the relationship between MG53 and CAV1 in fibrosis, we overexpressed MG53 in rat atrial fibroblasts via adenovirus vector transfection. Both the mRNA and protein expression levels of MG53 in the Ad-MG53 group were significantly higher than those in the control group and Ad-GFP group (Figure 2 (a,c)), indicating that the overexpression model was successful. The relative abundance of CAV1 was decreased in the atrial fibroblasts transfected with Ad-MG53 (Figure 2(b,c)). Higher TGF-β1, P-SMAD2, collagen I and α-SMA expression levels were observed in the MG53-overexpressing group (Attachment 2). These results suggest that MG53 overexpression inhibits the expression of CAV1 and activates the expression of the TGF-β1/SMAD2 signaling pathway, which is consistent with the expression results in the human tissues.

Attachment 2. (a) The relative abundance of TGF-β1, SMAD2, COL1A1, COL1A2 and ACTA2 was analyzed by RTQ-PCR in atrial fibroblasts transfected with Ad-MG53 or Ad-GFP. (b) The relative abundance of CAV1, TGF-β1, P-SMAD2, collagen I and α-SMA in atrial fibroblasts transfected with Ad-MG53 or Ad-GFP was assessed by western blotting. The symbol α indicates comparisons with the Ad-GFP group, α$p<0.05$, αα$p<0.01$, ααα$p<0.001$.

MG53 depletion and its effects in atrial fibroblasts

To further explore the effects of MG53 from another perspective, siRNA was used to deplete MG53 expression in atrial fibroblasts. The atrial fibroblasts transfected with MG53-si-2 RNA showed significant downregulation at the mRNA

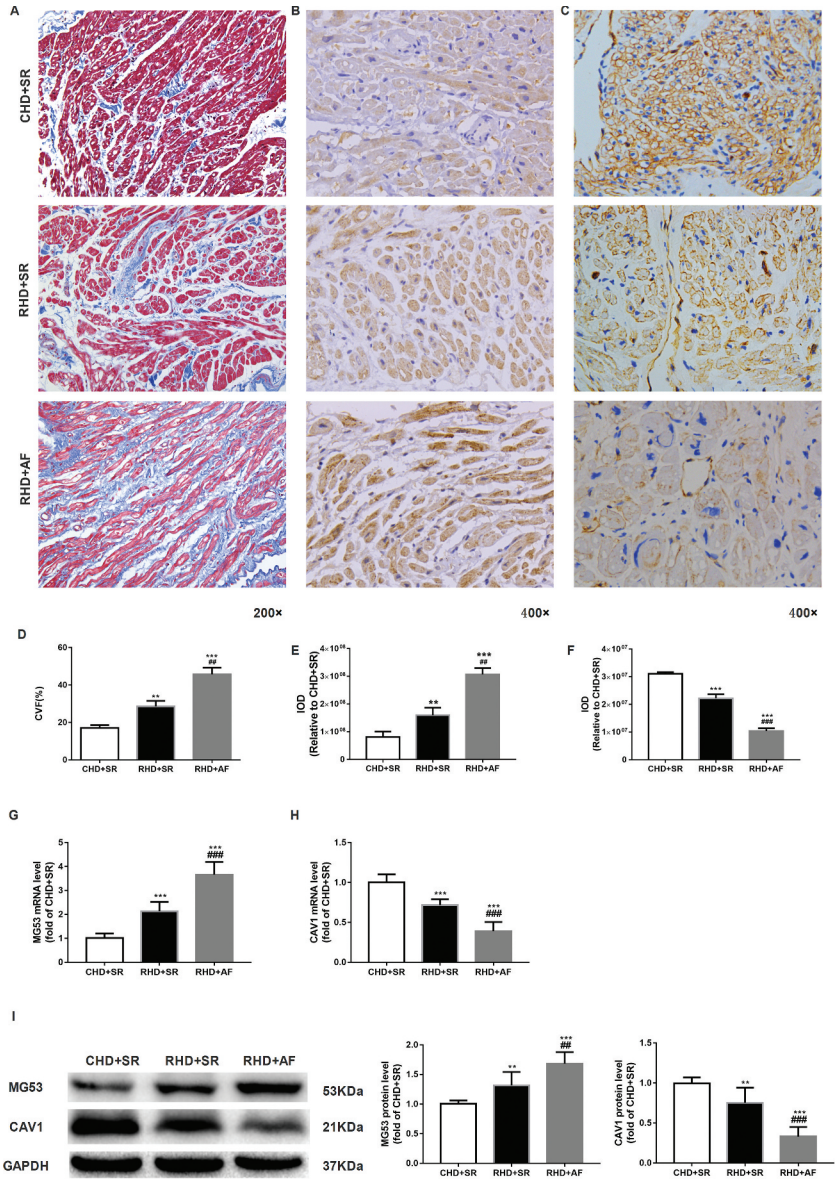

Figure 1. Atrial fibrosis and MG53 and CAV1 expression levels in the human atrium in the CHD+SR, RHD+SR and RHD+AF groups. (a) Representative images (200× magnification) of Masson's trichrome staining and the relative quantification of fibrosis (d) in the atrium of different groups (n = 3 per group). Immunohistochemical staining (400× magnification) was used to evaluate the expression and relative quantification of MG53 (b and e) and CAV1 (c and f) in the atrium in different groups (n = 3 per group). (g and h) The relative abundance of MG53 and CAV1 mRNA levels in the atrium in different groups was analyzed by RTQ-PCR (n = 6 per group). (i) The MG53 and CAV1 expression levels in the atrium were analyzed by western blotting (n = 8 per group). The data are represented as the mean±SD. The symbol * indicates comparisons with the CHD+SR group, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; and the symbol # indicates comparisons with the RHD+SR group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$.

and protein levels compared to the negative control and normal control cells (Figure 3(a,c)). The relative abundance of CAV1 in the atrial fibroblasts transfected with MG53-si-2 RNA was higher than that in the negative control cells according the RTQ-PCR and western blot analyses (Figure 3 (b,c). The relative expression of TGF-β1, P-SMAD2, collagen I and α-SMA was downregulated in the MG53-si-2 RNA group compared with that in the NC-si group (Attachment 3), suggesting that MG53 may regulate the CAV1/TGF-β1/SMAD2 signaling pathway.

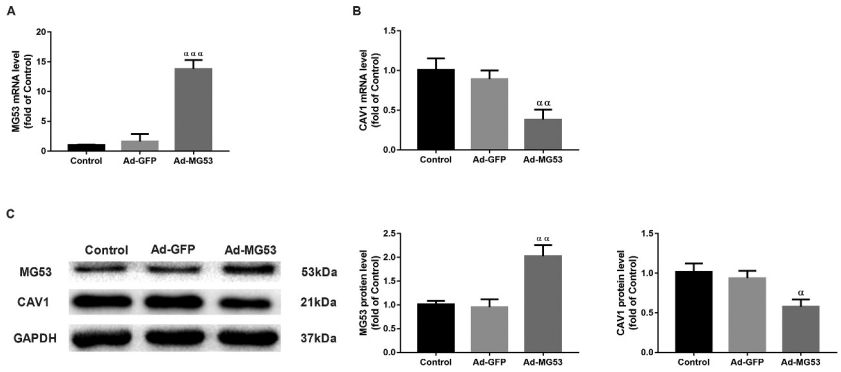

Figure 2. Effects of MG53 overexpression in rat atrial fibroblasts. (A, B and C) The relative abundance of MG53 and CAV1 in atrial fibroblasts transfected with Ad-MG53 or Ad-GFP was determined by RTQ-PCR and western blotting. The data are represented as the mean±SD. The symbol α indicates comparisons with the Ad-GFP group, $^{\alpha}p<0.05$, $^{\alpha\alpha}p<0.01$, $^{\alpha\alpha\alpha}p<0.001$.

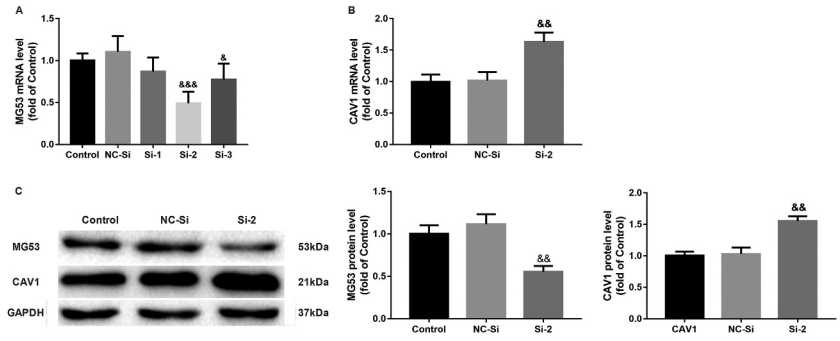

Figure 3. Effects of MG53 knockdown in rat atrial fibroblasts. (A, B and C) The relative abundance of MG53 and CAV1 in atrial fibroblasts transfected with MG53-siRNA or NC-siRNA was determined by RTQ-PCR and western blotting. NC = Negative control. The data are represented as the mean±SD. The symbol & indicates comparisons with the NC-si group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

Attachment 3. (a) The relative abundance of TGF-β1, SMAD2, COL1A1, COL1A2 and ACTA2 in atrial fibroblasts transfected with MG53-siRNA or NC-siRNA was analyzed by RTQ-PCR. (b) The relative abundance of TGF-β1, P-SMAD2, collagen I and α-SMA in atrial fibroblasts was detected by western blotting. NC = Negative control. The symbol & indicates comparisons with the NC-si group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

CAV1 inactivates the TGF-β1/SMAD2 signaling pathway

Subsequently, we explored the relationship between CAV1 and the TGF-β1 signaling pathway by using siRNA to deplete CAV1 expression. The atrial fibroblasts transfected with CAV1-si'-3 RNA exhibited significant mRNA and protein downregulation compared to the negative control and normal control cells (Figure 4(a,b,c)). CAV1 has been previously reported to negatively regulate TGF-β1/SMAD2 signaling pathway activation [10]. Our experiment further confirmed this hypothesis. In this study, we observed that the CAV1 knockdown caused TGF-β1, P-SMAD2, collagen I and α-SMA upregulation at the mRNA and protein levels (Figure 4(d,e,f)). These results suggest that CAV1 inactivates the TGF-β1/SMAD2 signaling pathway.

Ang II induced MG53 and TGF-β1 signaling pathway upregulation and CAV1 downregulation

Finally, we studied the effect of Ang II–induced pathological injury on the MG53/CAV1/TGF-β1

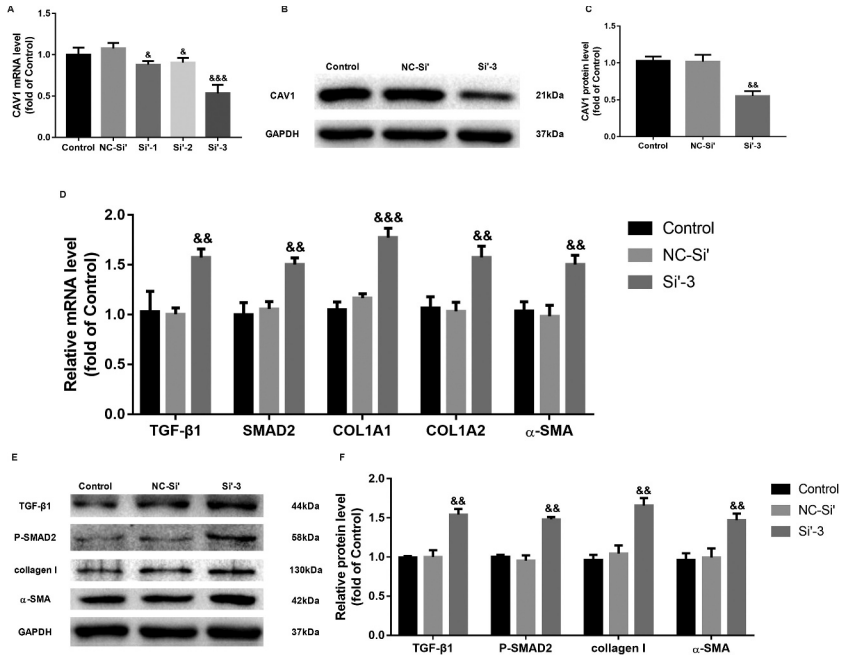

Figure 4. Effects of CAV1 knockdown on the TGF-β1/SMAD2 signaling pathway in rat atrial fibroblasts. (A, B and C) The relative abundance of CAV1 in atrial fibroblasts transfected with CAV1-si'RNA or NC-si'RNA was determined by RTQ-PCR and western blotting. (d) The mRNA levels of TGF-β1, SMAD2, COL1A1, COL1A2 and ACTA2 in atrial fibroblasts transfected with CAV1-si'-3 RNA or NC-si'RNA were analyzed by RTQ-PCR. (e and f) Western blotting was used to detect the expression of TGF-β1, P-SMAD2, collagen I and α-SMA. NC = Negative control. The data are represented as the mean±SD. The symbol & indicates comparisons with the NC-si' group, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

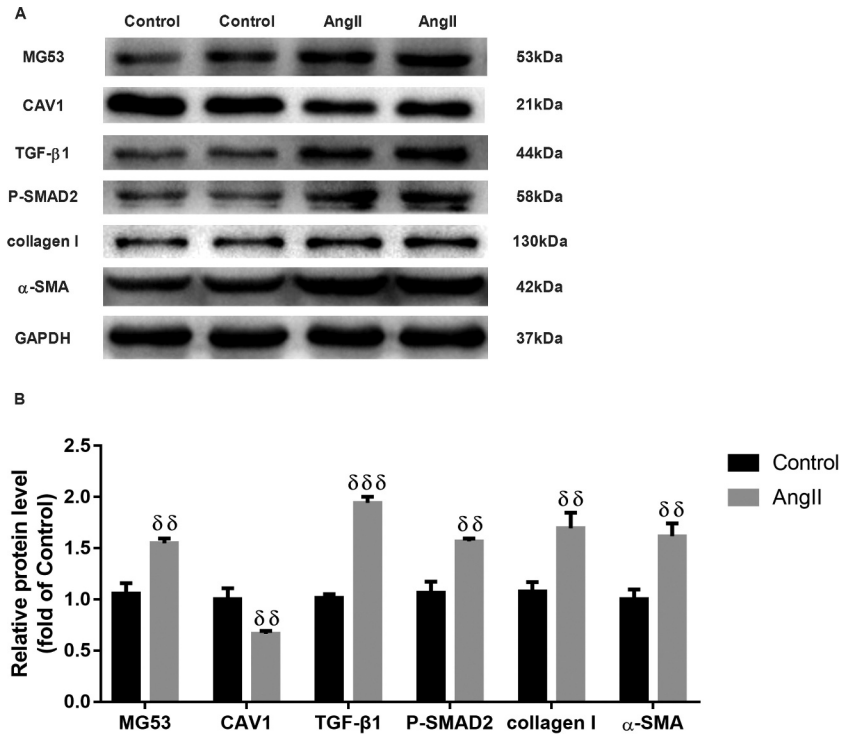

Figure 5. Effect of Ang II–induced pathological injury on the MG53/CAV1/TGF-β1 signaling pathway. (a and b) The relative abundance of MG53, CAV1, TGF-β1, P-SMAD2, collagen I and α-SMA in atrial fibroblasts was detected by western blotting. The data are represented as the mean±SD. The symbol δ indicates comparisons with the control group, $^{\delta\delta}p<0.01$, $^{\delta\delta\delta}p<0.001$.

signaling pathway. MG53 expression induced by Ang II in atrial fibroblasts was higher than that in the control group (Figure 5(a,b)). In contrast, the expression of CAV1 was downregulated. The western blot analysis also showed that TGF-β1, P-SMAD2, collagen I and α-SMA expression was significantly upregulated (Figure 5(a,b)). These results are consistent with the human results, suggesting that the MG53 F-β1/CAV1/TGF signaling pathway is involved in the process of fibrosis.

Discussion

This study showed that CAV1 plays critical roles in atrial fibrosis. CAV1 expression in patients with AF was significantly lower than that in SR patients and inversely related to the expression of MG53 and the degree of fibrosis. In atrial fibroblasts, CAV1 inactivates the TGF-β1/SMAD2 signaling pathway. Moreover, CAV1 is an important bridge connecting MG53 and the TGF-β1/SMAD2 signaling pathway. CAV1 can be regulated by MG53 in atrial fibroblasts by MG53 knockdown and overexpression. When atrial fibroblasts were treated with Ang II to induce fibrotic injury, the results were consistent with those found using human tissues. Overall, this study revealed that MG53 could regulate TGF-β1/SMAD2 signaling pathway-induced atrial fibrosis via the molecular target of CAV1.

Previous studies have demonstrated that the TGF-β1/SMAD2 signaling pathway plays an important role in tissue fibrosis and that the phenotype of fibroblasts transformed into myofibroblasts, especially atrial cells, is more susceptible [15]. Here, we found that the TGF-β1/SMAD2 pathway is activated in RHD+AF patients with severe fibrosis, which is consistent with a previous report [17]. In the present study, we found that TGF-β1, P-SMAD2, collagen I and α-SMA upregulation was positively correlated with increased collagen distribution in the myocardium and was significantly increased in patients with AF compared with patients with SR. These results show that the TGF-β1/SMAD2 signaling pathway is the most important central link in atrial fibrosis of AF. The higher the expression of the TGF-β1 signaling pathway, the more severe the fibrosis, which increases the risk of AF.

CAV1 is a major protein in the caveolae structure, and previous studies have confirmed that CAV1 plays an important role in tissue fibrosis [27]. Lu J and Zhang GY et al. demonstrated that CAV1 can regulate liver and dermal fibrosis by inhibiting the TGF-β1/SMAD2 signaling pathway [25,28]. Our results demonstrate that CAV1 is expressed in human atrial tissue and is downregulated in RHD+AF patients with severe fibrosis. TGF-β1, P-SMAD2, α-SMA and collagen I expression was highly expressed in AF patients and negatively correlated with CAV1. To assess the relationship between CAV1 and the TGF-β1 signaling pathway, siRNA was used to deplete CAV1 expression. The expression levels of TGF-β1, P-SMAD2, α-SMA and collagen I were increased in the atrial fibroblasts with CAV1-siRNA knockdown. Miyasato SK et al also confirmed that compared to wild-type mice, the TGF-β1 signaling pathway was significantly activated in CAV1 knockout mice [24]. Therefore, this result suggests that CAV1 can negatively regulate TGF-β1/SMAD2 signaling pathway activation in fibrotic tissues.

Our previous experiments confirmed that MG53, which is a tripartite motif-containing protein, is expressed in human atrial tissue [12]. In the present study, we also found that compared with SR patients, MG53 is highly expressed in AF patients. Chen X et al found that MG53 expression was increased in TAC mice and that silencing MG53 reversed TAC-induced cardiac fibrosis [29]. Similarly, mice overexpressing MG53 exhibited symptoms of diabetes-like cardiomyopathy, such as insulin resistance, ventricular hypertrophy, and fibrosis, at 20 weeks of age [9]. Interestingly, MG53 exerts myocardial protective effects by activating RISK signaling pathways (mainly the PI3K-AKT-GSK3β and ERK1/2 signaling pathways) during myocardial ischemia and reperfusion [30]. Because atrial fibrosis has long-term and slow-growing pathophysiological characteristics, MG53 may exhibit different biological functions in acute and chronic lesions. In atrial tissue, we observed

that MG53 expression was negatively correlated with CAV1 but positively correlated with TGF-β1/SMAD2 signaling pathway activation and the tissue collagen content. To explore the role of MG53, we used two cell models. After MG53 overexpression, the atrial fibroblasts showed lower CAV1 expression. In contrast, CAV1 expression was increased in the MG53 depleted atrial fibroblasts. Based on these studies, we found that MG53 can regulate CAV1 expression. Our study also found that in atrial fibroblasts, TGF-β1, P-SMAD2, α-SMA and collagen I expression was decreased following the MG53 knockdown, whereas this expression increased following MG53 overexpression via adenovirus. Based on these studies, we found that MG53 can regulate the TGF-β1/SMAD2 signaling pathway. As previously described, CAV1 depletion can inhibit the expression of the TGF-β1/SMAD2 signaling pathway. Therefore, MG53 can activate the TGF-β1/SMAD2 signaling pathway by inhibiting CAV1 expression.

Ang II can induce fibrosis injury, which increases the susceptibility to AF [31]. In atrial fibroblasts treated with Ang II, TGF-β1 P-SMAD2, α-SMA and collagen I expression was increased. However, Ang II downregulates CAV1 expression, which is consistent with IshizakaN's study in vascular smooth muscle cells [32]. More importantly, we confirmed that Ang II induced MG53 expression upregulation, which is consistent with previously described results. In addition, MG53 depletion can attenuate the proliferation and migration of fibroblasts [28,33]. Thus, MG53 participates in fibrosis by regulating the CAV1/TGF-β1 signaling pathway.

Conclusion

In summary, AF is the most common arrhythmia in the clinic. Atrial fibrosis is considered the most important pathologic change in AF and plays a key role in AF formation. TGF-β1, which is the strongest ECM deposition promoter, is crucial for the development of atrial fibrosis. CAV1 can inhibit TGF-β1/SMAD2 signaling pathway activation, which regulates atrial fibrosis. MG53 is a newly discovered tripartite motif-containing protein. This study demonstrates that MG53 can inhibit CAV1 expression, which can regulate TGF-β1/SMAD2 signaling pathway-induced fibrosis in clinical and cell experiments. It may be possible to interfere with the expression of atrial MG53 to regulate fibrosis and whether the upregulation of the CAV1 expression or exogenous CAV1 to inhibit the occurrence of fibrosis. Thus, targeting MG53 and CAV1 is a potential treatment strategy for TGF-β1/SMAD2 signaling pathway-induced atrial fibrosis in AF.

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Availability of data and materials

All relevant data are included in the paper.

Disclosure statement

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