European Review for Medical and Pharmacological Sciences 2020; 24: 6270-6278

Effects of Secreted frizzled-related protein 1 on inhibiting cardiac remodeling

H. WANG[1], Y. LIU[2], X. LIANG[1], G. YANG[1], Y. LIU[1], F. LI[1], Y.-T. GUO[3], H.-J. WANG[1], H.-B. LIU[1], L. CHEN[4]

[1]Department of Cardiology, Second Medical Center; National Clinical Research Center for Geriatric Diseases, Chinese PLA General Hospital, Beijing, China
[2]Department of Critical Care Medicine, People's Hospital of Lezhi county of Sichuan Province, Ziyang, China
[3]Department of Nephrology, First Medical Center, Chinese PLA General Hospital, Beijing, China
[4]Department of Emergency, First Medical Center, Chinese PLA General Hospital, Beijing, China

Hao Wang and Yu Liu contributed equally to this work

Abstract. – OBJECTIVE: To investigate the effect of Secreted frizzled-related protein 1 (Sfrp1) on myocardial fibroblasts through Wnt/β-catenin signaling pathway.

MATERIALS AND METHODS: Rat myocardial fibroblasts were cultured and divided into control group, proliferation group (TGF-β1 group), and Sfrp1 transfection group (TGF-β1 + Ad-Sfrp1 group). The control group received no treatment. The TGF-β1 group was stimulated with TGF-β1 10 ng/mL for 12 h to establish a proliferation model. The TGF-β1 + Ad-Sfrp1 group was first transfected with Ad-Sfrp1 virus. On day 3, TGF-β1 was added at 10 ng/mL to stimulate 12 h. The β-catenin and the marker protein α-SMA of myofibroblast (MyoFB) differentiation were detected by Western blotting method. In addition, we used MTT to test cell proliferation and flow cytometry to test cell cycle. At the same time, we used enzyme-linked immunosorbent assay (ELISA) to detect the collagen I and collagen III content of the cell supernatant and used quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) to test the expression of apoptotic factors and Dvl-1 and Cyclin D1.

RESULTS: In TGF-β1 group, the β-catenin, and α-SMA protein expressions were all upregulated, the OD value and collagen I and collagen III contents were increased, but the apoptosis rate was decreased. On the contrary, the expression of β-catenin and α-SMA proteins in the TGFβ1 + Ad-Sfrp1 group were all downregulated, the OD value, collagen I and collagen III content, and percentage of S-phase cells were reduced, but the percentage of G0/G1, G2/M-phase cells, and the apoptotic rate increased.

CONCLUSIONS: Sfrp1 can effectively inhibit myocardial fibroblast proliferation, collagen synthesis, promote fibroblast apoptosis, and inhibit the transformation of fibroblasts into myofibroblasts by inhibiting Wnt/β-catenin signaling pathway.

Key Words:
Wnt/β-catenin signaling pathway, Secreted frizzled-related protein 1, Myocardial fibrosis, Cell proliferation, Apoptosis.

Introduction

At present, cardiovascular disease (CVD) is still a major disease endangering human health, and pathological repair after myocardial injury is the main pathophysiological process[1]. The mammalian heart is a terminally differentiated organ and cannot be regenerated after myocardial necrosis. And necrotic myocardium has inflammatory response, extracellular matrix (ECM) deposition, collagen formation, and finally myocardial fibrosis (MF)[2]. However, the factors and mechanisms affecting MF have not been fully elucidated. On the one hand, it is committed to studying myocardial regeneration and providing broad prospects for the clinical solution of myocardial regenerative diseases. Zuppo and Tsang[3] have suggested that lower vertebrates, such as adult zebrafish can regenerate completely after removing about 20% of their hearts. Lam and Sadek[4] have found that newborn mouse hearts have a short-term regenerative ability that disappears after 1 week of birth. However, the results

Corresponding Author: Li Chen, MD; e-mail: chenli-china@163.com

have not yet been transformed into clinical trials. Stem cell therapy was previously believed to promote myocardial regeneration but is still questioned. On the other hand, cardiac fibroblast (CFs) is activated and proliferates and differentiates in large quantities after cardiac injury and other stimulation or is transformed into MyoFB with stronger secretory activity and smooth myolike contraction characteristics, which is the most important pathological process of MF. Both CF and MyoFB can secrete a large amount of ECM, and the essence of the process of MF is caused by the excessive accumulation and degradation of ECM[5]. Therefore, any factor that inhibits CF activation, trans-differentiation into MyoFB, and factors that promote ECM degradation may inhibit MF. The related influencing factors found, and regulatory genes will be of great benefit in preventing and treating such diseases.

The Wnt signal pathway is a highly conserved signal pathway in biological evolution, and plays an important physiological function during embryonic development, development, cell growth, and proliferation[6]. Therefore, the abnormality of the Wnt signal pathway is closely related to the development of CVD. Stylianidis et al[7] have found that the Wnt/β-catenin signaling pathway can be reactivated after myocardial infarction (MI) and is involved in regulating the pathophysiological processes, such as ventricular remodeling, cardiomyocyte apoptosis, and MF. Bowin et al[8] have shown that when the Wnt signaling pathway is activated, Wnt ligand binds to the Frizzled protein (Fz) receptor and low-density lipoprotein receptor-related proteins (LRP-5/6) with the participation of dishevelled protein (DVL), resulting in an increase of β-catenin in the cytoplasm, an increase the β-catenin into the nucleus, and an interaction with the T-cell-specific transcription factor (TCF)/lymphoid enhancer factor (LEF) family to promote the expression of downstream target genes.

The members of the Sfrps family play a negative regulatory role in the Wnt signaling pathway and are a class of secreted glycoprotein. The Sfrps family has five members (Sfrps1-5), which are composed of more than 300 amino acids, all of which contain a signal sequence, a Fz - like cysteine-rich domain (CRD), an n-terminus and a c-terminus of a hydrophilic region[9]. CRD has 10 conserved cysteine residues, which share sequence homology with the partially curled receptor CRD domain outside the cell. This structure increases the likelihood that Sfrps molecules bind to Fz receptors and Wnt ligands[10]. Sklepkievicz et al[11] have found that Sfrp1 overexpression can effectively inhibit the Wnt/β-catenin pathway activity in aged mice, effectively delay the process of chronic ventricular remodeling in the aged, and then, reduce the incidence and mortality of chronic heart failure in the aged mice, but whether the inhibition of Wnt/β-catenin signaling pathway can effectively delay MF is unclear.

From this, we speculate whether overexpressed Sfrp1 in MFs, can inhibit the effects of external factors on myocardial fibroblasts apoptosis and fibrosis.

Materials and Methods

Drug Preparation

TGF-β_1 (Tianpu, Guangzhou, China) was prepared with 10 mM citric acid (Natural, Shenyang, China) and stored at 4°C after PH adjustment.

Cell Culture

CFs (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Life Technology, Wuhan, China) medium containing 5% fetal bovine serum (FBS; Life Technology, Wuhan, China) and 1% penicillin/streptomycin (Life Technology, Wuhan, China) in an incubator at a constant temperature of 37°C, 5% CO_2 and 95% air. When the cell density reached 70-80%, the cells were digested with trypsin (Life Technology, Wuhan, China) for passage, and the supernatant was replaced every 2-3 days.

Cell Grouping and Intervention Methods

The cells were divided into control group, proliferation group (TGF-β_1 group), and Sfrp1 transfection group (TGF-β_1 + Ad-Sfrp1 group). The control group received no treatment. TGF-β_1 group: CFs were replaced with serum-free DMEM medium, and TGF-β_1 10 ng/mL was added to stimulate for 12 h to establish a proliferation model. TGF-β_1 + Ad-Sfrp1 group: we transfected CFs with Ad-Sfrp1 (Hanbio, Shanghai, China), and on the 3th day, we established a proliferation model. The proliferation model was established in the same way as the TGF-β_1 group.

MTT Detects Cell Proliferation

We added MTT reagent (Jian Cheng, Nanjing, China) to ensure the final concentration of cells in each group was 5 mg/mL. After incubation at

37°C in the dark for 4 h, we added 150 μL DMSO reagent (Jian Cheng, Nanjing, China) and 25 μL glycine buffer. After mixing, we recorded the absorbance at 490 nm (OD) value. The blank group serves as a reference.

Cell Cycle Detection

We collected the cells of three groups, added pre-cooled 70%-80% ethanol (Jian Cheng, Nanjing, China), and vortex to mix the cells thoroughly, and then, we protected them from light at 4°C overnight. Then, the cells were washed with PBS and staining solution (Walvax, Kunming, China) to remove ethanol. Then, we added 500 μL Propidium Iodide (PI) comprehensive staining solution (Walvax, Kunming, China), and stained at 20°C for 10 minutes, then, we detected the cell cycle by flow cytometry.

Western Blotting (WB) Technology

Cells from each group were collected, and the total protein was extracted after lysis (Camilo Biological, Nanjing, China). We used the bicinchoninic acid (BCA) method (Camilo Biological, Nanjing, China) to determine the protein concentration. After the protein was quantified, the sample was added (40 μg total protein) and diluted with equal volume of loading buffer (Camilo Biological, Nanjing, China). Then, the dispersed protein was then transferred to a polyvinylidene difluoride (PVDF, Thermo Fisher Scientific, Waltham, MA, USA) membrane at 4°C. After the membrane transferred, the membrane was washed with 0.05% Tris-Buffered Saline-Tween (TBST) for 5 s, and then, blocked it with 5% skim milk for 1 h. Then, we incubated the corresponding primary and secondary antibody (goat anti-rabbit IgG antibody, YifeiXue, Nanjing, China), and then, we added the color development solution (Camilo Biological, Nanjing, China) and put it into the luminometer (Viamech, Suzhou, China) for exposure. Image lab was used to analyze Western blotting pictures, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) protein content was used as a reference. The antibodies designed in this study are as follows: (Bcl-2, Abcam, Cambridge, MA, USA, Mouse, 1:2000; Bax, Abcam, Cambridge, MA, USA, Mouse, 1:2000; α-SMA, Abcam, Cambridge, MA, USA, Rabbit, 1:3000; Collagen I, Abcam, Cambridge, MA, USA, Rabbit, 1:3000; Collagen III, Abcam, Cambridge, MA, USA, Rabbit, 1:3000; Sfrp1, Abcam, Cambridge, MA, USA, Rabbit, 1:3000; GAPDH, Proteintech, Rosemont, IL, USA, 1:5000).

RNA Isolation and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The cells of three groups were collected, and total RNA was extracted by TRIzoI method (Thermo Fisher Scientific, Waltham, MA, USA). The total RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) using a reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, USA), PCR primers were added and placed on an ABI 7500 PCR instrument (Applied Biosystems, Foster City, CA, USA) for amplification reaction, and GAPDH was used as an internal reference. The reaction conditions were: pre-denaturation at 95°C for 2 min, 1 cycle; denaturation at 95°C for 30 s, refolding at 58°C for 30 s, extension at 72°C for 30 s, 40 cycles, extension at 72°C for 10 min. We used the $2^{-\Delta\Delta Ct}$ method to calculate the relative expression of the target gene. The primers used were shown in Table I.

Table I. qRT-PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Bax	TGAAGACAGGGGCCTTTTTG	AATTCGCCGGAGACACTCG
Bcl-2	GTCGCTACCGTCGTGACTTC	CAGACATGCACCTACCCAGC
Collagen I	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Collagen III	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Dvl-1	TCTGTACCCTGGCCCTTG	TGCTCTTGCTCCCTTCACT
Cyclin D1	GCGTACCCTGACACCAATCTC	CTCCTCTTCGCACTTCTGCTC
Sfrp1	CAACGTGGGCTACAAGAAGAT	GGCCAGTAGAAGCCGAAGAAC
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

qRT-PCR, quantitative reverse-transcription polymerase chain reaction.

Enzyme Linked Immunosorbent Assay (ELISA)

The content of collagen I and collagen III in the cell supernatant was detected. The supernatants of CFs from each group were collected and operated according to the instructions of the collagen I and collagen III ELISA kits (Elabscience, Wuhan, China).

TUNEL Staining

The CFs of each group were fixed in 15 groups with 4% paraformaldehyde (Walvax, Kunming, China), washed with phosphate-buffered saline (PBS) to remove paraformaldehyde, and then, we added 50 μL of biotin labeled solution (Walvax, Kunming, China), and incubated them at 37°C in the dark for 1 h. Then, 50 μL of labeled reaction stop solution (Walvax, Kunming, China) was added dropwise and incubated at 20°C for 10 min. After washing with PBS, 10 μL 4',6-diamidino-2-phenylindole (DAPI) (Walvax, Kunming, China) stained nucleus was added dropwise, and after incubation at room temperature for 10 min, the positive cells were observed under a fluorescence microscope (Viamech, Suzhou, China).

Statistical Analysis

We used the Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM Corp., Armonk, NY, USA). The measurement data were expressed as mean ± SD (standard deviation). The differences between the two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Count data was expressed as rates, and the comparisons between groups were tested by chi-square. $p<0.05$ was considered statistically significant.

Results

Effect of TGF-β_1 on Cardiac Fibroblasts

According to literature reports, we selected 10 ng/mL TGF-β_1 to stimulate CFs. To verify the effect of TGF-β_1 on CFs proliferation and cycle, we used MTT method and flow cytometry to test each group (Figure 1A and 1B). The results indicated that that after TGF-β_1 treatment of CFs, its proliferation activity, as well as the percentage of cells in S phase were significantly increased, while the percentage of cells in G0/G1 phase and G2/M phase was decreased. Second, we used WB to teste the fibrosis-related proteins in the two groups and found that the expression of Collagen I and Collagen III in the TGF-β_1 group was dramatically increased (Figure 1C). Both qRT-PCR (Figure 1D) and ELISA (Figure 1E and 1F) results were similar to WB. Then, we performed TUNEL staining on the two groups of cells and found that TGF-β_1 treatment induced an increase in the apoptosis rate of CFs (Figure 1G). At the same time, WB detection of Bax, and Bcl-2 protein expression also confirmed that TGF-β_1 can inhibit Bax expression and promote Bcl-2 expression (Figure 1H). At the same time, similar results were obtained with qRT-PCR (Figure 1I). Then, we detected the expression of α-SMA and Sfrp1 in the two groups by WB and found that in the TGF-β_1 group, the expression of Sfrp1 protein was remarkably reduced, while the expression of α-SMA was significantly increased (Figure 1J).

Overexpression of Sfrp1 Inhibits CFs Proliferation and Fibrosis

To verify our conjecture, we first transfected CFs with Ad-Sfrp1 virus. By qRT-PCR detection, CFs reached the peak expression of the target gene on the 3th day of transfection (Figure 2A). Next, we used MTT method (Figure 2B) and flow cytometry (Figure 2C) to test cell proliferation and cycle. The results indicated that the TGF-β_1 + Ad-Sfrp1 group had reduced proliferation and percentage of cells in S phase, while the percentage of cells in G0/G1 and G2/M phases increased. In addition, we detected the Collagen I and Collagen III expression by WB (Figure 2D) and qRT-PCR (Figure 2E). Both results indicated that when CFs overexpressed Sfrp1, the expressions of Collagen I and Collagen III were remarkably reduced. ELISA tests also obtained similar results (Figure 2F and 2G). Secondly, we detected MyoFB differentiation protein α-SMA by WB. The results confirmed that TGF-β_1 can promote the expression of α-SMA in myofibroblasts. When we overexpressed Sfrp1, we found that the effect of TGF-β_1 on the expression of α-SMA in myofibroblasts was significantly inhibited (Figure 2H).

Overexpression of Sfrp1 Inhibits CFs Apoptosis

At the same time, we explored whether the overexpression of Sfrp1 can promote the apoptosis of CFs induced by TGF-β_1. Similarly, we first tested the apoptotic rate between groups by TUNEL staining (Figure 3A). It was found that CFs overexpressed Sfrp1, and the apoptosis rate

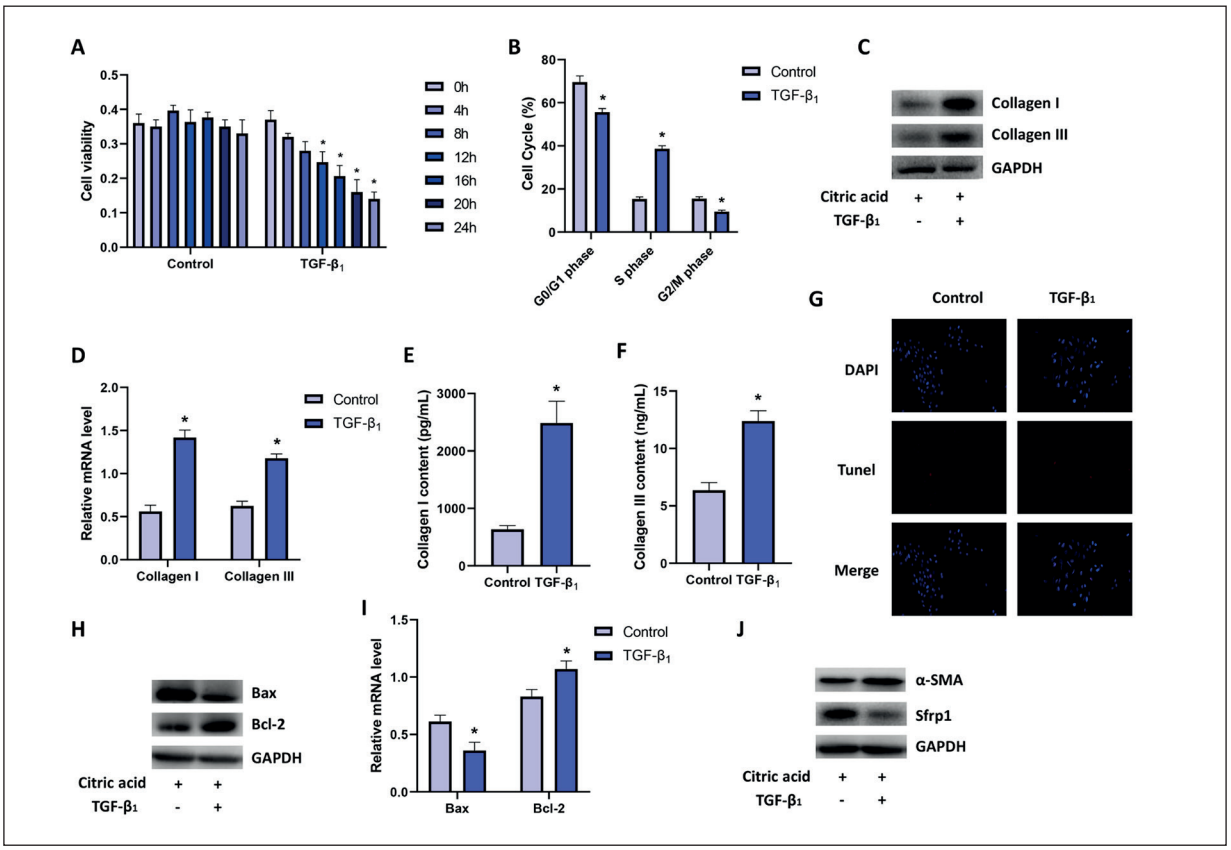

Figure 1. Effect of TGF-β_1 on cardiac fibroblasts. **A,** MTT assay detected the cell viability. **B,** Flow cytometry detected the cell cycle. **C,** WB detected the expression of Collagen I and Collagen III. GAPDH is used as an internal control. **D,** qRT-PCR detected the expression of Collagen I and Collagen III. **E,** and **F,** Elisa method detected the contents of Collagen I and Collagen III in the cell supernatant. **G,** TUNEL staining. (magnification: 400×) **H,** WB detected the Bax and Bcl-2 expression. GAPDH is used as an internal control. **I,** qRT-PCR detect the Bax and Bcl-2 expression. **J,** WB detected the α-SMA and Sfrp1 expression. GAPDH is used as an internal control. ("*" indicates statistical difference from the control group $p<0.05$).

was remarkably increased. Second, we used WB to detect the apoptosis-related proteins Bax and Bcl-2 in 3 groups and it was found that the expression of Bcl-2 in the TGF-β_1 + Ad-Sfrp1 group was significantly lower than that in the TGF-β_1 group and the TGF-β_1 + Ad-NC group, while the expression of Bax was highest in the 3 groups (Figure 3B). QRT-PCR also obtained similar results (Figure 3C). From the above results, we confirmed that the overexpression of Sfrp1 can regulate the apoptosis of CFs.

Overexpression of Sfrp1 Inhibits Wnt Signaling Pathway Activation

First, we tested β-catenin protein expression. The results indicated that the expression of β-catenin in the TGF-β_1 group was significantly increased, while the β-catenin protein expression was lower in the TGF-β_1 + Ad-Sfrp1 group than in the TGF-β_1 group. Second, studies have indicated that β-catenin in normal cells can be phosphorylated by GSK3β and can be rapidly degraded. When the Wnt pathway is abnormally activated, GSK3β phosphorylation is reduced, resulting in more β-catenin accumulation in the cytoplasm and promoting into the nucleus, while interacting with the TCF/LEF family, and started the gene transcription process. Therefore, we detected the expression of p-β-catenin protein and found that the expression of p-β-catenin protein in TGF-β_1 group significantly decreased, while the expression of p-β-catenin protein in TGF-β_1+ Ad-sfrp1 group significantly increased (Figure 4A and 4B). Then, we detected the expression of Dvl-1 and Cyclin D1 by qRT-PCR (Figure 4C). The results indicated that after TGF-β_1 stimulated CFs, the expression of Dvl-1 and Cyclin D1 increased, while the expression of Dvl-1 and Cyclin D1 significantly decreased when Sfrp1 was overexpressed.

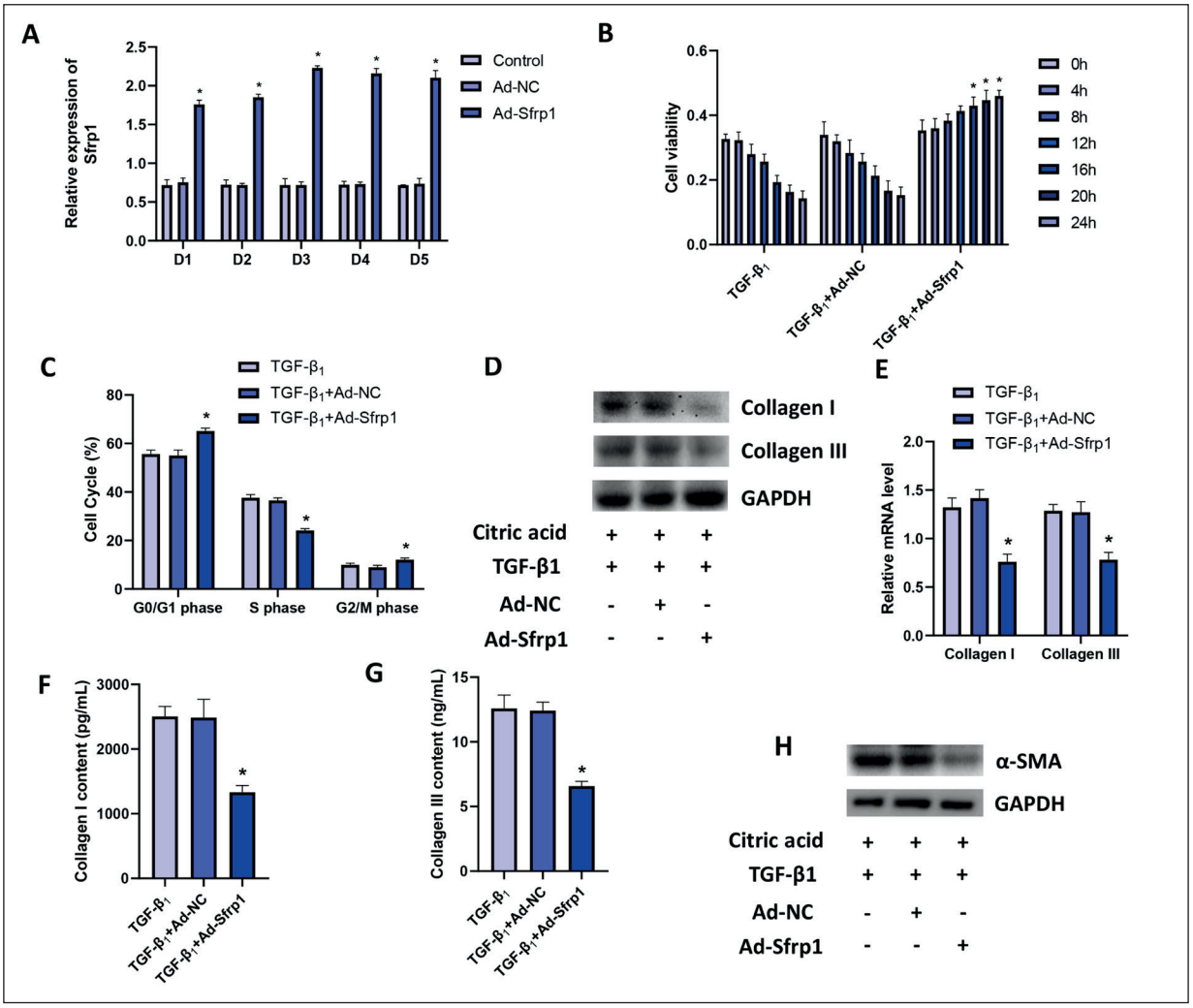

Figure 2. Overexpression of Sfrp1 inhibits CFs proliferation and fibrosis. **A,** qRT-PCR detected the Sfrp1 expression. **B,** MTT assay detected the cell viability. **C,** Flow cytometry detected the cell cycle. **D,** WB detected the Collagen I and Collagen III expression. GAPDH is used as an internal control. **E,** qRT-PCR detected the expression of Collagen I and Collagen III. **F,** and **G,** Elisa method detected the contents of Collagen I and Collagen III in the cell supernatant. **H,** WB detected the α-SMA expression. GAPDH is used as an internal control. ("*" indicates statistical difference from the TGF-β_1 group $p<0.05$).

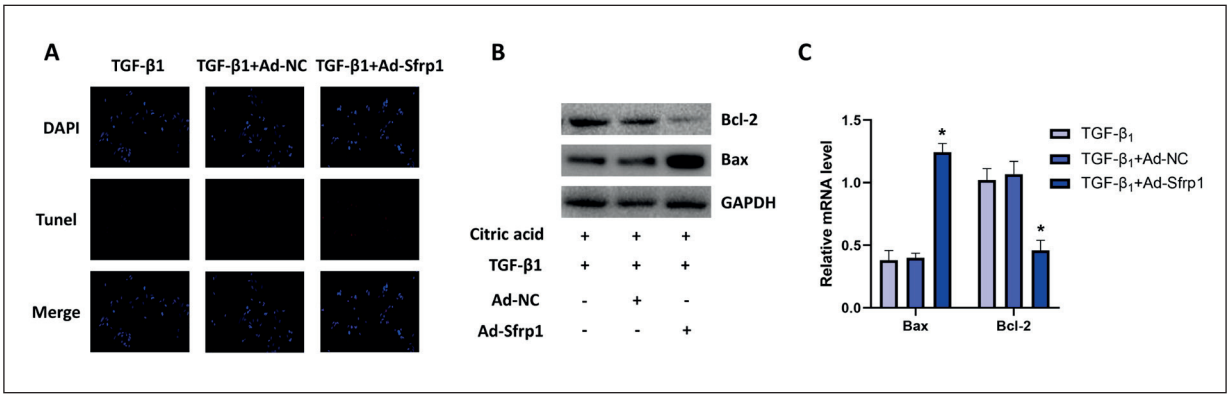

Figure 3. Overexpression of Sfrp1 inhibits CFs apoptosis. **A,** TUNEL staining (magnification: 400×). **B,** WB detected the Bax and Bcl-2 expression. GAPDH is used as an internal control. **C,** qRT-PCR detect the Bax and Bcl-2 expression. ("*" indicates statistical difference from the TGF-β_1 group $p<0.05$).

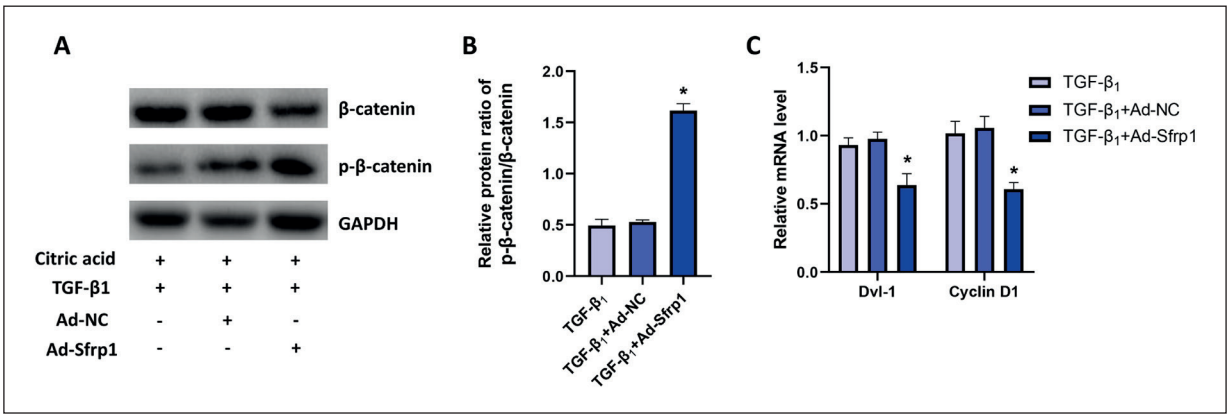

Figure 4. Overexpression of Sfrp1 inhibits Wnt signaling pathway activation. **A,** WB detected the β-catenin and p-β-catenin expression. GAPDH is used as an internal control. **B,** Protein analysis: p-β-catenin/β-catenin ratio. **C,** qRT-PCR detect the Dvl-1 and Cyclin D1 expression. ("*" indicates statistical difference from the TGF-β_1 group $p<0.05$).

Discussion

MF is an adaptive proliferation of the heart to various stimuli and is the main manifestation of myocardial remodeling. MF manifests as myocardial fibroblast proliferation, collagen deposition, phenotypic transformation of myofibroblasts, etc., which can increase myocardial stiffness, reduce its compliance, limit the contractile and diastolic functions of the heart, and is a common pathological change of many CVDs[2]. Therefore, looking for drugs that can effectively inhibit the proliferation of myocardial fibroblasts, the collagen secretion and the transformation of myofibroblasts may effectively delay or retard the occurrence and development of MF. At the same time, it has been reported that the loss of Sfrp1 will lead to the deterioration of cardiac function in young rats[12], and the exogenous administration of Srfp2 can significantly reduce fibrosis and improve cardiac function in MI model rats[13]. Therefore, we speculate that Sfrp1 has a protective effect on cardiac function and myocardial remodeling. After MI, the proliferation of CFs is an important factor leading to MF and myocardial remodeling. Given all this, we explored whether Sfrp1 could inhibit MF and remodeling via inhibiting the proliferation of CFs.

At present, there are different results on the regulation of Sfrp1 on various cells *in vitro*. Barandon et al[14] showed that transgenic mice with overexpression of Sfrp1 could induce the activation of GSK3 protein and reverse the beneficial effect of ischemic preconditioning after MI. On the other hand, transgenic mice with overexpression of Sfrp1 could remarkably reduce the area of MI, prevent heart rupture, and improve myocardial function. Salazar et al[15] showed that Sfrp1 markedly inhibited the proliferation of lung fibroblasts. We found that the overexpression of Srfp1 can significantly inhibit the proliferation of CFs, which was consistent with previous studies. The overexpression of Wnt/β-catenin signaling pathway members in fibroblasts can regulate cell proliferation, migration, and differentiation. Hahn et al[16] found that the overexpression β-catenin can stimulate the proliferation and differentiation of neonatal rat myocardial fibroblasts and induce hypertrophic responses in myocardial cells. Furthermore, β-catenin is a key protein molecule in the Wnt signaling pathway, and its localization change is a marker of activation of this pathway.

The balance between Sfrp1 and β-catenin plays an important role in the development of CVD[17]. In this research, we found that, compared with the control group, the expression of β-catenin, Dvl-1, and Cyclin D1 in TGF-β1 group were upregulated, suggesting that after TGF-β1 stimulation, the Wnt/β-catenin signaling pathway in CFs was activated, the expression of Bcl-2 was promoted, and the expression of Bax was inhibited, thus promoting the proliferation of CFs. At the same time, compared with TGF-β1+ AAV-Sfrp1 group, the expression of β-catenin, Dvl-1, Cyclin D1, and Bcl-2 were all downregulated, and the expression of Bax was increased, suggesting that Sfrp1

overexpression could inhibit the activation of Wnt/β-catenin signaling pathway, thus promoting the apoptosis of CFs.

In addition, the research found that after TGF-β1 stimulation, the expression of α-SMA increased and CFs were transformed into myofibroblasts. Enhanced expression of α-SMA is a marker of MyoFB differentiation[18]. This study found that, after TGF-β_1 stimulation, the myocardial fibroblasts proliferated, collagen synthesis increased, and fibroblasts were transformed into myofibroblasts. The overexpression Sfrp1 can effectively reduce the proliferation of myocardial fibroblasts, reduce DNA synthesis, significantly reduce the percentage of myocardial fibroblasts in the S phase, and increase the percentage of G0/G1 and G2/M phases. In terms of collagen synthesis, it can inhibit the collagen synthesis of fibroblasts, and weaken the expression of collagen I and collagen III, the main component of extracellular matrix of muscle cells. In addition, Sfrp1 remarkably reduced the expression of MyoFB marker protein α-SMA and inhibited the transformation of fibroblasts into myofibroblasts.

Based on the above results, we speculated that Srfp1 plays an important role in regulating MF and remodeling. This will also provide a new research basis for the treatment of MF and remodeling induced by pathological factors (hypertrophic cardiomyopathy[19], MI, etc.). Next, we will also carry out *in vivo* experiments and continue to explore the role of Sfrp1. According to previous reports, Sfrp2 is upregulated after MI, but its effect on MF can only be partially suppressed. Thus, we speculate that Sfrp1 may also have a similar phenomenon.

Conclusions

Sfrp 1 can inhibit the Wnt/β-catenin signaling pathway activity of FCs, inhibit myocardial fibroblast proliferation, collagen synthesis, and MyoFB differentiation, and effectively alleviate the progress of pathological MF, thereby providing new targets for the treatment of MF and remodeling.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding Acknowledgements

The Military Commission Logistics Support Department Health Special Subject (18BJZ27); the Central Medical Ethic Committee of Chinese PLA General Hospital, Beijing, China (approval No. S2014-065-01) and Local Institutional Review Boards.

References

1) Fares MA. Introduction: Challenges and advances in cardiovascular disease. Cleve Clin J Med 2017; 84: 11.
2) Talman V, Ruskoaho H. Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration. Cell Tissue Res 2016; 365: 563-581.
3) Zuppo DA, Tsang M. Zebrafish heart regeneration: Factors that stimulate cardiomyocyte proliferation. Semin Cell Dev Biol 2020; 100: 3-10.
4) Lam NT, Sadek HA. Neonatal heart regeneration: comprehensive literature review. Circulation 2018; 138: 412-423.
5) Nagaraju CK, Dries E, Gilbert G, Abdesselem M, Wang N, Amoni M, Driesen RB, Sipido KR. Myofibroblast modulation of cardiac myocyte structure and function. Sci Rep 2019; 9: 8879.
6) Ruiz-Villalba A, Hoppler S, van den Hoff MJ. Wnt signaling in the heart fields: Variations on a common theme. Dev Dyn 2016; 245: 294-306.
7) Stylianidis V, Hermans K, Blankesteijn WM. Wnt signaling in cardiac remodeling and heart failure. Handb Exp Pharmacol 2017; 243: 371-393.
8) Bowin CF, Inoue A, Schulte G. WNT-3A-induced beta-catenin signaling does not require signaling through heterotrimeric G proteins. J Biol Chem 2019; 294: 11677-11684.
9) Huang A, Huang Y. Role of Sfrps in cardiovascular disease. Ther Adv Chronic Dis 2020; 11: 1754193766.
10) Perumal V, Dharmarajan AM, Fox SA. The Wnt regulator SFRP4 inhibits mesothelioma cell proliferation, migration, and antagonizes Wnt3a via its netrin-like domain. Int J Oncol 2017; 51: 362-368.
11) Sklepkiewicz P, Shiomi T, Kaur R, Sun J, Kwon S, Mercer B, Bodine P, Schermuly RT, George I, Schulze PC, D'Armiento JM. Loss of secreted frizzled-related protein-1 leads to deterioration of cardiac function in mice and plays a role in human cardiomyopathy. Circ Heart Fail 2015; 8: 362-372.
12) Sklepkiewicz P, Shiomi T, Kaur R, Sun J, Kwon S, Mercer B, Bodine P, Schermuly RT, George I, Schulze PC, D'armiento JM. Loss of secreted frizzled-related protein-1 leads to deterioration of cardiac function in mice and plays a role in human cardiomyopathy. Circ Heart Fail 2015; 8: 362-372.
13) He W, Zhang L, Ni A, Zhang Z, Mirotsou M, Mao L, Pratt RE, Dzau VJ. Exogenously administered secreted frizzled related protein 2 (Sfrp2) reduces fibrosis and improves cardiac function in a rat model of myocardial infarction. Proc Natl Acad Sci U S A 2010; 107: 21110-21115.

14) Barandon L, Dufourcq P, Costet P, Moreau C, Allieres C, Daret D, Dos SP, Daniel LJ, Couffinhal T, Duplaa C. Involvement of FrzA/sFRP-1 and the Wnt/frizzled pathway in ischemic preconditioning. Circ Res 2005; 96: 1299-1306.

15) Salazar KD, Lankford SM, Brody AR. Mesenchymal stem cells produce Wnt isoforms and TGF-beta1 that mediate proliferation and procollagen expression by lung fibroblasts. Am J Physiol Lung Cell Mol Physiol 2009; 297: L1002-L1011.

16) Hahn J-Y, Cho H-J, Bae J-W, Yuk H-S, Kim K-I, Park K-W, Koo B-K, Chae I-H, Shin C-S, Oh B-H, Choi Y-S, Park Y-B, Kim H-S. Beta-catenin overexpression reduces myocardial infarct size through differential effects on cardiomyocytes and cardiac fibroblasts. J Biol Chem 2006; 281: 30979-30989.

17) Tao J, Abudoukelimu M, Ma YT, Yang YN, Li XM, Chen BD, Liu F, He CH, Li HY. Secreted frizzled related protein 1 protects H9C2 cells from hypoxia/re-oxygenation injury by blocking the Wnt signaling pathway. Lipids Health Dis 2016; 15: 72.

18) Lofdahl A, Rydell-Tormanen K, Muller C, Martina HC, Thiman L, Ekstrom G, Wenglen C, Larsson-Callerfelt AK, Westergren-Thorsson G. 5-HT2B receptor antagonists attenuate myofibroblast differentiation and subsequent fibrotic responses in vitro and in vivo. Physiol Rep 2016; 4: e12873.

19) Raman B, Ariga R, Spartera M, Sivalokanathan S, Chan K, Dass S, Petersen SE, Daniels MJ, Francis J, Smillie R, Lewandowski AJ, Ohuma EO, Rodgers C, Kramer CM, Mahmod M, Watkins H, Neubauer S. Progression of myocardial fibrosis in hypertrophic cardiomyopathy: mechanisms and clinical implications. Eur Heart J Cardiovasc Imaging 2019; 20: 157-167.