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MicroRNA-199a regulates myocardial fibrosis in rats by targeting SFRP5

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Abstract. – OBJECTIVE: Myocardial fibrosis seriously affects normal heart function. This study focused on the role of microRNA-199a in regulating rat myocardial fibrosis by targeting secreted frizzled-related protein 5 (SFRP5).

MATERIALS AND METHODS: The *in vitro* myocardial fibrosis model was established by 10 μM isoproterenol (ISO) induction in cardiac fibroblasts (CFs) for 24 h. Expression levels of microRNA-199a, collagen I and α smooth muscle actin (α-SMA) were detected by quantitative real-time polymerase chain reaction (qRT-PCR). Protein levels of SFRP5 and transforming growth factor-β1 (TGF-β1) in CFs were detected by Western blot. The bindi dition between microRNA-199a and SFR verified by luciferase reporter gene assay er transfection of microRNA-199a inhibitor or S 5 overexpression plasmid, proliferative and mi tory rates of CFs were determined by cell counti kit-8 (CCK-8) and transwell assa tively.

RESULTS: ISO treatment re regulated microRNA-199a expressi CFs. T sfection of microRNA-199a inhibit uld inh liferation, migration and ca ibrob broblast transformati CM Luciferase reporter gene assay firmed nding of microRNA-199a to S 5 3'UTR. M r. SFRP5 overexpression d the effects icroRNA-199a inhibi on eration, migration, and CMT of CFs.

CONCLU NS: MicroR a deficiency can inhibit th oliferative and mi y potentials of CFs, a l as C T by targeting SFRP5, thus exertin protec effect on myocardial fibrosis.

Key W
Microri a, M dial fibrosis, SFRP5.

Introduction

The main features of myocardial fibrosis are ive secretion and deposition of extracellular ma ECM, mainly collagen fibers) in the myocardial tissues. These pathological changes may result in card ysfunction, disorder, myocardial enhancemen diac systolic dysfu n, a duction of coronary blood flow. Uncontrolled c injuries finally lead to mali rrhythmia a cardial infarction[1-3]. fibroblasts (CF are important sourc- f ECM during the fibrotic progression. The notype of ac ted and proliferated CFs si- aneously t forms into myofibroblasts, w is called T. ECM is abundantly secreted a during CMT, in which type I and type collagen are excessively synthesized t less degraded[4,5]. Massive deposition of ECM leads to the proliferation of CFs. The ain ector cells of myocardial fibrosis are myofibroblasts with a great abundance of α smooth muscle actin (α-SMA), and they also secrete a large amount of collagen, cytokines, growth factors, etc.[6-8]. Therefore, CFs exert a key role in the development of fibrosis.

MicroRNAs are a class of endogenous, non-coding RNAs consisting of approximately 22 bases. They participate in the post-transcriptional regulation of mRNA translation or degradation by binding to the 3'UTR of the target microRNA, thus mediating various biological processes, such as cell proliferation, differentiation, and apoptosis[9-11]. In recent years, microRNA is confirmed to be closely related to fibrosis. Certain microRNAs can regulate most of the fibrosis-related signaling pathways, such as transforming growth factor-β (TGF-β), mitogen-activated protein kinase (MAPK) and epithelial-mesenchymal transition (EMT) pathways[12-14]. Therefore, microRNAs may be utilized as key regulators in the development of cardiac fibrosis-related diseases.

Overexpression of miR-24 in CFs can inhibit expressions of fibrosis-related genes, and CMT[15]. Moreover, miR-214, miR-29, and miR-133a are reported to alleviate myocardial fibrosis by inhibiting the target genes TGFβ1, matrix

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metalloprotein-2 (MMP2), and connective tissue growth factor (CTGF), respectively[16-18]. Celastrol-induced downregulation of miR-21 and phosphorylated extracellular signal-regulated kinase (ERK) inhibits the miR-21/ERK axis, thus preventing myocardial fibrosis[19]. MiR-1 has a high myocardial specificity and shows a highest abundance relative to other microRNAs in the heart, accounting for 40%. A relative study[20] found higher serum level of miR-1 in rats with myocardial fibrosis, suggesting that miR-1 may serve as a novel hallmark for clinical evaluation of acute myocardial infarction.

The pathogenesis of myocardial fibrosis is complicated and has not been completely explained. Effective treatment for myocardial fibrosis is still lacked[21]. Therefore, it is urgent to develop a novel approach for prevention, diagnosis, and treatment of myocardial fibrosis. Current studies on the function of microRNA-199a in myocardial fibrosis are rarely reported. In this paper, we found out that microRNA-199a was highly expressed in rat myocardium with myocardial remodeling. We aim to elucidate the molecular mechanism of microRNA-199a in regulating myocardial fibrosis.

Materials and Methods

Reagents

Isoproterenol (ISO) adm (South-west Pharmaceutical, Co D, ngqing, China); Dulbecco's Mo d Eag dium (DMEM; Thermo Fishe nti MA, USA); Fetal bo se BS; GIBCO BRL, Grand Islan Y, USA sin (Wisent, Canada); ectamine™ 2 TRIzol (Invitrogen, CA, USA), MicroRNA-199a inh or, ov RP5 (GenePharma, Shanghai ina).

Isola and ulture of Primary CF om N atal Rats

with days old were sacrificed a horaci ity were cut open in an ted T pex was taken and quickly ed in p d phosphate-buffered saline (PB or 3-5 ti s. Apex tissues were cut in 1 m for 6-8 times with 5 min each, t rmi d with DMEM containing 10% S. The filtered suspension was centrifuged g for 5 min. The precipitate was cultured in tle for 90 min, and those adherent cells were CFs. Cell passage was performed until 80% of confluence. Fourth-generation CFs were harvested for establishing the myocardial fibrosis model with 10 μM ISO treatme This study was approved by the A al Ethi Committee of Southwest Med University Animal Center.

Transfection

50 pmol of microR 99a inhibitor μL of LipoFiterTM mixed 250 μL serum-free mediu y we ixed together and stand at roo te or 20 . The mixture was s ed fo cultu d fresh medium was aced 4-6 h

Cell Co ng (CCK-8)

Transfected CFs h were incubated with 10 μ for another These cells were in- o d in the 96-well pla with 1×10^4 cells per and subjected to viability determination at appointed ti points. Before determination 0 nm wavel h, 10 μL of CCK-8 (Dojindo, K moto, Jap was supplied per well for 3 h incu

Transwell Assay

fected CFs for 36 h were incubated th μM ISO for another 24 h and prepared for suspension. 100 μL of cell suspension was applied in the transwell chamber, which was inserted in a 24-well plate with DMEM containing 20% FBS. At 48 h later, methanol fixation for 30 min and violet crystal dye for 20 min were performed for those penetrating cells, which were finally captured using a microscope for counting.

Luciferase Reporter Gene Assay

HEK293 cells were co-transfected with 20 nmol/L microRNA-199a or control and 600 ng FRP5 3'UTR-pmirGLO for 36 h, respectively. Relative luciferase unit of Firefly (RLU-1) and Renilla (RLU-2) was determined for calculating the luciferase intensity as RLU-1/RLU-2.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA from tissues or cells were extracted by TRIzol, reversely transcribed into complementary deoxyribose nucleic acid (cDNA) and amplified by qRT-PCR. Relative levels of microRNA-199a, SFRP5, Collagen I, Vimentin, DDR2, α-SMA, and Tensin were calculated.

Western Blot

Total protein from cells or tissues was extracted using radioimmunoprecipitation assay (RIPA) (Beyotime, Shanghai, China) and loaded for electrophoresis. After transferring on a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) at 300 mA for 100 minutes, it was blocked in 5% skim milk for 2 hours, incubated with primary antibodies at 4°C overnight and secondary antibodies for 2 hours. Bands were exposed by enhanced chemiluminescence (ECL) and analyzed by Image Software (NIH, Bethesda, MD, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for all statistical analysis, and GraphPadPrism5.0 (La Jolla, CA, USA) was used for figure editing. Data were represented as mean ± SD. The *t*-test and chi-square test were used for analyzing measurement and categorical data, respectively. $p<0.05$ indicated the significant difference.

Results

MicroRNA-199a Expression Inc in ISO-Treated CFs

Here we established the *in vitro* myocardial fibrosis model by ISO t in CFs. QRT-PCR was conducted to deter mRNA levels of microRNA-19 SFRP5, I, and α-SMA in CFs ed with or ISO. MicroRNA-19 expres increas while SFRP5 expr n de sed af r ISO treatment (Figu Moreov brosis-related gen collag nd α A were upregulated SO treatm e 1C). To further ex biological tion of microRNA a, w structed microRNA-199a inhibitor and con its transfection efficacy s (Figure a fibrosis-related g protein level of GF-β1 was markedly gulated by ISO treatment, but was inhibby the t fection of microRNA-199a itor (Figur).

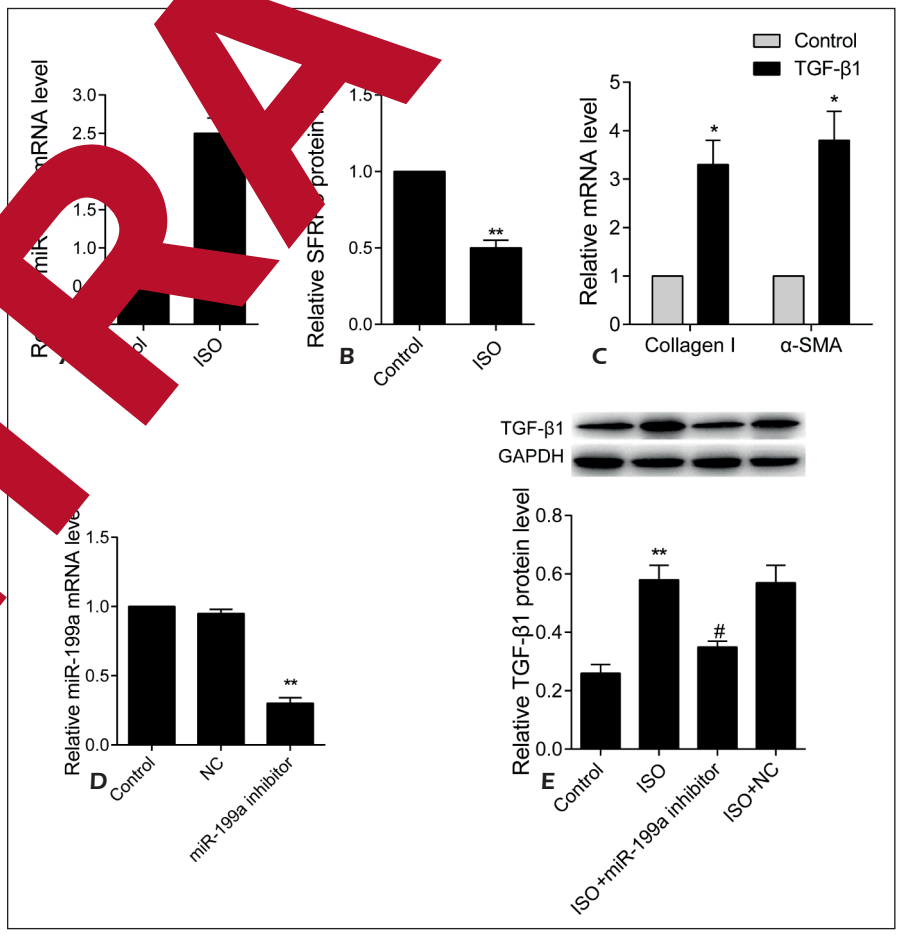


Figure 1. MiR-1 exp increased in ISO ated CFs Relative mR vel of miR-199a in CFs tr with or without ISO. **B** tive m NA level of SFR CFs tr with or with O. **C**, e mRN level and α-S in CFs th or w t **D**, Tra eff of inhit s. **E**, Wes lot analys GF-β1 in C SO-treated CFs, ISO-tre d with miR-i inhibitor ISO-treated transfected with negative l. $^{**}p<0.01$ compared with c roup; #$p<0.05$ compared with group. $^{*}p<0.05$; ISO, isoproterenol.

Knockdown of MicroRNA-199a Attenuated Proliferative, Migratory Rates and CMT of CFs

Rat CFs transfected with negative control or microRNA-199a inhibitor were treated with ISO for 0, 24, 48, and 72 h, respectively. Viability curve showed the remarkable proliferative inhibition in CFs transfected with microRNA-199a inhibitor compared with controls (Figure 2A). Subsequently, mRNA levels of fibrotic genes in CFs with ISO treatment for 72 h were determined. As the data revealed, mRNA levels of Vimentin and DDR2 increased, while mRNA levels of α-SMA and Tensin decreased by microRNA-199a knockdown, suggesting a reverse of CMT (Figure 2B). Transwell assay demonstrated that CFs transfected with microRNA-199a inhibitor showed a fewer migratory CFs than controls, indicating the inhibited migratory potential (Figure 2C, 2D).

SFRP5 Was the Target Gene of MicroRNA-199a

Both protein and mRNA levels of SFRP5 were downregulated in CFs transfected with microRNA-199a inhibitor (Figure 3A, 3B). We spec[illegible] that SFRP5 may be a potential target gene of microRNA-199a. By constructing the wild-t[illegible]nd mutant-type SFRP5 plasmids, lucifer[illegible] gene assay showed a remarkable r[illegible]tion in f[illegible] ciferase intensity of wild-type gr[illegible] confirming the binding of microRNA-199a [illegible]3'UTR of SFRP5 (Figure 3C). Hence, we c[illegible]d that SFRP5 was the target gen[illegible] microR[illegible]a.

SFRP5 Overexpres[illegible] Rev[illegible]d the Regulatory [illegible]tions of MicroRNA-1[illegible] in C[illegible]

To elucidate[illegible] poss[illegible]nctio[illegible] SFRP5 in CFs, over[illegible]ssion plasmi[illegible]RP5 (over-SFRP5) wa[illegible]ted. The CCK[illegible]say indicated no signif[illegible] di[illegible]a in cell viability between CFs co-transfected w[illegible]er-SFRP5 and microRNA-19[illegible]hibitor with [illegible] of controls (Figure 4A). However, mRNA leve[illegible] of Vimentin, DDR2, [illegible]MA, and Tensin did not markedly change after [illegible]ransfection o[illegible]r-SFRP5 and microRNA-199a [illegible]itor in CFs ([illegible]re 4B). Transwell assay failed t[illegible]l the dif[illegible]ce in the migratory rate of CFs co-t[illegible] over-SFRP5 and microRNA-199a inhibitor [illegible] those of controls (Figure 4C, 4D).

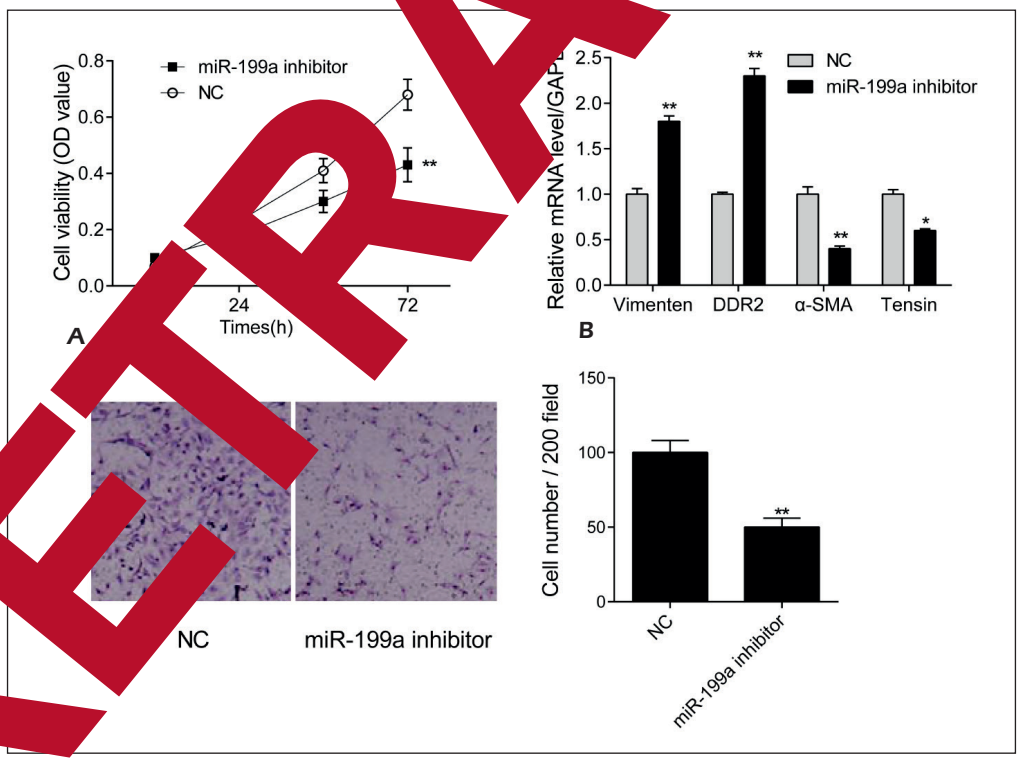
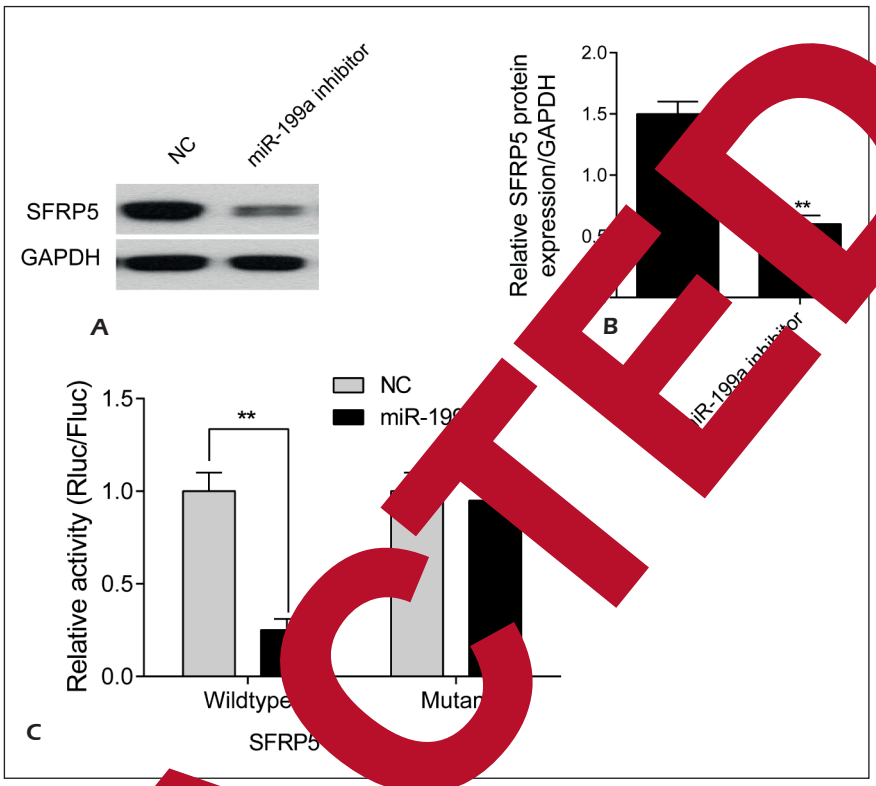


Figure 2. Knockdown of miR-199a attenuated proliferative, migratory rates and CMT of CFs. **A**, Transfection of miR-[illegible]ibitor inhibited ISO-induced proliferation in CFs. **B**, Transfection of miR-199a inhibitor increased mRNA levels of V[illegible]tin and DDR2, while decreased mRNA levels of α-SMA and Tensin in CFs. **C**, Transfection of miR-199a inhibitor inhibited ISO-induced migration in CFs. n=3, $^{**}p<0.01$ compared with NC group.



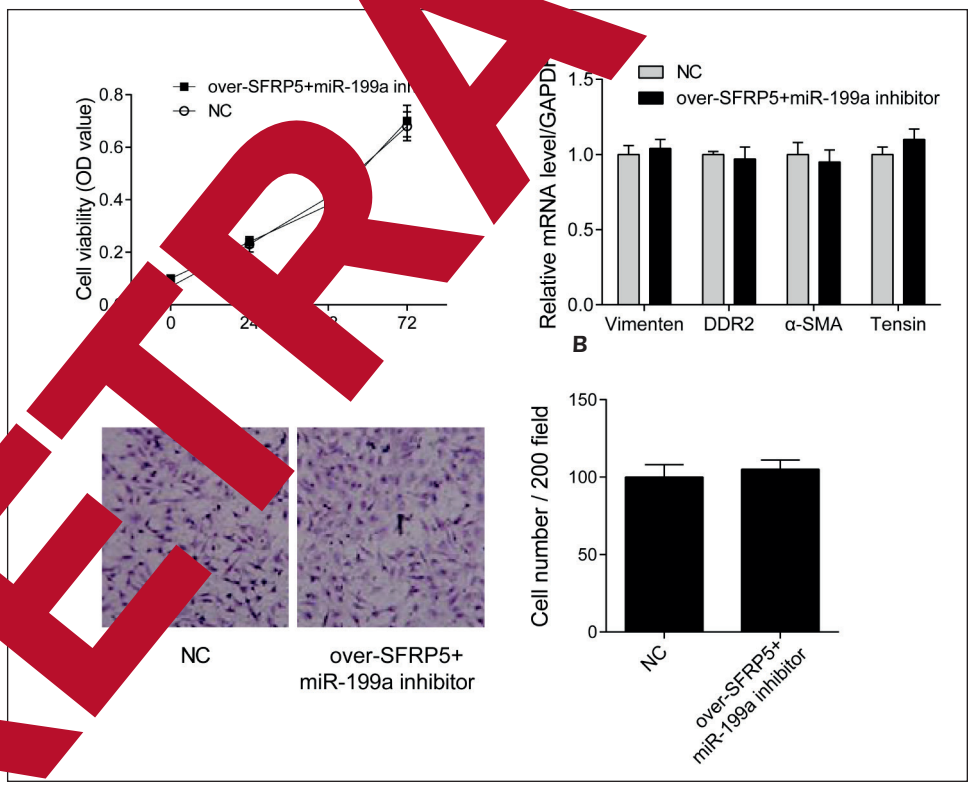


Figure 4. SFRP5 overexpression reversed the regulatory functions of miR-199a inhibitor in CFs. **A**, CCK-8 assay showed viability in CFs co-transfected with over-SFRP5+miR-199a inhibitor and controls. **B**, The mRNA levels of Vimentin, DDR2, α-SMA and Tensin in CFs co-transfected with over-SFRP5+miR-199a inhibitor and controls. **C**, Transwell assay showed migration in CFs co-transfected with over-SFRP5+miR-199a inhibitor and controls. n=3, $^{**}p<0.01$ compared with NC group.

The above data suggested that the inhibitory effects of microRNA-199a knockdown on proliferative, migratory rates and CMT of CFs were reversed by SFRP5 overexpression.

Discussion

Previous studies[22,23] have shown that microRNA-199a is downregulated in myocardial tissue of hypoxic preconditioning rats. Mice overexpressing microRNA-199a present significant cardiac hypertrophy and inhibition of cardiomyocyte autophagy. Scholars[24-26] have reported that microRNA-199a exerts its biological function by targeting the GSK3β/mTOR pathway. Srf-induced microRNA-199a knockdown suppresses phenotypic transformation and migratory potential in high-glucose treated cells[27]. Fornari et al[28] found that microRNA-199a promotes cardiomyocyte proliferation in rats with myocardial infarction. In the myocardial fibrosis, however, the specific mechanism of microRNA-199a has been rarely reported.

This work indicated that microRNA-199a was highly expressed in ISO-induced CFs, the myocardial fibrosis model. We speculate that microRNA-199a may be involved in the development of myocardial fibrosis. Subsequently, we found that TGF-β1 expression was remarkably downregulated after microRNA-199a knockdown in CFs, suggesting that microRNA-199a may promote myocardial fibrosis. Luciferase reporter gene assay further verified the binding of microRNA-199a to SFRP5 3'UTR, suggesting that SFRP5 was a target gene for microRNA-199a. Knockdown of microRNA-199a downregulate myocardial fibrosis markers Vimentin and DDR2, but upregulated fibroblast markers α-SMA and Tensin. Moreover, microRNA-199a knockdown suppressed CMT, proliferative and migratory potentials of CFs, thus protecting myocardial fibrosis.

SFRPs are antagonists of the Wnt pathway, including members in three subgroups based on sequence homology. In the SFRPs family, SFRP1, SFRP2 and SFRP5 belong to the first subgroup, SFRP3 and SFRP4 are the second subgroup, Sizzled, Sizzled2, and Crescent belong to the third subgroup[29-31]. Researches[32,33] have shown that SFRP2 regulates the Wnt pathway by competing with Frizzled, the specific receptor of the Wnt pathway, through the homologous CRD (cysteine rich domain). Chatani et al[34] found that Wnt5a enhances the proliferative and migratory rates of hepatic stellate cells, which are inhibited by SFRP5. In addition, CCL4 decreased expression and plasma level of Wnt5a, while SFRP5 knockdown greatly enhanced the degree of CCL4-induced liver fibrosis. We may conclude that SFRP5 improves fibrosis of the liver by inhibiting the Wnt5a/F axis. In our study, microRNA-199a inhibitor markedly decreased SFRP5 expression in CFs. Most importantly, SFRP5 overexpression reversed the inhibitory effects of microRNA-199a inhibitor on proliferative, migratory potentials and CMT of CFs.

To sum up, our results indicate that microRNA-199a could directly bind to SFRP5 3'UTR. Besides, microRNA-199a knockdown suppressed SFRP5 expression, and proliferative and migratory rates, and inhibited CMT of CFs, thereafter alleviating the secretion and deposition of ECM. It is believed that microRNA-199a inhibitor exerted anti-fibrosis role by targeting SFRP5.

Conclusions

We found that microRNA-199a deficiency can decrease proliferative and migratory potentials of CFs as well as CMT by targeting SFRP5, thus exerting a protective effect on the myocardial fibrosis.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Vagos M, van Herck I, Sundnes J, Arevalo HJ, Edwards AG, Koivumaki JT. Computational modeling of electrophysiology and pharmacotherapy of atrial fibrillation: recent advances and future challenges. Front Physiol 2018; 9: 1221.
2) Iwasaki YK, Nishida K, Kato T, Nattel S. Atrial fibrillation pathophysiology: implications for management. Circulation 2011; 124: 2264-2274.
3) Yue L, Xie J, Nattel S. Molecular determinants of cardiac fibroblast electrical function and therapeutic implications for atrial fibrillation. Cardiovasc Res 2011; 89: 744-753.
4) Rotini A, Martinez-Sarra E, Pozzo E, Sampaolesi M. Interactions between microRNAs and long non-coding RNAs in cardiac development and repair. Pharmacol Res 2018; 127: 58-66.
5) Tao L, Bei Y, Zhou Y, Xiao J, Li X. Non-coding RNAs in cardiac regeneration. Oncotarget 2015; 6: 42613-42622.

6) Ma ZG, Yuan YP, Wu HM, Zhang X, Tang QZ. Cardiac fibrosis: new insights into the pathogenesis. Int J Biol Sci 2018; 14: 1645-1657.
7) Penas FN, Carta D, Dmytrenko G, Mirkin GA, Modenutti CP, Cevey AC, Rada MJ, Ferlin MG, Sales ME, Goren NB. Treatment with a new peroxisome proliferator-activated receptor gamma agonist, pyridinecarboxylic acid derivative, increases angiogenesis and reduces inflammatory mediators in the heart of trypanosoma cruzi-infected mice. Front Immunol 2017; 8: 1738.
8) Koitabashi N, Arai M, Kogure S, Niwano K, Watanabe A, Aoki Y, Maeno T, Nishida T, Kubota S, Takigawa M, Kurabayashi M. Increased connective tissue growth factor relative to brain natriuretic peptide as a determinant of myocardial fibrosis. Hypertension 2007; 49: 1120-1127.
9) Zhang ZC, Wang GP, Yin LM, Li M, Wu LL. Increasing miR-150 and lowering HMGA2 inhibit proliferation and cycle progression of colon cancer in SW480 cells. Eur Rev Med Pharmacol Sci 2018; 22: 6793-6800.
10) Yang KC, Yamada KA, Patel AY, Topkara VK, George I, Cheema FH, Ewald GA, Mann DL, Nerbonne JM. Deep RNA sequencing reveals dynamic regulation of myocardial noncoding RNAs in failing human heart and remodeling with mechanical circulatory support. Circulation 2014; 129: 1009-1021.
11) Liu X, Luo G, Bai X, Wang XJ. Bioinformatic[illegible]sis of microRNA biogenesis and functio[illegible] proteins in eleven animal genomes. J[illegible]net Genomics 2009; 36: 591-601.
12) Li Y, Lu J, Bao X, Wang X, Wu J, Li X, [illegible] W. MiR-499-5p protects cardiomyocytes aga[illegible] ischaemic injury via anti-apopt[illegible]by targeti[illegible] PDCD4. Oncotarget 2016; 7[illegible]317.
13) Dakhlallah D, Zhang J, Yi[illegible]Marsh[illegible]Angelos MG, Khan M. MicroRN[illegible]a engi[illegible]ed mesenchymal stem cells[illegible]ent ca[illegible] and cell survival in the i[illegible]ea[illegible] Pharmacol 2015; 6[illegible]41-2[illegible]
14) Derrien T, Johnso[illegible]Bussotti G[illegible]A, Djebali S, Tilgner H, Gue[illegible], Martin D, M[illegible]Knowles DG, Lagarde[illegible]lli L, Ruan X,[illegible]Y, Lassmann T, Ca[illegible]i P,[illegible]JB, Lipovich L, Gonzalez JM, Thomas[illegible], Davis C[illegible]hattar R, Gingeras TR, Hubba[illegible], Notredame C,[illegible]w J, Guigo R. The GEN[illegible]DE v7 catalog of h[illegible]long noncoding RN[illegible]analysi[illegible]of their gene structure, evolution, expres[illegible]Genome Res 2012; 22: 1775-[illegible]
15) [illegible]ng W, X[illegible]Nie Y, Cao X, Meng J, Xu X, [illegible]eng Z. M[illegible]RNA-24 regulates cardiac fibrosis[illegible]m[illegible]rdial infarction. J Cell Mol Med 2012;[illegible]0-2160.
16) Cai X,[illegible]Y, Wang L, Wang S, Li Y, Fu Y, [illegible]Y, Su G. Adiponectin upregulates miR-133a [illegible]ypertrophy through ampk activation and reduced ERK1/2 phosphorylation. PLoS One 2016; 11: e148482.
17) Yang HY, Tu YS, Long J, Zhang HQ, Qi CL, Xie XB, [illegible]H, Zhang YJ. SRF-miR29b-MMP2 axis inhibits [illegible]CLC invasion and metastasis. Int J Oncol 2015; 47: 641-649.
18) Khan QE, Sehic A, Khuu C, Risnes S, Osmundsen H. Expression of Clu and Tgfb1 during murine tooth development: effects of in-viv[illegible]tion with anti-miR-214. Eur J Oral[illegible]303-312.
19) Cheng M, Wu G, Song Y, Wang[illegible]u L, Zhang L, Zhang C. Celastrol-induced s[illegible]sion of the miR-21/ERK signalling pathway a[illegible]s cardiac fibrosis and dysfunctio[illegible]Cell Phy[illegible]chem 2016; 38: 1928-1938.
20) Qiu J, Wang A, Xu Y[illegible]o S, A[illegible]J, Li H, [illegible]C. [Role of micro[illegible]1-medi[illegible]AMP-activated protein kinas[illegible]way i[illegible]diac fibroblasts induced by high g[illegible]i[illegible]8]. Zhon[illegible]a Wei Zhong Bing[illegible]Yi[illegible]8; 30: 1[illegible]50.
21) Fang L, Mi[illegible]AJ, Dart[illegible]cli[illegible]perspective of a[illegible]rotic therapie[illegible]diovascular diseas[illegible]harmacol 201[illegible]186.
22) Zhan[illegible]Zhu[illegible]g C, Liu W, Zhu J. [miR-199a-5p in[illegible]its the p[illegible]tion of rat airway smooth muscle cells and t[illegible]pression of hypoxia inducible factor 1 alpha[illegible]hypoxia conditions]. [illegible]Bao Yu Fen Zi Mian Yi Xue Za Zhi 2015; 31: 1183-1188.
[illegible] Gonsalves C[illegible]alra VK. Hypoxia-mediated expression of 5[illegible]xygenase-activating protein involves HIF-1[illegible]a and NF-kappaB and microR-[illegible]135a[illegible]199a-5p. J Immunol 2010; 184: [illegible]
24) Fu J,[illegible]Li L, Tian Y, Liu Y, Gu Y, Wu J. miR-199a-3p is involved in estrogen-mediated autophagy through the IGF-1/mTOR pathway in osteocyte-like MLO-Y4 cells. J Cell Physiol 2018; 233: 2292-2303.
25) Zhang Y, Huang B, Wang HY, Chang A, Zheng X. Emerging role of microRNAs in mTOR signaling. Cell Mol Life Sci 2017; 74: 2613-2625.
26) Li Z, Song Y, Liu L, Hou N, An X, Zhan D, Li Y, Zhou L, Li P, Yu L, Xia J, Zhang Y, Wang J, Yang X. miR-199a impairs autophagy and induces cardiac hypertrophy through mTOR activation. Cell Death Differ 2017; 24: 1205-1213.
27) Che M, Shi T, Feng S, Li H, Zhang X, Feng N, Lou W, Dou J, Tang G, Huang C, Xu G, Qian Q, Sun S, He L, Wang H. The microRNA-199a/214 cluster targets E-cadherin and Claudin-2 and promotes high glucose-induced peritoneal fibrosis. J Am Soc Nephrol 2017; 28: 2459-2471.
28) Fornari F, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L, Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2010; 70: 5184-5193.
29) Cheng YY, Mok E, Tan S, Leygo C, McLaughlin C, George AM, Reid G. SFRP tumour suppressor genes are potential plasma-based epigenetic biomarkers for malignant pleural mesothelioma. Dis Markers 2017; 2017: 2536187.
30) Maekawa T, Kulwattanaporn P, Hosur K, Domon H, Oda M, Terao Y, Maeda T, Hajishengallis G. Differential expression and roles of secreted frizzled-related protein 5 and the wingless homolog wnt5a in periodontitis. J Dent Res 2017; 96: 571-577.

31) Fontenot E, Rossi E, Mumper R, Snyder S, Siamakpour-Reihani S, Ma P, Hilliard E, Bone B, Ketelsen D, Santos C, Patterson C, Klauber-DeMore N. A novel monoclonal antibody to secreted frizzled-related protein 2 inhibits tumor growth. Mol Cancer Ther 2013; 12: 685-695.
32) Li X, Lu P, Li B, Zhang W, Luo K. Effects of iodine-125 seeds on the methylation of SFRP2 and P16 in colorectal cancer. Exp Ther Med 2013; 6: 1225-1228.
33) Liu LB, Chen XD, Zhou XY, Zhu Q. The Wnt antagonist and secreted frizzled-related protein 5: implications on lipid metabolism, in[illegible] and type 2 diabetes mellitus. Bio[illegible] 38: BSR20180011.
34) Chatani N, Kamada Y, Kizu T, Ogur[illegible]uruta K, Egawa M, Hamano M, Ezaki H, Kiso S, [illegible]A, Ouchi N, Yoshida Y, Takehara T. Secreted friz[illegible]ted protein 5 (Sfrp5) decreases h[illegible]tic stella[illegible]ctivation and liver fibrosis. Li[illegible]t 2015; 35: 2[illegible]