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Original Article

Role of SDF-1 and Wnt signaling pathway in the myocardial fibrosis of hypertensive rats

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Abstract: Objective: To investigate the effects of stromal cell-derived factor-1 (SDF-1) and Wnt signaling pathway on the bioactivities of myofibroblasts (MFs) and the expressions of SDF-1 and components of Wnt signaling pathway in the myocardium of spontaneously hypertensive rats (SHR). Methods: BMSCs were induced to differentiate into MFs *in vitro*, and SDF-1 and Wnt signaling pathway were independently or simultaneously blocked. Then, the migration of MFs and the secretion of Col I and α-SMA were determined in MFs. Heart function, progression of myocardial fibrosis and structure of the heart were evaluated. The expression of SDF-1 and components of Wnt signaling pathway in SHR was detected. Results: TGF-β could induce the differentiation of BMSCs into B-MFs; Blocking SDF-1/CXCR4 axis and/or Wnt signaling pathway was able to inhibit the MFs migration and Col I secretion; Blocking Wnt signaling pathway inhibited the differentiation of BMSCs into MFs; Serum SDF-1 increased with the increase in blood pressure, and serum β-catenin elevated with the fluctuation of blood pressure; Protein and mRNA expressions of SDF-1 in the myocardium increased, and those of DKK-1 (an inhibitor of Wnt signaling pathway) and GSK-3 reduced in SHR. Conclusion: SDF-1 and Wnt signaling pathway are involved in the differentiation of BMSCs into MFs, as well as the migration and collagen secretion of MFs; Hypertension affects the expressions of SDF-1 and components of Wnt signaling pathway. In the myocardium of SHR, SDF-1 expression increases, but the expression of inhibitor of Wnt signaling pathway reduces.

Keywords: Stromal cell-derived factor-1, myofibroblasts, Wnt signaling pathway, spontaneous hypertensive rat, myocardial fibrosis

Introduction

A variety of studies have confirmed that persistent hypertension may increase heart load and cause myocardial hypertrophy and sclerosis, resulting in diastolic heart failure (DHF) characterized by normal ejection fraction [1, 2].

In the hypertension induced DHF, myocardial fibrosis is an important pathology of heart disorders, and long-lasting hypertension may significantly increase the afterload and the oxygen consumption of the heart, and cause myocardial hypertrophy, leading to pathological features (such as oxygen insufficiency of the coronary artery). Moreover, pro-inflammatory cytokines and chemokins increase markedly, which together with above pathology may further cause compensatory myocardial fibrosis [3, 4]. Thus, to investigate the pathogenesis of hypertension induced myocardial fibrosis is clinically important to delay the progression of heart failure and improve the prognosis of DHF patients.

Stromal cell-derived factore-1 (SDF-1) is able to bind to its receptor CXCR4, which is involved in numerous physiological processes [5, 6]. Under the pathological conditions, SDF-1 has a potent capability to induce cell chemotaxis, which may induce the migration of CXCR4 positive cells to the injured sites for tissue repair. Studies have confirmed that SDF-1/CXCR4 axis plays an important role in the angiogenesis, myocardial repair and myocardial fibrosis. Wingless related protein (Wnt) [7] signaling pathway is crucial for the proliferation and differentiation of cells. A variety of studies demonstrate that Wnt signaling pathway plays a key role in the heart development and myocardial fibrosis. In patients

with heart failure, the expression of components of Wnt signaling pathway reduces.

To date, few studies have been conducted to investigate the role of both Wnt signaling pathway and SDF-1/CXCR4 in the pathogenesis of myocardial fibrosis and their interaction. In the present study, BMSCs were induced *in vitro* to differentiate into myofibroblasts (MFs), and the migration and secretion of Col I and α-SMA were determined in bone marrow myofibroblasts (B-MFs) following blocking of SDF-1 and/or Wnt signaling pathway. In addition, the progression of myocardial fibrosis and its influence on the heart structure and function were observed in hypertensive rats, and the effects of hypertension on the protein expressions of SDF-1 and components of Wnt signaling pathway as well as the MFs were also explored in rats.

Materials and methods

Experimental animals

SD rats aged 3 weeks, spontaneously hypertensive rats (SHR) aged 6 weeks and healthy Wistar rats with normal blood pressure (Wistar Kyoto rat, WKY) were purchased from Shanghai SLAC Experimental Animal Co., Ltd (license No: SCXK[Hu]2004-2005; use No: SYXK[Hu]2007-0007).

All the animals were housed in specific pathogen free (SPF) animal center of the Affiliated Ninth People's Hospital of Shanghai Jiaotong University. All animal procedures were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals of China.

Methods

Culture and identification of bone marrow mesenchymal stem cells (BMSCs): SD rats aged 3 weeks were sacrificed by cervical dislocation, and the femur and tibia were collected and flushed with L-DMEM (Hyclone, USA). The filtrates were filtered and centrifuged at 1500 rpm/min at 37°C for 5 min. Cells were collected and re-suspended in L-DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 U/ml streptomycin, followed by incubation at 37°C in a humidified environment with 5% CO_2. Cells of the 3rd passage were subjected to flow cytometry for CD29, CD45 and CD90 (BDbiosciences, USA) for cell identification.

Induced differentiation of BMSCs into MFs and identification of MFs: BMSCs of the 3rd passage were seeded into 6-well plates at a density of 5×10^4/well. For induced differentiation, cells were incubated with H-DMEM containing 20% FBS and 5 μg/L TGF-β1 for 2 weeks. In control group, cells were incubated with L-DMEM containing 10% FBS for 2 weeks. Cells were identified by immunofluorescence staining. The primary antibody was anti-rat α-SMA antibody (Abcam, USA; 1:100), and the fluorescent secondary antibody was kindly provided by the Tissue Engineering Laboratory of the Affiliated 9th People's Hospital of Shanghai Jiaotong University. Cells were observed under an inverted fluorescence microscope.

Scratch wound healing assay: B-MFs were maintained in dishes (0.5×10^6) in DMEM containing 1% FBS and then divided into 6 groups: (1) blank control group: cells were treated with PBS; (2) cells were treated with 100 ng/ml SDF-1; (3) cells were treated with 100 ng/ml SDF-1 and 0.5 μg/ml AMD3100 (an inhibitor of CXCR4); (4) cells were treated with 100 ng/ml SDF-1 and 0.5 μg/ml ICG-001 (an inhibitor of Wnt signaling pathway); (5) cells were treated with 100 ng/ml SDF-1, 0.5 μg/ml AMD3100 and 0.5 μg/ml ICG-001. At 0, 12, 24, 36 and 48 h after incubation, the migration of B-MFs and wound healing were observed and photographed under a microscope, and data were compared at 0 h and 48 h.

Detection of mRNA expressions of Col I and α-SMA in MFs: Following induction, BMSCs were processed for the detection of mRNA expressions of Col I and α-SMA. Primers used for PCR were as follows: (1) Col I (182 bp): F 5'-GGAGAGAGTGCCAACTCCAG-3'; R 5'-GTGCTTTGGAAAATGGTGCT-3'; (2) a-SMA (120 bp): F 5'-CCGAGATCTCACCGACTACC-3'; R 5'-TCCAGAGCGACATAGCACAG-3'. PCR conditions were as follows: Pre-denaturation at 95°C for 3 min, 32 cycles of denaturation at 95°C for 45 s, annealing at 59°C for 45 s and extension at 72°C for 60 s, and a final extension at 72°C for 10 min.

Measurement of blood pressure: Non-invasive arterial blood pressure measurement and analysis system (PowerLab) was used to measure the blood pressure of the tail vein of rats.

Measurement was done thrice and an average was obtained.

Heart ultrasonography of SHR and WKY rats: Echocardiography was performed in the Cardiovascular Department of Medical School of Fudan University to monitor the structure and function of the heart. Blood pressure was measured once every 2 weeks until 20 weeks old.

Masson staining of myocardium and measurement of collagens: Rats aged 20 weeks were anesthetized with chloral hydrate at 3 ml/kg, and the heart was collected and rapidly washed with normal saline. The apical heart was harvested and fixed in 4% paraformaldehyde over night. Tissues were embedded in paraffin and cut into 4-μm consecutive sections, followed by Masson staining.

Measurement of collagen area: Five fields were randomly selected from each section at a magnification of 100×, and images were captured for analysis with Image J analysis system. The mean proportion of collagen area was calculated: (collagen area/myocardial area) ×100%.

Detection of serum β-catenin and SDF-1 by enzyme-linked immunosorbent assay (ELISA): Blood (2 ml) was collected from SHR and WKY rats once every 2 weeks since week 6, and serum contents of β-catenin and SDF-1 were measured by ELISA according to the manufacturer's instructions (USCN, China).

Detection of mRNA expressions of Col I and α-SMA by PCR: Total RNA was extracted according to the manufacturer's instructions. The primers and conditions for PCR were described above.

Detection of mRNA expressions of SDF-1, β-catenin, DKK-1 and GSK-3β by Western blot: Tissues were lysed according to the manufacturer's instructions. The primary antibodies included rabbit anti-rat SDF-1 polyclonal antibody (Abcam, USA), rabbit anti-rat β-catenin monoclonal antibody (CST, USA), rabbit anti-rat DKK-1 monoclonal antibody (Epitomics, USA), and rabbit anti-rat GSK-3βmonoclonal antibody (CST, USA) (1:1000). The secondary antibody was horseradish peroxidase conjugated goat anti-rabbit IgG (Tissue Engineering Laboratory of the Affiliated 9th Hospital of Shanghai Jiaotong University).

Statistical analysis

Data are expressed as mean ± standard deviation ($\bar{X} \pm sd$). Intergroup comparisons were done with one way analysis of variance, and comparisons between two groups with LSD-t test. If heterogeneity of variance was present, Kruskal-WallisH test was used for comparisons among groups and Mann-Whitney U for comparisons between two groups. Multiple testing correction was done with Bonferroni correction. A value of $P<0.05$ was considered statistically significant. Statistical analysis was done with SPSS version 16.0.

Results

Culture and identification of BMSCs

CD29 and CD90 are specific antigens on MSCs. Flow cytometry showed BMSCs of the 3rd passage showed high expressions of CD29 and CD90. The proportions of CD29 and CD90 positive cells were 84.3% and 94.2%, respectively. However, the proportion of CD45 (a surface antigen of hematopoietic stem cells) positive cells was only 6.2%. These findings suggest that the purity of BMSCs meets the requirements of a study.

Induced differentiation of BMSCs into B-MFs and identification of B-MFs

BMSCs have the pluripotent potential. TGF-β and serum at a high concentration may induce the differentiation of BMSCs into MFs. Immunofluorescence staining showed the proportion of α-SMA positive cells was up to 95% in cells after TGF-β treatment, which was significantly higher than in control group ($P<0.05$). qRT-PCR showed the mRNA expressions of Col I and α-SMA in TGF-β treated cells were dramatically higher than in untreated BMSCs ($P<0.05$). These findings indicate that BMSCs are successfully induced to differentiate into B-MFs (**Figure 1**).

Role of SDF-1/CXCR4 and Wnt signaling pathway in the migration of B-MFs

At 48 h after scratching a wound, SDF-1 significantly promoted the migration of MFs as compared to untreated cells (*$P<0.05$). In addition, after blocking of SDF-1/CXCR4 with AMD3100,

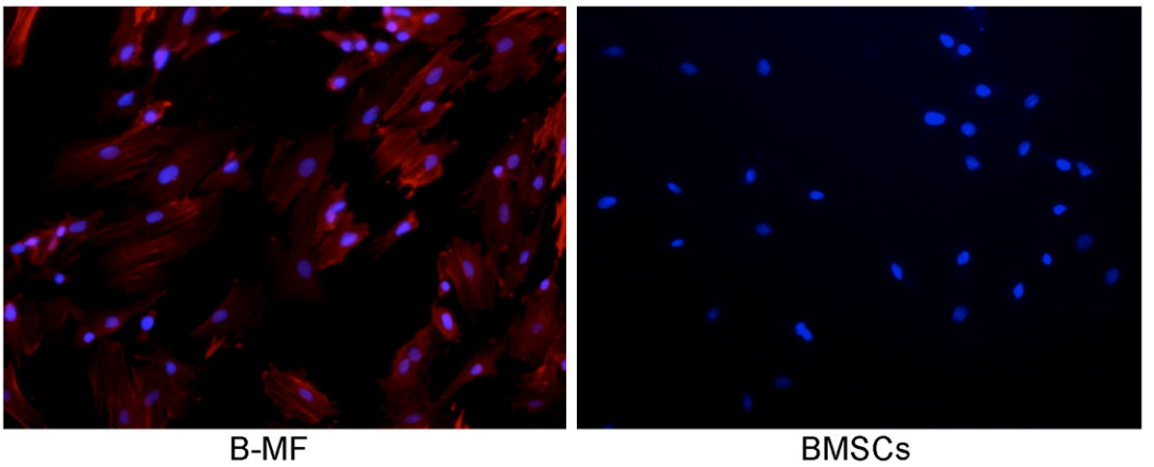

Figure 1. TGF-β1 induced differentiation of BMSCs into MFs (immunofluorescence staining; 100×).

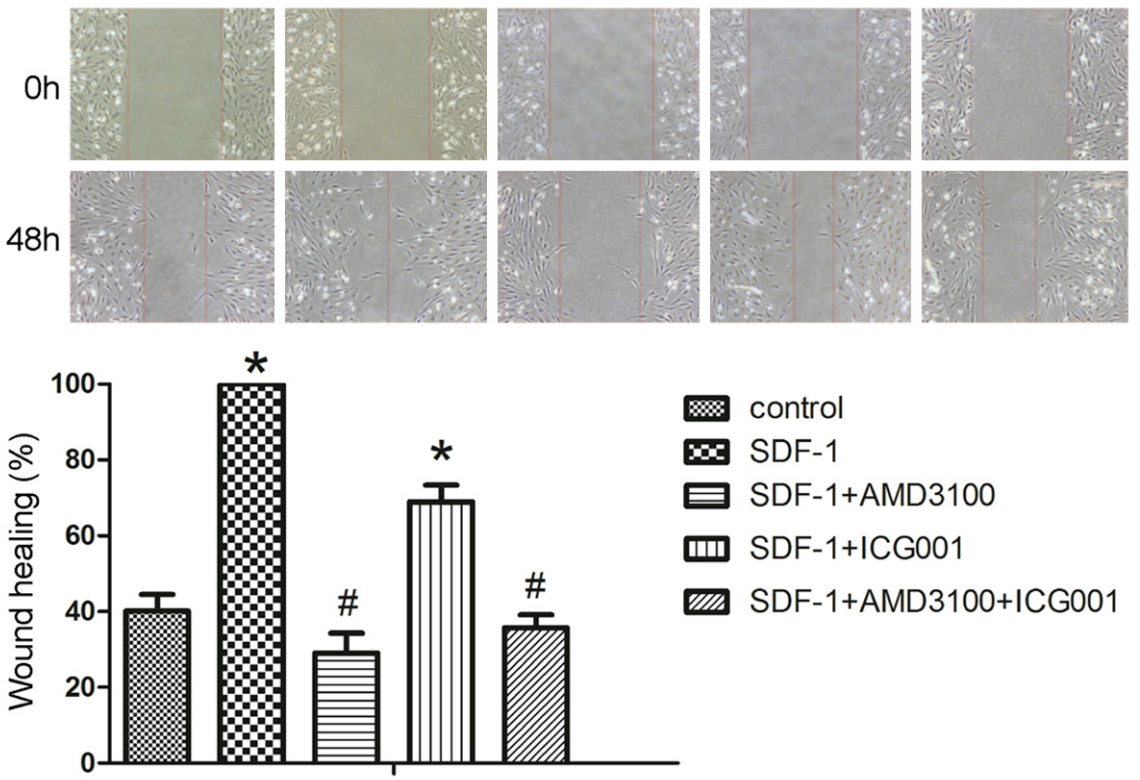

Figure 2. Cell migration determined by scratch wound healing assay (100×). *P<0.05, #P<0.01.

the cell migration was inhibited markedly (#P<0.01); following blocking of Wnt signaling pathway with ICG-001, the migration of B-MFs was also inhibited significantly (*P<0.05), but the inhibitory effect of ICG-001 on the cell migration was inferior to that of AMD3100 (**Figure 2**).

Effects of SDF-1/CXCR4 and Wnt signaling pathway on the differentiation of BMSCs into B-MFs and sectetion of collagens in B-MFs

qRT-PCR was employed to detect the Col I mRNA expression. When compared with control group, AMD3100 or ICG-001 treatment signifi-

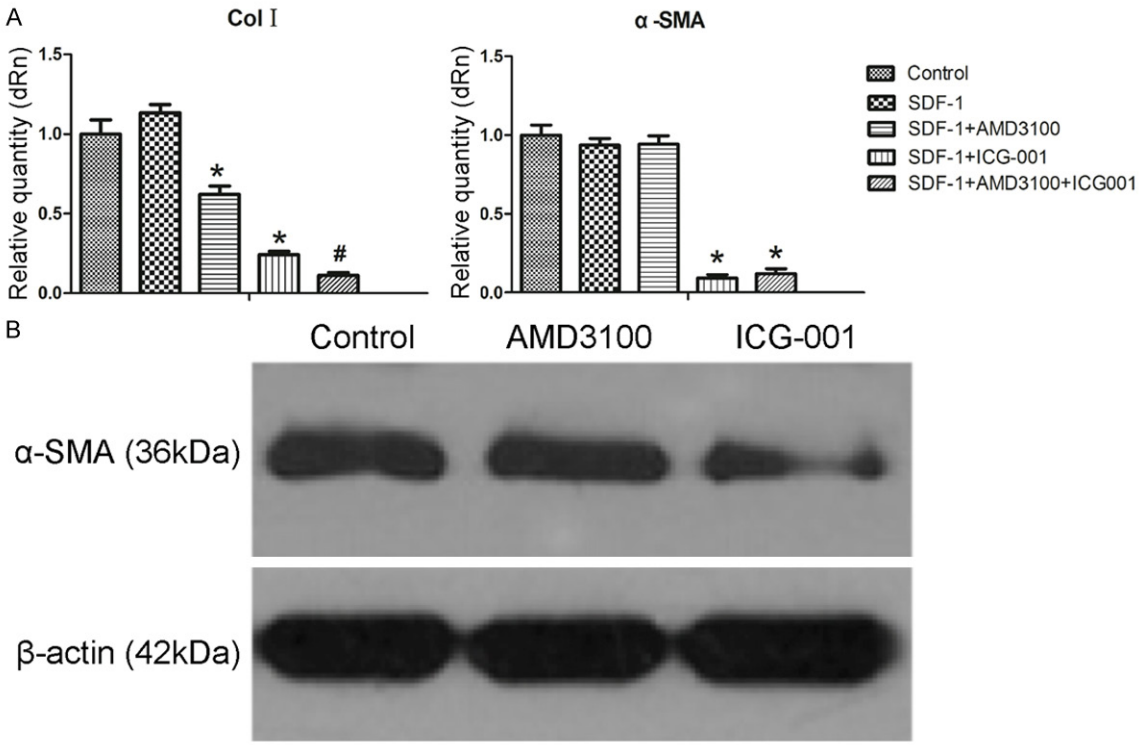

Figure 3. A. mRNA expressions of α-SMA and Col I in B-MFs following blocking of SDF-1/CXCR4 and/or Wnt signaling pathway. *P<0.05, #P<0.01. B. α-SMA protein expression in B-MFs following blocking of SDF-1/CXCR4 and/or Wnt signaling pathway.

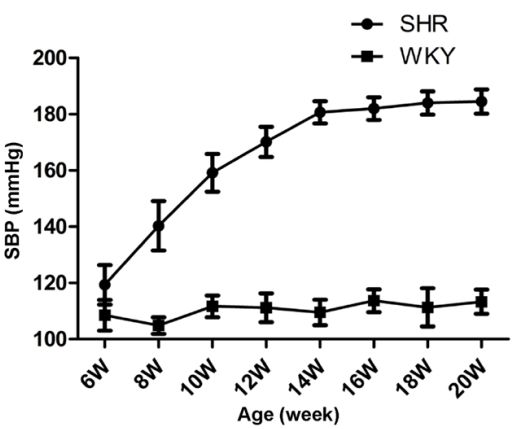

Figure 4. Systolic blood pressure in SHR and WKY rats.

cantly reduced the mRNA expression of Col I (*P<0.05), and this reduction was more obvious in cells treated with both AMD3100 and ICG-001 (#P<0.01). In addition, ICG-001 treated cells showed significantly reduced α-SMA mRNA expression when compared with control group (*P<0.05), but α-SMA mRNA expression remained unchanged following AMD3100 treatment (**Figure 3A**). Western blot assay displayed similar findings to PCR: α-SMA expression in ICG-001 treated cells reduced signifiacntly (**Figure 3B**).

Systolic blood pressure in SHR

Non-invasive monitoring of blood pressure of the tail vein showed the systolic blood pressure was 119 mmHg and 109 mmHg in SHR aged 6 weeks and WKY rats aged 6 weeks, respectively, showing no marked difference. Since week 8, the blood pressure increased in SHR and reached a peak at week 14, but the systolic blood pressure remained stable in WKY rats without significant fluctuation (**Figure 4**).

Effects of chronic hypertension on the structure and function of the heart and the myocardial fibrosis in rats

Structure and function of the heart: The structure and function of the SHR aged 6 weeks and WKY rats aged 6 weeks were comparable. At

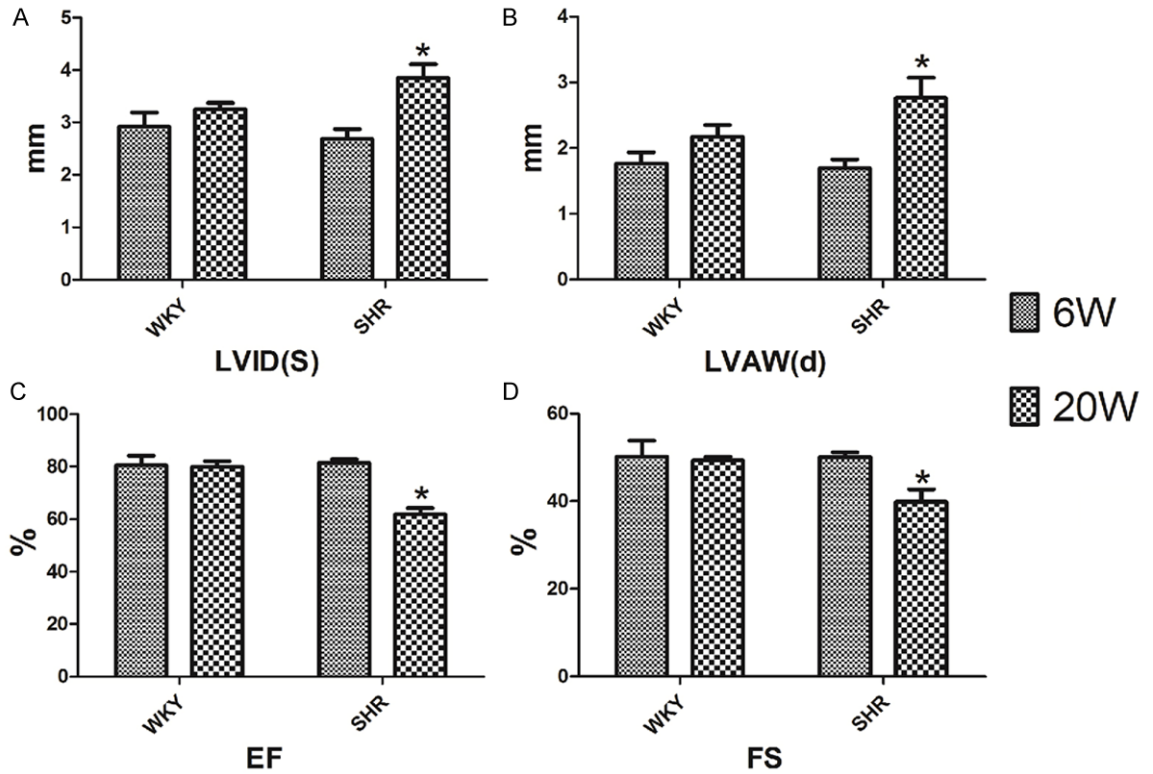

Figure 5. Structure and function of the heart of SHR and WKY. A. LVID(s) of WKY rats and SHR at weeks 6 and 20; B. LVAW(d) of WKY rats and SHR at weeks 6 and 20; C. EF of WKY rats and SHR at weeks 6 and 20; D. FS of WKY rats and SHR at weeks 6 and 20.

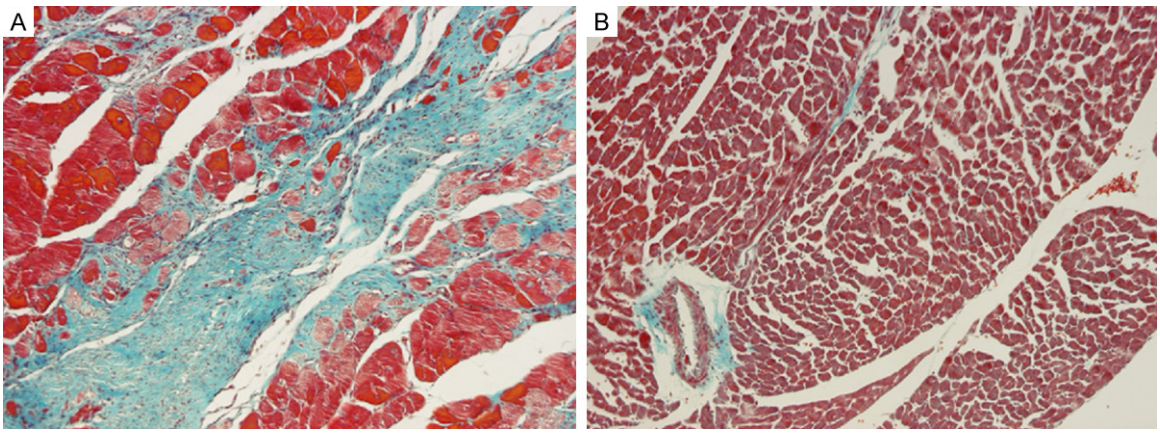

Figure 6. A. Masson staining of the myocardial fibers of SHR (100×); B. Masson staining of the myocardial fibers of WKY rats (100×).

week 20, the left ventricular internal diameter in the end of systole (LVID[s]) increased, left ventricular anterior wall in the end of diastole (LVAD[d]) elevated, ejection fraction (EF) reduced and fraction shortening (FS) decreased significantly in SHR when compared with WKY rats (*P<0.05) (**Figure 5**).

Myocardia fibrosis: The heart was collected and processed for Masson staining. In WKY rats aged 20 weeks, the myocardial fibers showed regular and dense arrangement and only a few collagens in the myocardial interstitium. In SHR, the myocardial fibers were enlarged and showed disordered arrangement,

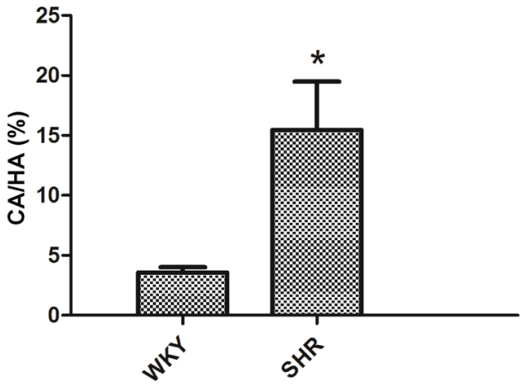

Figure 7. Proportion of collagen area to myocardial area in SHR and WKY rats. *P<0.05.

and a large amount of collagens was found in the myocardial interstitium. IPP image analysis system was employed to determine the proprotion of collagen area in the myocardium (Collagen Area/Heart Area, CA/HA). Results showed the CA/HA in SHR aged 20 weeks was significantly higher than in WKY rats (*P<0.05) (**Figures 6** and **7**).

Serum levels of SDF-1 and β-catenin determined by ELISA: Serum was collected from SHR and WKY rats at different ages, and ELISA was employed for the detection of serum contents of SDF-1 and β-catenin. Results showed serum SDF-1 content in SHR was significantly higher than in WKY rats (*P<0.05). Of note, the serum SDF-1 level increased over age in both SHR and WKY rats (**Tables 1** and **2**; **Figure 8**). Serum β-catenin level remained relatively stable in SHR aged between 6 weeks and 12 weeks, but was significantly higher than in WKY rats (*P<0.05). Since week 14, the serumβ-catenin level reduced and had no significant difference as compared to WKY rats (**Figure 8**).

Protein expressions of SDF-1 and components of Wnt signaling pathway in SHR: Western blot assay was employed to detect the protein expressions of SDF-1 and components of Wnt signaling pathways. Results showed the SDF-1 protein expression in the myocardium of SHR aged 20 weeks was signifiantly higher than in WKY rats aged 20 weeks, but β-catenin protein expression was comparable between them. However, the protein expressions of DKK-1 and GSK-3β (inhibitors of Wnt signaling pathway) in the myocardium reduced significantly in SHR as compared to WKY rats (**Figure 9A**).

Real time PCR was employed to detect the mRNA expressions of SDF-1 and components of Wnt signaling pathway. Results showed the mRNA expressions of SDF-1 and β-catenin in SHR aged 20 weeks increased significantly when compared with WKY rats aged 20 weeks (P<0.05). However, the protein expressions of DKK-1 and GSK-3β in the myocardium reduced significantly in SHR as compared to WKY rats (**Figure 9B**).

Discussion

Heart failure (HF) is a major disease threatening the human health in the 21st century. In China, about 6 million people is affected by HF, the mortality of hospitalized patients with HF accounts for 40% of deaths due to cardiovascular diseases, and the mortality of HF patients is significantly higher than the overall mortality of patients with cardiovascular diseases. Although HF is a final outcome of some cardiovascular diseases, persistent hypertension is one of important causes of HF. Long-lasting hypertension may increase the heart load and lead to myocardial hypertrophy and myocardial sclerosis, resulting in DHF characterized by normal ejection fraction [8]. The clinical manifestations of DHF are insidious and thus DHF is usually neglected in clinical practice. Usually, DHF deteriorates during the therapy of other diseases, which significantly affects the quality of life of these patients.

Myocardial fibrosis serves as a compensatory response to myocardial injury [9] and may be found in a variety of physiological and pathological processes. Myocardial fibrosis is characterized by increased secretion of extracellular matrix (ECM), excess deposition of ECM between myocytes, and significant increase in MFs in the myocardium. ECM in the myocardium is composed of 5 types of collagens of which type I and III collagens are the major ones. Type I collagen (Col I) accounts for 80% of collagens in the myocardium and type III collagen for 11%. Myocardial fibrosis may increase the ventricular stiffness, reduce the ventricular compliance and limit the diastolic filling, resulting in heart dysfunction and final DHF [10].

MFs are a group of fibroblast-like cells expressing α-smooth muscle actin (α-SMA). They possess the characteristics of both smooth muscle cells (contraction) and fibroblasts (secretion of

Table 1. Serum contents of SDF-1 (ng/ml) in WKY rats and SHR (ELISA; $\bar{X} \pm sd$)

Age (W)	WKY	SHR
6 W	1.430101±0.107454	1.571563±0.200642*
8 W	1.508579±0.137066	2.108485±0.138686*
10 W	1.657477±0.190492	2.105343±0.370706*
12 W	1.742595±0.151389	2.338425±0.594352*
14 W	1.441101±0.237388	2.522901±0.168887*
16 W	2.052045±0.31335	2.294769±0.307902*
18 W	1.813395±0.443052	2.096089±0.169467*
20 W	2.264304±0.272403	2.969256±0.301703*

Note: *P<0.05 vs WKY rats.

Table 2. Serum contents of β-catenin (ng/ml) in WKY rats and SHR (ELISA; $\bar{X} \pm sd$)

Age (W)	WKY	SHR
6 W	17.51193±1.323788	25.9589±0.51796*
8 W	17.15826±0.820629	23.75289±1.255015*
10 W	14.156360±1.2466	26.99778±1.529335*
12 W	13.27907±0.619304	25.50112±0.442347*
14 W	14.9939±1.479936	16.58322±1.023058
16 W	17.19586±1.346594	15.31136±0.969431
18 W	18.13818±0.898122	15.84358±1.125505
20 W	18.18858±1.257726	18.89522±0.81333

Note: *P<0.05 vs WKY rats.

ECM such as type I collagen). *In vitro*, BMSCs may be induced to differentiate into MFs in the presence of serum at a high concentration and TGF-β1; *in vivo*, some pathological conditions such as hypoxia/ischemia and excess pressure load may alter the internal environment leading to the increased secretion of inducible factors and chemokins, which may induce the differentiation of BMSCs into MFs. These MFs may colonize in the myocardium, and synthesize and secret Col I, resulting in myocardial fibrosis, which not only causes damage to the myocardial structure, but significantly compromises the cardiac function. Thus, it is necessary to take measures to inhibit the differentiation of BMSCs into MFs, the migration of B-MFs into target tissues and the secretion of Col I by B-MFs, which are helpful to delay or treat fibrosis and improve the cardiac function. Thus, they are important signals [11]. SDF-1 is also known as chemokine ligand 12 (CXCL12) and one of chemokine members. SDF-1 has a high homology between human and mouse. SDF-1 exerts its biological effects via binding to its receptor CXCR4. AMD3100 is a synthetic small molecule non-peptide blocker and serves as an antagonist of CXCR4. It is effective to block the binding of SDF-1 to CXCR4, and thus CXCR4 is not activated. Under pathological conditions, SDF-1 has a potent chemotaxic activity and can induce a variety of CXCR4 positive cells to move to injured sites for tissue repair, which plays important roles in the angiogenesis, myocardial repair and myocardial fibrosis. However, the myocardial fibrosis also affects the cardiac function.

Wnt signaling pathway is an important pathway for the regulation of proliferation and differentiation of cells [12]. The Wnt signaling pathway can be divided into classic and non-classic pathways according to the patterns of signal transduction. β-catenin dependent pathway is an important classic pathway. Wnt binds to frizzled receptor and low-density lipoprotein receptor related proteins to form complexes which may induce the translocation of β-catenin into nucleus, resulting in the transcription of target genes [13].

Numerous studies have confirmed that Wnt signaling pathway play important roles in the heart development and myocardial fibrosis [14, 15]. In the heart development and myocardial hypertrophy, Wnt signaling pathway is activated [16]. In different hypertension rat models, studies have demonstrated a positive relationship between left ventricular weight and Frizzled-2 mRNA expression, and a negative relationship between ventricular weight and mRNA expression of Dickkopf-3 (an inhibitor of Wnt signaling pathway) [17]. In heart failure patients, Wnt signaling pathway is inhibited, and β-catenin expression reduces [18]. Following myocardial infarction, the expressions of frizzled-1/-2 and its downstream Dvl-1 were observed in MFs migrating into the infarct region, which is usually accompanied by myocyte apoptosis or reduction, increase in collagens, compromised thinning of the ventricular wall and improvement of cardiac function [19, 20]. Thus, Wnt signaling pathway may be a target in the therapy of myocardial fibrosis. However, the role of Wnt signaling pathway in the pathogenesis of fibrosis is still poorly understood, and available findings are still conflicting.

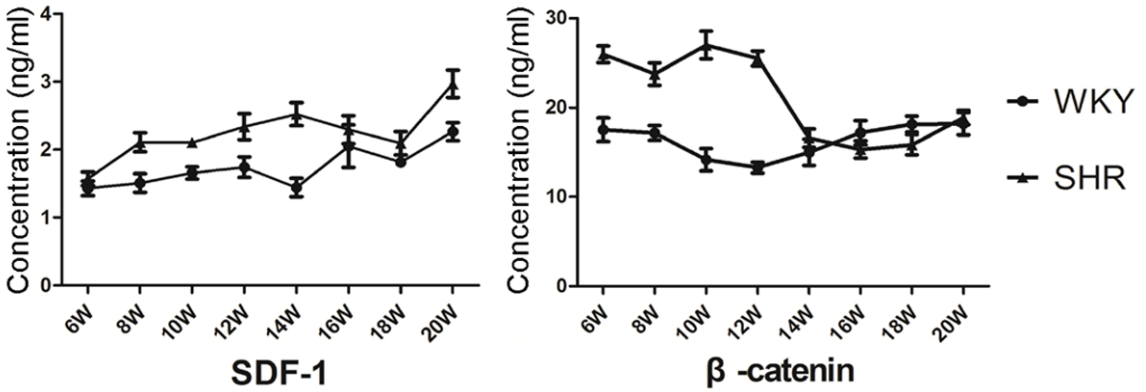

Figure 8. Serum contents of SDF-1 and β-catenin in SHR and WKY rats.

β-catenin is an important component of Wnt signaling pathway. Recently, there is evidence showing that β-catenin is also related to SDF-1/CXCR4 axis [21-23]. In the tumorogenesis and invasion of colon cancer, SDF-1/CXCR4 and Wnt/β-catenin may exert synergistic effects, and DDK-1 (an inhibitor of Wnt signaling pathway) is able to significantly compromise the CXCR4-mediated invasion of colon cancer. Thus, we speculate that, in hypertension induced myocardial fibrosis, SDF-1/CXCR4 may exert synergistic effects with Wnt signaling pathway.

Our study confirmed that SDF-1 was able to enhance the migration of B-MFs, which however was blocked by AMD3100 (an inhibitor of CXCR4). In addition, ICG-001 (an inhibitor of Wnt signaling pathway) partially inhibited the migration of B-MFs, and this effect was inferior to that of AMD3100. In addition, AMD3100 in combination with ICG-001 failed to exert synergistic effects to further attenuate cell migration. Thus, we postulate that Wnt related migration of B-MFs is regulated by SDF-1/CXCR4. In B-MFs, ICG-001 significantly inhibited the secretion of Col I, but AMD3100 partially suppressed the Col I secretion. Of note, combined use of AMD3100 and ICG-001 could exert synergistic effects to inhibit Col I mRNA expression. Thus, we postulate that SDF-1/CXCR4 and Wnt signaling pathway have synergistic activity to regulate Col I secretion.

Since week 8, the systolic blood pressure increased in SHR and reached a peak at week 14 (180-185 mmHg). Heart ultrasonography showed the structure and function of the heart were comparable between SHR aged 6 weeks and WKY rats aged 6 weeks. However, at week 20, the LVID increased significantly, LVAD elevated markedly, EF reduced dramatically, FS decreased significantly, myocardial hypertrophy became more obvious and cardiac dysfunction was more evident. Masson staining showed the myocardial fibers were enlarged and disordered, there was a large amount of collagens between myocytes, especially around the vessels, and CA/HA ratio increased markedly. These findings suggest that the persistent hypertension in SHR results in myocardial fibrosis and myocardial hypertrophy, which are pathological characteristics of myocardial remodeling and may significantly influence in the cardiac function.

In addition, our results also revealed that serum SDF-1 level in SHR was significantly higher than in WKY rats, suggesting that hypertension may increase serum SDF-1. However, since week 14, the blood pressure remained relatively stable in SHR, but SDF-1 still showed an increased tendency, which might be ascribed to aging. Thus, we speculate that the SDF-1 in SHR is affected by both blood pressure and age.

Wnt signals are a big family and there are several important components in the Wnt signaling pathway [24, 25]: β-catenin accumulates in the nucleus of fibroblasts, suggesting the activation of Wnt signaling pathway; GSK-3 is a negative regulator of myocardial hypertrophy, and inhibition of GSK-3 by LiCl is able to promote the pressure load induced myocardial hypertrophy. In addition, DKK-1 expression has been found to reduce in some fibrotic diseases [26].

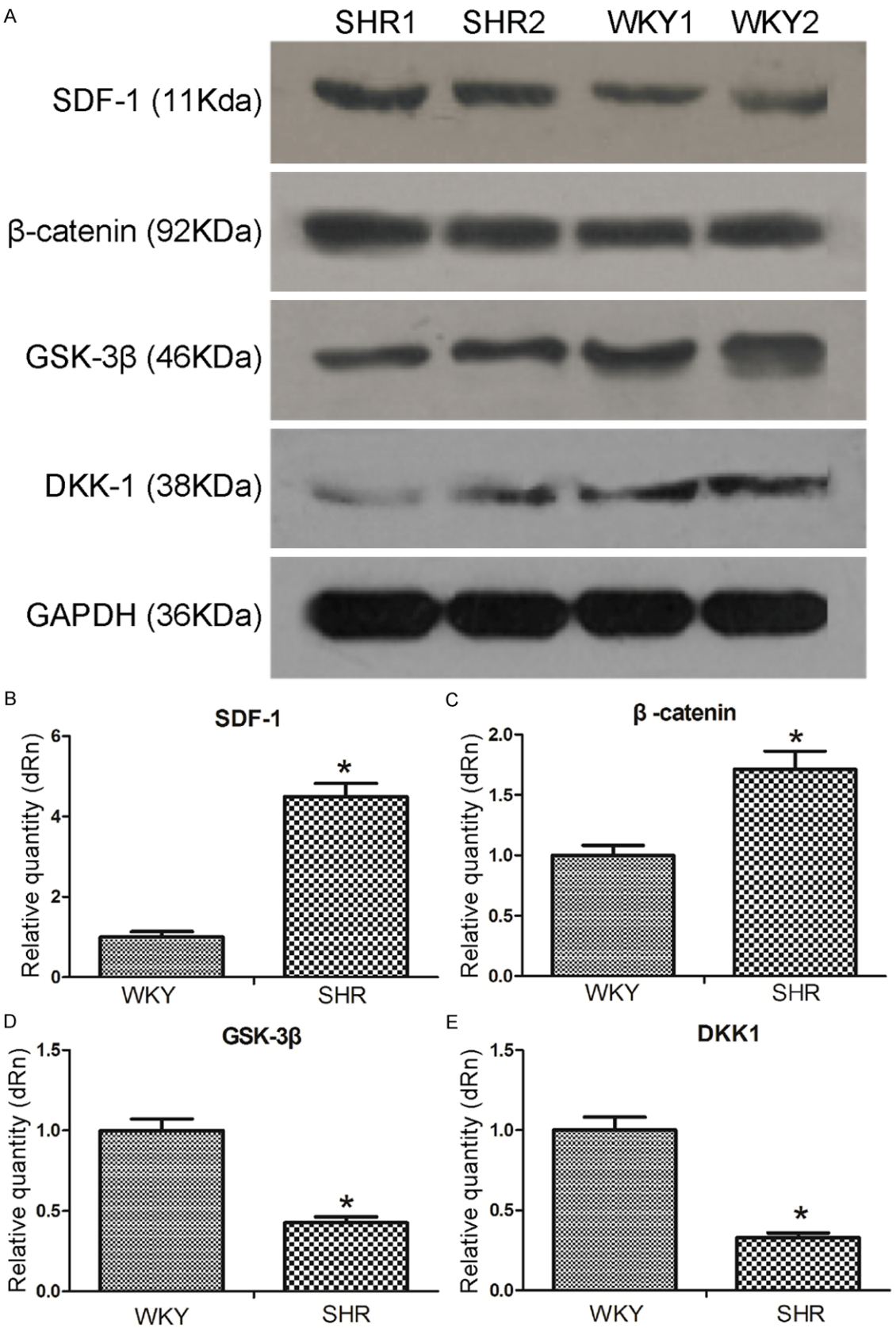
A
SHR1
SHR2
WKY1
WKY2
SDF-1 (11Kda)
β-catenin (92KDa)
GSK-3β (46KDa)
DKK-1 (38KDa)
GAPDH (36KDa)
B
SDF-1
Relative quantity (dRn)
WKY
SHR
C
β -catenin
Relative quantity (dRn)
WKY
SHR
D
GSK-3β
Relative quantity (dRn)
WKY
SHR
E
DKK1
Relative quantity (dRn)
WKY
SHR

Figure 9. A. Protein expressions of SDF-1 and components of Wnt signaling pathway in SHR and WKY rats; B-E. Protein expressions of SDF-1 and components of Wnt signaling pathway in SHR and WKY rats, *P<0.05. B. SDF-1 expression in the myocardium of rats aged 20 weeks; C. β-catenin expression in the myocardium of rats aged 20 weeks; D. GSK-3β expression in the myocardium of rats aged 20 weeks; E. DKK-1 expression in the myocardium of rats aged 20 weeks.

Our findings indicated that the protein and mRNA expressions of DKK-1 and GSK-3β in the myocardium of SHR aged 20 weeks were significantly lower than in WKY rats. Moreover, β-catenin increased with the fluctuation of blood pressure. Thus, we postulate that, in spontaneously hypertensive patients, the SDF-1 expression increases gradually over time and with the increase in blood pressure, which may drive the migration of circulating CXCR4+ cells to the myocardium, participating in myocardial fibrosis. In early stage of blood pressure fluctuation, Wnt signaling pathway is activated, β-catenin expression increases and proteins of Wnt signaling pathway are released into intercellular space. When the blood pressure reaches a stable level, β-catenin expression becomes nearly normal, but the expressions of GSK-3β and DKK-1 (two inhibitors of Wnt signaling pathway) reduce: the reduced GSK-3β leads to decreased degradation of β-catenin and further activation of Wnt signaling pathway; reduced DKK-1 also decrease the DKK-1 related inhibition of Wnt signaling pathway, resulting in persistent Wnt signaling pathway activation. The activation of Wnt signaling pathway may activate MFs, and increase the secretion of collagens in MFs, which facilitates the myocardial fibrosis. Thus, during the hypertension induced myocardial fibrosis, SDF-1 and Wnt signaling pathway may exert synergistic effects. However, Wnt signaling pathway is very complex and influenced by multiple factors in vivo. Thus, the interaction between SDF-1/CXCR4 and Wnt signaling pathway and its influence on the hypertension induced myocardial fibrosis are required to be further elucidated, which may provide a new target for the therapy of hypertension related HF.

Disclosure of conflict of interest

None.

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