Human umbilical cord mesenchymal stem cells alleviate interstitial fibrosis and cardiac dysfunction in a dilated cardiomyopathy rat model by inhibiting TNF-α and TGF-β1/ERK1/2 signaling pathways

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Abstract. Dilated cardiomyopathy (DCM) is a disease of the heart characterized by pathological remodeling, including patchy interstitial fibrosis and degeneration of cardiomyocytes. In the present study, the beneficial role of human umbilical cord-derived mesenchymal stem cells (HuMSCs) derived from Wharton's jelly was evaluated in the myosin-induced rat model of DCM. Male Lewis rats (aged 8-weeks) were injected with porcine myosin to induce DCM. Cultured HuMSCs ($1x10^6$ cells/rat) were intravenously injected 28 days after myosin injection and the effects on myocardial fibrosis and the underlying signaling pathways were investigated and compared with vehicle-injected and negative control rats. Myosin injections in rats (vehicle group and experimental group) for 28 days led to severe fibrosis and significant deterioration of cardiac function indicative of DCM. HuMSC treatment reduced fibrosis as determined by Masson's staining of collagen deposits, as well as quantification of molecular markers of myocardial fibrosis such as collagen I/III, profibrotic factors transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), and connective tissue growth factor (CTGF). HuMSC treatment restored cardiac function as observed using echocardiography. In addition, western blot analysis indicated that HuMSC injections in DCM rats inhibited the expression of TNF-α, extracellular-signal regulated kinase 1/2 (ERK1/2) and TGF-β1, which is a master switch for inducing myocardial fibrosis. These findings suggested that HuMSC injections attenuated myocardial fibrosis and dysfunction in a rat model of DCM, likely by inhibiting TNF-α and the TGF-β1/ERK1/2 fibrosis pathways. Therefore, HuMSC treatment may represent a potential therapeutic method for treatment of DCM.

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Introduction

Dilated cardiomyopathy (DCM) is characterized by dilatation of the ventricles, patchy interstitial fibrosis and degenerated cardiomyocytes. Along with genetic abnormalities, myocarditis has been considered to be a major factor that leads to the development of DCM (1). There is evidence for the role of the immune system in the pathogenesis of myocarditis and subsequent development of DCM (2,3). Experimental autoimmune myocarditis (EAM), which mimics human fulminant myocarditis in the acute phase and human DCM in the chronic phase, is induced by immunization of rats with cardiac myosin (4).

DCM is a progressive disorder, and despite available state-of-the art treatment such as diuretic or cardiac resynchronization therapy (CRT), it is characterized by high morbidity and mortality rates (5). Mesenchymal stem cell (MSC) therapy may be a potential novel approach for treatment of cardiovascular injury and for promotion of tissue regeneration (6). However, various stem cell trials for cardiovascular indications have been disappointing, possibly due to of the use of autologous stem cells (7). Cardiovascular disease patients typically belong to the older age groups, where numerous risk factors may compromise stem cell function (8,9). Allogeneic MSCs may be easily scaled and quality-controlled, and are

immunologically relatively well-tolerated, allowing their use for stem cell trials that has exceed the feasibility of autologous strategies. Therefore, previous studies using allogeneic stem cells have been established, including the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis in patients with DCM trial revealed that the rate of major adverse cardiac events was significantly lower in patients treated with allogenic vs. autologous stem cells (10-12). An additional source of MSCs are human umbilical cord-derived mesenchymal stem cells (HuMSCs). They are generally discarded as medical waste after delivery, thus, their use is of little ethical concern. There are two arteries and a vein in the umbilical cord which are surrounded by a hyaluronic acid-rich extracellular matrix (ECM) also termed Wharton's jelly (WJ). MSCs from umbilical cord WJ are easily isolated and cultured *in vitro*. Additionally, they can be differentiated *in vitro* into several tissue types (13). There are several distinct advantages for HuMSCs over other MSCs: i) They have low immunogenicity, attributable to low expression of human leukocyte antigen major histocompatibility complex I (MHC I); and ii) they lack MHC II molecules and co-stimulatory antigens, such as CD80 and CD86. Therefore, HuMSCs are regarded as immunologically safe for use in allogeneic clinical therapies (14,15). Previous studies demonstrated that HuMSCs possess many potential advantages for cell-based treatment of diseases, such as systemic lupus erythematosus (16), rheumatoid arthritis (17), diabetes (18) and myocardial ischemia (19,20). However, the potential beneficial effects of HuMSCs on DCM and the underlying signaling events remain speculative and remain to be fully elucidated.

A variety of signal transduction pathways are involved in myocardial fibrogenesis leading to DCM. For example, activation of the ERK/transforming growth factor-β1 (TGF-β1) pathway was associated with upregulated collagen deposition contributing to myocardial fibrosis (21-23).

In the present study, a DCM rat model was established in order to investigate the therapeutic efficiency of HuMSCs in DCM rats and to analyze the potential signaling mechanisms.

Materials and methods

Animals. Lewis rats (male, 8-weeks old; weight, 180-200 g, n=24) were obtained from Vital River Laboratories (Beijing, China) and maintained in an air-conditioned animal facility at Shantou University Medical College (Shantou, China) under 25˚C and 70% humidity conditions with a 12-h light/dark cycle. Throughout the experiments for the current study, all animals were treated in accordance with the institutional guidelines for animal experiments. The Animal Care and Use Committee of the Shantou University Medical College approved all experimental procedures.

Preparation of HuMSCs. HuMSCs were prepared as previously described (24). Briefly, human umbilical cords from pregnant women (12 volunteers; age, 25-35 years; recruited from February 2012 to November 2013) who underwent full-term Caesarian sections were collected from the Second Affiliated Hospital of Shantou University Medical College immediately, washed, and cut into 2-3-cm thick sections. Written informed consent was obtained from all participants. After separating the arteries and veins, WJ was sliced into smaller fragments and transferred to 75 cm^2 flasks containing Dulbecco's modified Eagle's medium/F12 media (Sigma-Aldrich; EMD Millipore, Billerica, MA, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 μg/ml penicillin/streptomycin (Shanghai Bioscience, Shanghai, China), 1 g/ml amphotericin B (Gilead Sciences, Inc., San Dimas, CA, USA), 5 ng/ml epidermal growth factor (Invitrogen; Thermo Fisher Scientific, Inc.), and 5 ng/ml basic fibroblast growth factor (Sigma-Aldrich; EMD Millipore). HuMSC were cultured for 5-7 days at 37˚C in 5% CO_2 to allow migration of cells from the explants. After three passages the cells were used for subsequent experiments. Ethical approval was obtained from the Institutional Review Board of Shantou University Medical College.

Generation of DCM rat model. Lewis rats were injected in the footpads with antigen-adjuvant emulsion in accordance with a procedure described previously (4). Briefly, purified porcine cardiac myosin (Sigma-Aldrich; EMD Millipore) was dissolved in 10 mM PBS and emulsified with an equal volume of complete Freund's adjuvant with 10 mg/ml *Mycobacterium tuberculosis* (Sigma-Aldrich; EMD Millipore). On day 0, rats received a single immunization with a total of 0.2 ml emulsion per rat at two subcutaneous sites (both footpads). At 28 days after immunization, surviving DCM rats (n=16) were divided into two treatment groups: i) 0.2 ml PBS only (vehicle control group, n=8), or ii) 0.2 ml HuMSCs ($1x10^6$ cells/animal; experimental group, n=8). HuMSCs or vehicle (PBS) was administered intravenously via the tail vein. Age matched Lewis rats without immunization were used as negative controls (negative control group, n=8). The echocardiography and myocardial pathological section were used to confirm the success of the DCM rat model (25).

Echocardiographic studies. Two-dimensional echocardiography was performed 56 days after myosin injections under isoflurane anesthesia (1.5-2.0% volume in air), and using a 13-MHz transducer linked to an ultrasound system (Acuson Antares, Siemens, Healthineers, Erlangen, Germany). M-mode images were used to obtain measurements of the left ventricular end systolic dimension (LVEDs), left ventricular end diastolic dimension (LVEDd), interventricular septal thickness (IVS), left ventricular posterior wall thickness (LVPW) and fractional shortening (FS %). The average of three beats was used for each parameter. FS (%) was calculated as follows [(LVEDd - LVEDs)/LVEDd] x100. All echocardiography analysis was performed offline and investigators were blinded to the treatment groups.

Histopathological studies. Following echocardiographic analysis, rats were sacrificed using cervical dislocation 56 days after myosin injection. The hearts were excised and weighed to calculate the heart/body weight (HW/BW) ratio. The hearts were subsequently fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at 4-μm thickness. These sections were stained with either hematoxylin and eosin (H&E) for infiltration of inflammatory cells, or Masson's trichrome stain for collagen fibers. Slides were viewed under a high-power light microscope. The area of myocardial fibrosis (blue color)

Table I. List of quantitative polymerase chain reaction primers.

Primer	Forward (3'-5')	Reverse (3'-5')
Col I	CGTGGAAACCTGATGTATGCT	CCTATGACTTCTGCGTCTGG
Col III	GATCCTAACCAAGGCTGCAA	ATCTGTCCACCAGTGCTTCC
TGF-β1	ATTCCTGGCGTTACCTTGG	AGCCCTGTATTCCGTCTCCT
TNF-α	GCTCCCTCTCATCAGTTCCA	GCTTGGTGGTTTGCTACGAC
GAPDH	AGAAGGCTGGGGCTCATTTG	AGGGGCCATCCACAGTCTTC

in left ventricular (LV) tissue sections following Masson's staining was quantified using a color image analyzer (Mac Scope; Mitani Co., Fukui, Japan) and measured as the collagen volume fraction (CVF) = (area of the collagen/area of field of vision) x100. Ten randomly selected sections (magnification, x100) from each rat were analyzed and the results averaged.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Collagen I, III, TGF-β1 and tumor necrosis factor-α (TNF-α) mRNA expression levels in myocardial tissue were detected using RT-qPCR. The total RNA was isolated from 50 mg heart tissue using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The primers were designed and synthesized by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). The primer sequences are presented in Table I. Primer concentration was 200 pM. RNA (500 ng) in a 10 μl reaction mixture was reverse transcribed using the PrimeScript RT reagent kit with gDNA Eraser (Perfect Real-Time; Takara Biotechnology Co., Ltd., Dalian, China). The reactions were incubated first at 37˚C for 15 min, followed by inactivation at 85˚C for 5 sec and finally held at 4˚C. The qPCR reaction was performed using SYBR Premix Ex Taq™ II (Tli RNaseH Plus; Takara Biotechnology Co., Ltd.) and detection was performed with the CFX96™ PCR detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The reaction cycles were: Denaturation at 95˚C for 30 sec, followed by 40 cycles of denaturation at 95˚C for 5 sec and annealing and extension at 60˚C for 30 sec. The relative level of gene expression was normalized to the expression of the housekeeping gene GAPDH using the $2^{-\Delta\Delta Cq}$ method (26).

Western blot analysis. Heart tissues were treated in radioimmunoprecipitation assay lysis buffer [100 mM NaCl, 20 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0), 0.5% Triton X-100, 0.5% Nonidet P-40] to extract total protein, which were quantified using bicinchoninic acid method (Beyotime Institute of Biotechnology, Haimen, China). Total protein (30 μg) was separated by 10% SDS-PAGE gel and transferred electrophoretically to polyvinylidene difluoride membranes (EMD Millipore) for western blot analysis. Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline [20 mM Tris (pH 6.8), 137 mM NaCl] with 0.1% Tween-20, washed, and incubated at 4˚C for 16 h with the following primary antibodies: GAPDH (catalog no. D4C6R, 1:10,000), extracellular signal-regulated kinase (ERK)-1/2 (catalog no. 9258, 1:1,000), phosphorylated (p)-ERK-1/2 (catalog no. 4668, 1:2,000), p38 mitogen activated protein kinase (MAPK) (catalog no. 8690, 1:1,000) and p-p38 MAPK (catalog no. 4511, 1:1,000) were all purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA). Collagen III (catalog no. ab7778, 1:5,000) and connective tissue growth factor (CTGF, catalog no. ab6992, 1:1,000) were purchased from Abcam (Cambridge, UK). Membranes were washed and incubated with a 1:2,000 dilution of horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody (catalog no. 4050-05; Southern Biotechnology Associates, Inc., Birmingham, AL, USA) for 1 h at room temperature. Immunoreactive protein bands were visualized using the ECL Plus chemiluminescence kit (EMD Millipore). Bands were analyzed using Image Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA) and protein expression quantities were determined according to the following calculation: Integrated optical density (IOD)=density (mean) x area.

Statistical analysis. Data are expressed as mean ± standard deviation. Analyses of the differences between groups were performed using one-way analysis of variance followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant.

Results

Effects of HuMSCs on myocardial fibrosis and cardiac function in DCM rats. HuMSCs treatment significantly reduced myocardial fibrosis (Fig. 1A and B). HW/BW was significantly greater in vehicle control rats compared with negative control rats. HuMSC treatment significantly reduced HW/BW when compared with those in vehicle control rats (Fig. 1C, Table II, $P<0.01$ vs. normal, $P<0.05$ vs. control). Echocardiographic analyses revealed a significant impairment in systolic and diastolic function (Fig. 1A). DCM rats demonstrated LV remodeling with increased LVEDd, LVEDs and reduced FS in vehicle-treated DCM rats compared with untreated rats (Fig. 1C, Table II, $P<0.01$ vs. normal), indicating impaired myocardial function. HuMSC treatment significantly reversed these changes (Fig. 1C, Table II, $P<0.01$ vs. control).

Histopathological examination revealed that the hearts of DCM rats showed severe fibrosis compared with negative control rats (Fig. 1A). Inflammatory cellular infiltration was not observed in the hearts of the three groups as identified by H&E staining (Fig. 1A). The area of myocardial fibrosis as quantified by Masson's staining of collagen deposits was approaching 30% in DCM rats (Fig. 1B).

Effects of HuMSCs on molecular markers of myocardial fibrosis in DCM rats. To confirm the positive effect of HuMSCs on myocardial fibrosis, RT-qPCR analysis on molecular

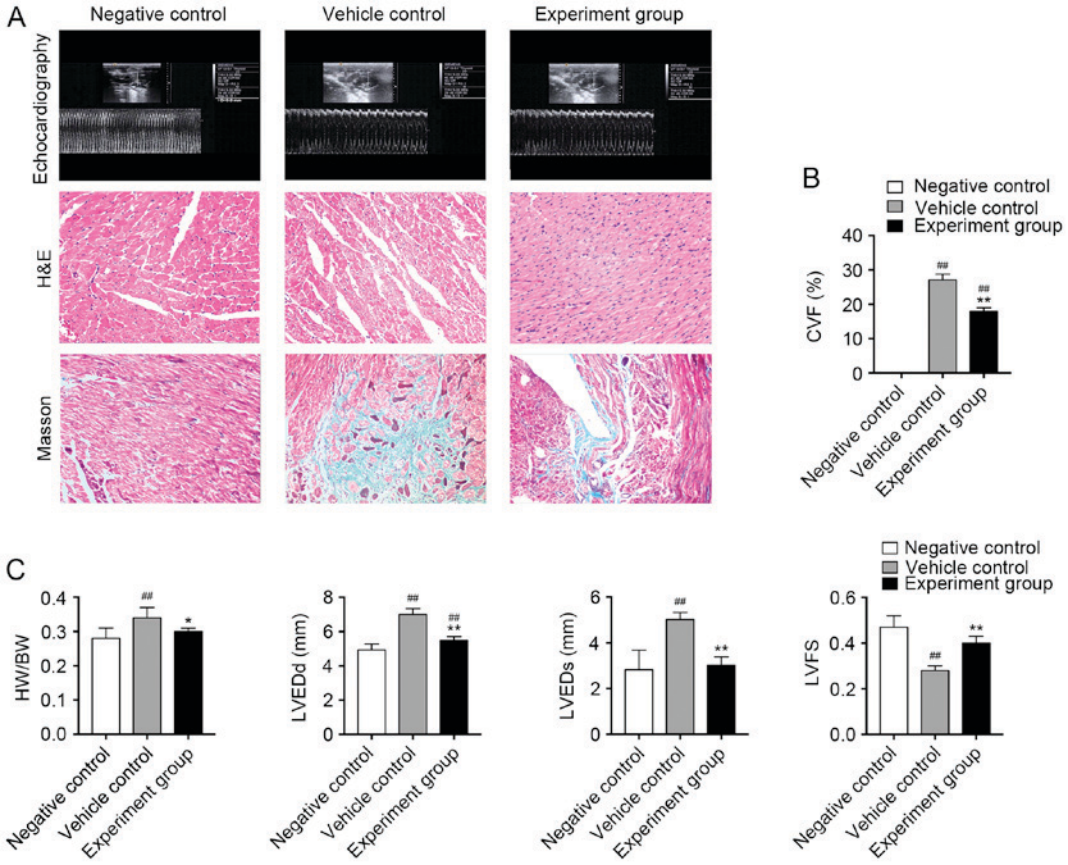

Figure 1. (A) H&E and Masson's staining in left ventricular tissue slices in age-matched untreated rats, immunized rats treated with vehicle and immunized rats treated with HuMSCs. Magnification, x100. (B) CVF for Masson's staining in left ventricular tissue slices from the different treatment groups. $^{\#\#}$P<0.01 vs. negative control; *P<0.05, **P<0.01 vs. vehicle control, n=8. (C) Heart cavity measurements. $^{\#\#}$P<0.01 vs. negative control; *P<0.05, **P<0.01 vs. vehicle control, n=8. H&E, hematoxylin and eosin; HuMSCs, human umbilical cord-derived mesenchymal stem cells; CVF, collagen volume fraction; HW/BW, heart weight/body weight; LVEDd, left ventricular dimension in end diastole; LVEDs, left ventricular dimension in end systole.

Table II. Echocardiographic parameters 56 days after treatment of DCM rats with HuMSCs in.

Characteristic	Negative control	Vehicle control	Experiment
HW/BW	0.28±0.03	0.34±0.03[a]	0.30±0.01[b]
LVEDd (mm)	4.93±0.35	7.00±0.34[a]	5.48±0.22[a,c]
LVEDs (mm)	2.82±0.86	5.02±0.31[a]	3.02±0.36[c]
IVS (mm)	2.16±0.20	2.12±0.19	2.10±0.20
LVWP (mm)	2.34±0.11	2.12±0.26	2.22±0.15
LVFS	0.47±0.05	0.28±0.02[a]	0.40±0.03[c]

[a]P 0.01 vs. normal; [b]P<0.05 vs. control; [c]P<0.01 vs. control. HW/BW, heart weight/body weight; LVEDd, left ventricular dimension in end diastole; LVEDs, left ventricular dimension in end systole; IVS, interventricular septal thickness; LVPW, left ventricular posterior wall thickness; LVFS, left ventricular fractional shortening; DCM, dilated cardiomyopathy; Negative control, age-matched untreated rats; Vehicle control, DCM rats treated with vehicle; Experiment, DCM rats treated with HuMSCs.

markers of myocardial fibrosis was performed (Fig. 2). Collagen I, III and the profibrotic factors TGF-β1 and TNF-α were significantly upregulated in vehicle-treated DCM rats, compared with negative control rats (Fig. 2, P<0.01). By contrast, treatment with HuMSCs significantly reduced mRNA expression of collagen-I, III, TGF-β1 and TNF-α when compared with vehicle-treated DCM rats (Fig. 2, P<0.01). At the protein level, CTGF and collagen-III were significantly upregulated in vehicle-treated DCM rats compared with negative control rats (Fig. 3, P<0.05, P<0.01). However, treatment with HuMSCs significantly reduced the myocardial protein expression of collagen-III and CTGF (Fig. 3, P<0.05, P<0.01).

Effects of HuMSCs on activation of MAPK signaling in DCM rats. TGF-β1 activates numerous non-canonical signaling pathways, including MAPK pathways. Therefore, in order to investigate the signaling mechanisms, which HuMSCs use to reduce myocardial fibrosis in DCM rats, activation of p38-MAPK and ERK1/2 was quantified (Fig. 4). Myocardial protein expression of p38-MAPK, ERK1/2, p-p38-MAPK and p-ERK1/2 were quantified using western blot analysis. P-ERK1/2 levels significantly increased in vehicle control rats compared with negative control rats, whereas p-p38-MAPK did not change (Fig. 4B, P<0.01). HuMSCs treatment alleviated these changes in DCM rats. Protein expression of p-ERK1/2; however, not p-p38-MAPK was attenuated significantly in the HuMSCs treated DCM rats compared with vehicle-treated

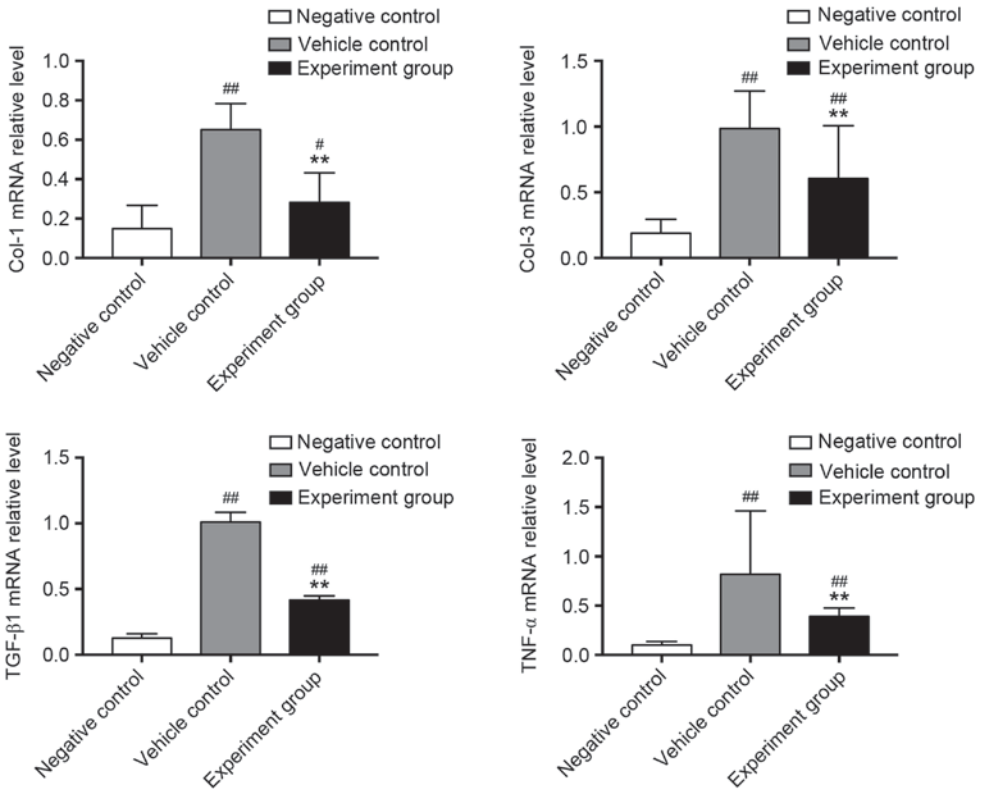

Figure 2. Analysis of myocardial mRNA levels of the extracellular matrix components collagen I and III, and the profibrotic factors TGF-β1 and TNF-α by reverse transcription-quantitative polymerase chain reaction of tissues from negative control, vehicle control, and experiment group rats. Relative expression levels are presented. GAPDH was used as the internal standard. $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. negative control. *P<0.05; **P<0.01 vs. vehicle control, n=8. TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.

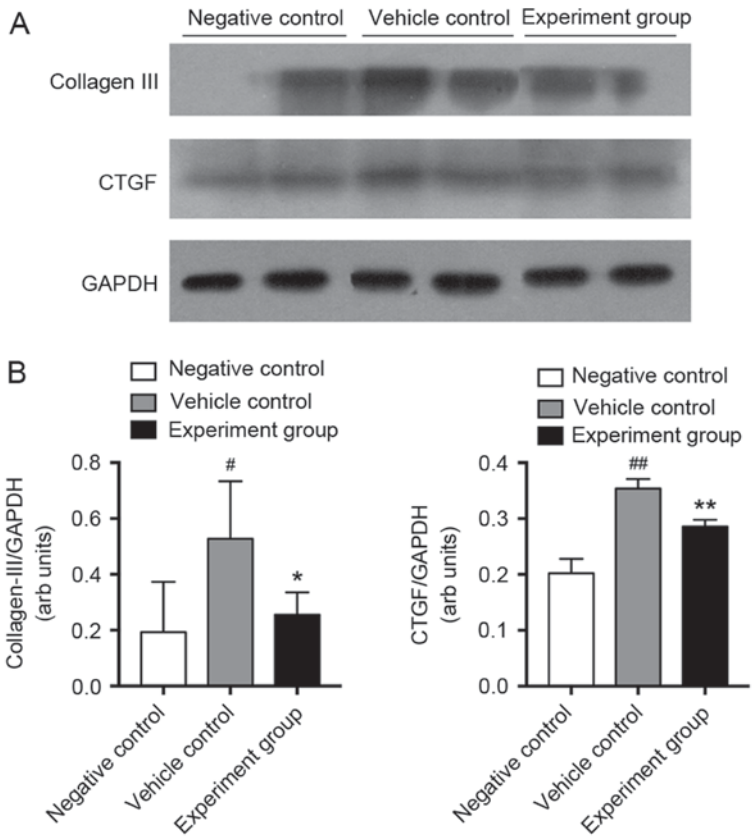

Figure 3. Western blot analysis of CTGF and collagen III expression in negative control, vehicle control, and experimental rats. (A) Western blot analysis of immunolabeled bands for CTGF and collagen III, with GAPDH as an internal control. (B) Ratio of the mean density values of CTGF and Col III relative to GAPDH. $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs. negative control; *P<0.05, **P<0.01 vs. vehicle control, n=8. CTGF, connective tissue growth factor.

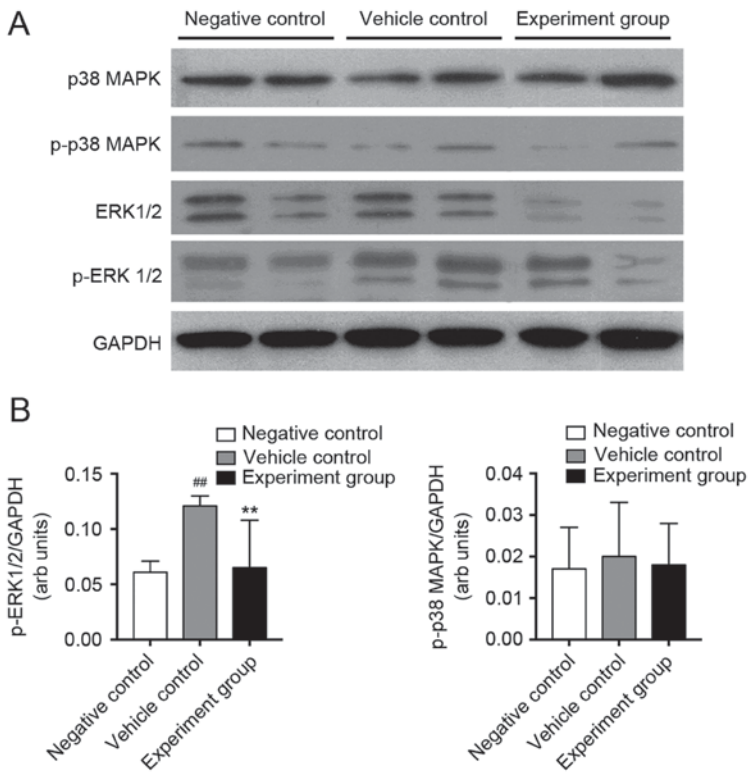

Figure 4. Western blot analysis of p38 MAPK and ERK1/2 phosphorylation in negative control, vehicle control, and experimental rats. (A) Representative western blotting showing immunolabeled bands for p38-MAPK, p-p38 MAPK, ERK1/2, p-ERK1/2, and GAPDH was used as an internal control. (B) Mean density values of p-ERK1/2 and p-p38 MAPK expressed as the ratio relative to GAPDH expression. GAPDH used as an internal control. $^{\#\#}$P<0.01 vs. negative control; **P<0.01 vs. vehicle control, n=8. p38 MAPK, p38 mitogen-activated protein kinase; p-p38 MAPK, phosphorylated-p38 MAPK; ERK1/2, extracellular-signal regulated kinase 1/2; p-ERK1/2, phosphorylated-ERK1/2.

DCM rats (Fig. 4B, P<0.01), indicating that HuMSCs may alter myocardial fibrosis via TNF-α, TGF-β and ERK1/2 activation.

Discussion

In the present study, reduced cardiac function in a DCM rat model was associated with LV remodeling, as well as increased myocardial collagen deposition and upregulated myocardial type I and III collagen expression. Treatment with HuMSCs significantly improved LV remodeling, LV systolic function and reduced collagen deposition, likely by inhibiting the TGF-β/TNF-α, ERK1/2 signaling pathways.

A loss of cardiomyocytes and an increase in interstitial fibrosis is characteristic for DCM (27). The degree of cardiac fibrosis, leading to passive ventricular stiffness and reduced cardiac function, may be determined by measuring the myocardial collagen volume fraction. Masson's staining revealed a significant increase in collagen deposition in DCM rats. The abnormal deposition of collagen within myocardial tissues was significantly reduced following treatment with HuMSCs. Cardiac fibroblasts, the cells that form the interstitial tissue within the healthy myocardium, are considered to be the major source of collagen and fibrosis after cardiac injury (28). The major fibrillar collagens of the adult heart are type I and III collagens (29,30) and if the level of collagen within the myocardium increases, ventricular compliance may be reduced. The data of the present study indicated that type I and III collagen was upregulated in myocardial tissues of DCM rats; however, their expression levels were significantly reduced following treatment with HuMSCs.

CTGF is another fibrosis-associated molecule that may induce fibrocyte differentiation into a myofibroblast phenotype and increase ECM deposition in the myocardium (31). The data of the present study indicated that CTGF was upregulated in myocardial tissues of DCM rats; however, the expression level was significantly reduced following treatment with HuMSCs.

A previous study determined that TGF-β is upregulated in several models of myocardial infarction and DCM (32). It has been previously suggested that TGF-β1 is a master switch for inducing myocardial fibrosis and may upregulate the expression of procollagen genes to promote synthesis of ECM components, subsequently leading to fibrosis (32). TGF-β1-overexpressing mice had significant cardiac hypertrophy along with interstitial fibrosis (33). TGF-β1 levels in dilated and infarcted hearts were reduced following treatment with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers (34,35). A previous study suggested that the antifibrotic mechanism of HuMSCs in lung fibrogenesis may be associated with TGF-β1 downregulation, which may lead to suppression of fibrosis following lung injury (36). The present study determined that HuMSCs downregulated myocardial TGF-β1 mRNA expression and led to the suppression of myocardial fibrosis in DCM rats.

TGF-β1 activates various non-canonical signaling pathways, including the MAPK and phosphatidylinositol-3

kinase/Akt pathways (37,38). The progression of FasL-induced DCM and congestive heart failure was prevented by blocking the ERK1/2 pathway, with reductions in fibrosis, inflammation and apoptosis (39). Isoproterenol and cAMP signaling attenuated the profibrotic effect of TGF-β1 in cardiac fibroblasts by suppressing ERK1/2 phosphorylation. Additionally, the MEK/ERK pathway is involved in interleukin-17-mediated cardiac fibrosis in EAM (40). In the present study, HuMSC administration significantly reduced ERK1/2 phosphorylation in DCM rats. However, phosphorylation of p38-MAPK was not altered in the three experimental groups. These findings support previous observations (37,38), that the TGF-β/ERK1/2 pathway is an important mediator in cardiac fibrosis. The data of the present study revealed that ERK1/2, not p38-MAPK signaling, was involved with fibrosis in DCM and identified that HuMSCs treatment may effectively protect against cardiac fibrosis by attenuating the activation of the ERK1/2 pathway.

TNF-α, a proinflammatory cytokine with a wide range of biological effects, is involved in the pathophysiology of various cardiovascular diseases (41,42). TNF-α overexpression in transgenic mice may lead to adverse cardiac remodeling, characterized by increased total matrix metalloproteinase (MMP) activity and increased fibrosis (43). Inhibition of TNF-α may protect from myocardial inflammation and fibrosis through reduced ERK phosphorylation in experimental diabetic cardiomyopathy (44). In the present study, administration of HuMSCs effectively reduced myocardial mRNA expression of TNF-α in DCM rats. Therefore, it is possible that similar to the TGF-β/ERK1/2 pathway, HuMSCs alter TNF-α/ERK1/2 signaling and may alleviate cardiac fibrosis, which subsequently improves ventricular compliance and cardiac function.

In conclusion, the present findings demonstrated that injected HuMSCs improve cardiac function by attenuating myocardial collagen network remodeling via downregulation of TGF-β1 and TNF-α expression and activation of ERK1/2 signaling in DCM rats. Therefore, HuMSC treatment may be a potential therapeutic avenue for treatment of DCM.

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