Nesprin-1 plays an important role in the proliferation and apoptosis of mesenchymal stem cells

WENGANG YANG, HUI ZHENG, YONGYI WANG, FENG LIAN, ZHENGLEI HU and SONG XUE

Department of Cardiovascular Surgery, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200127, P.R. China

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Abstract. The aim of this study was to investigate the expression of nesprin-1 protein and its effects on rat bone marrow mesenchymal stem cells (MSCs). MSCs were cultured in DMEM and surface-associated antigens of MSCs were detected by flow cytometry. The protein expression of nesprin-1 was detected by immunofluorescence and western blot analysis. A lentiviral vector expressing small interfering RNA (siRNA) targeting nesprin-1 was constructed (LV-siNesprin-1) and the MSCs were subsequently transfected with this vector. Another group of MSCs was transfected with the LV-GFP vector and another group of untransfected cells was used as the controls (normal group). The protein expression level of nesprin-1 in the 3 groups of MSCs (LV-siNesprin-1, LV-GFP and normal group) was measured by western blot analysis. Cell proliferation was assessed by MTT assay, and the cell cycle and apoptosis were detected by flow cytometry. DAPI was used to stain the nucleus of the MSCs. The MSCs appeared spindle-shaped with irregular processes and were positive for CD90, CD29 and negative for CD45. Nesprin-1 protein was found in the nuclear membrane. The protein expression of nesprin-1 in the LV-siNesprin-1 group was lower than that in the LV-GFP (P=0.03) and normal group (P=0.028); this difference was significant (P<0.05). The cell proliferation of the MSCs transfected with LV-siNesprin-1 was reduced; the apoptotic rate was higher in the LV-siNesprin-1 group compared with the other 2 groups (LV-GFP and normal group) (P=0.032, P=0.025, respectively; P<0.05). The changes in the morphology of the nucleus in the LV-siNesprin-1 group included fusion and fragmentation. In conclusion, the data presented in this study indicate that nesprin-1 regulates the proliferation and apoptosis of MSCs; our results are consistent with those from previous studies. Thus, nesprin-1 protein plays an important role in the proliferation and apoptosis of MSCs.

Correspondence to: Professor Song Xue, Department of Cardiovascular Surgery, Renji Hospital, Shanghai Jiaotong University School of Medicine, 1630 Dongfang Road, Shanghai 200127, P.R. China
E-mail: xuesong64@163.com

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Introduction

Nesprin-1 is a protein isoform of the nesprin protein family that contains spectrin repeats similar to those in mAKAP, which forms homodimers and specifically targets the nuclear envelope through a KASH domain (1-5). Nesprin-1α is also a candidate for a mAKAP nuclear envelope receptor. It has been reported that mutations in the nesprin-1 gene may be responsible for adult cerebella ataxia and mutations of nesprin-1 which interact with lamin A/C may lead to at least 2 distinct human disease phenotypes, myopathic or neurologic, a feature similar to that found in laminopathies. Puckelwartz *et al* (6) reported Δ/Δ KASH mice expresses nesprin-1 without its carboxyl-terminal KASH domain; these Δ/Δ KASH mice have a normally assembled but dysfunctioning nuclear membrane complex and provide a model for nesprin-1 mutations and developing cardiomyopathy with associated cardiac conduction system disease.

Mesenchymal stem cells (MSCs) are bone marrow-derived cells that retain the capability to differentiate into various types of tissue cells and contribute to the regeneration of a variety of tissues, including bone, cartilage, muscle and adipose tissue (7-9). MSCs, after being transplantated into the ischemic myocardial tissue, secrete a variety of factors including vascular endothelial growth factor (VEGF). The cardioprotective effects of MSCs are known to be mediated not only by their differentiation into cardiomyocyte-like cells, but also by their ability to supply large amounts of angiogenic, anti-apoptotic and mitogenic factors (10-12). These findings suggest the therapeutic potential of MSCs for heart failure.

From the literature, we know that nesprin proteins exist only in multiple tissues (skeletal, cardiac and vascular smooth muscle) and not in stem cells (2). The study of nesprin-1 protein, which is speculated to be localized to the nuclear membrane, may aid in the understanding of the process through which MSCs differentiate into cardiomyocyte-like cells.

Materials and methods

Animals. Clean Sprague-Dawley (SD) rats, weighing 250-300 g, were obtained from the Experimental Animal Center of Shanghai Jiaotong University Medical School, Shanghai,

China (production license: scxk (hu)2004-0001; use license no. syxk (hu)2003-2009). The present study was reviewed and approved by the University Institutional Animal Care and Use Committee.

Reagents. Except where otherwise specified, all reagents were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Gibco (Grand Island, NY, USA), including cell culture medium [low-glucose Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS)]. The cardiac-specific antibodies (TNI, α-sarcomeric actin, desmin), FITC-conjugated goat anti-rat antibodies (CD45), PE-conjugated rabbit anti-rat antibodies (CD90), allophycocyanin (APC)-conjugated rabbit anti-rat antibodies (CD29) and FITC-conjugated rabbit anti-rat nesprin-1 antibodies, were purchased from Abcam (Cambridge, UK). The BLOCK-iT™ POLIImiR RNAi Expression Vector kit with EmGFP, pcDNA™ 6.2-GW/EmGFPmiR, *Escherichia coli (E. coli)* DH5, Lipofectamine® 2000, Opti-MEM, TRIzol reagent and pLenti6.3/V5-DEST were purchased from Invitrogen (Carlsbad, CA, USA). Restriction endonuclease, DNA ligase and the Large Plasmid DNA Extraction kit were purchased from Qiagen (Dusseldorf, Germany). The reagents and instruments for immunohistochemistry, immunofluorescence and western blot analysis were purchased from Gibco, Sigma-Aldrich Chemical Co. and Invitrogen, respectively.

Cell culture. Eight-week-old SD rats (250-300 g) were prepared as donors. The procedures were performed in accordance with the guidelines for animal experimentation of Shanghai Jiaotong University and approved by the institutional ethics committee. Under general anesthesia with ether (approximately 100 μl) bone marrow was aspirated from both the tibia and femur with a 20-gauge needle attached to a 10-ml syringe containing 0.5 ml DMEM with 40 U/ml heparin.

The concentration of the cells in suspension was adjusted to $5x10^5$ mononuclear cells/ml culture medium at 37˚C in a humidified atmosphere with 5% CO_2; the cells were then seeded on culture plates, without removal of the red blood cells. Since bone marrow-derived MSCs (BMSCs) grow initially in colonies and do not reach confluence over the entire culture dish, the cells were passaged 7 days after seeding, when half the colonies reached 70-80% confluence; the cells were then passaged weekly when the cells reached confluence. For subcultures, adherent BMSCs wer harvested using 0.125% trypsin and plated at a ratio of 1:3.

For flow cytometry, the cells were detached using accutase instead of trypsin, in order to achieve a better preservation of the cell surface molecules. 293T cells were maintained in MEM supplemented with 5% FBS and 50 mg/ml gentamycin. The cells were trypsinized by a 0.05% trypsin-0.5 mM EDTA solution.

Flow cytometry. Flow cytometry was performed using a FACSAria flow cytometer/cell sorter (BD Biosciences, San Jose, CA, USA). Following accutase treatment, the cells were resuspended at a density of $1x10^5$ cells/200 μl phosphate-buffered saline (PBS) and incubated with 2% FCS (PBS-FCS) on ice. The cells were stained with antibody, and incubated with FITC-conjugated CD45 monoclonal antibody, PE-conjugated CD90 monoclonal antibody and APC-conjugated CD29 monoclonal antibody (at concentrations indicated by the manufacturer) for 30 min at 4˚C in the dark, and then washed in PBS-FCS. After washing, the cells were analyzed in the cytometer. At least 5,000 events were analyzed for each sample. Negative controls, used to detect the unspecific bindings, included an irrelevant antibody or PBS-FCS alone. The acquired data were analyzed using Summit software (Cytomation, Inc., Fort Collins, CO, USA).

Immunofluorescence microscopy. BMSCs grown on glass coverslips were fixed by a 20-min incubation in 4% formaldehyde (freshly prepared from paraformaldehyde), rinsed in PBS, and stored in 70% ethanol at -20˚C. The fixed cells were blocked for 30 min in blocking solution (PBS supplemented with 2% goat serum, 1% BSA, 0.1% gelatin, 0.1% Triton X-100 and 0.05% Tween-10), and incubated overnight with the primary antibody (at the dilution indicated by the manufacturer) at 4˚C. After washing, the cells were incubated with the secondary antibody (FITC-conjugated anti-rat IgG for nesprin-1) for 30 min. Finally, the coverslips were washed, mounted in glycerol and examined under an epifluorescence microscope (Olympus, Tokyo, Japan).

Western blot analysis. After washing with PBS, the BMSCs were scraped off the culture dish and transferred to centrifuge tubes. Following centrifugation at 700 x g for 10 min at 4˚C, the pellets were lysed in hot Laemmli loading buffer (62.5 mmol/l Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.05% β-mercaptoethanol, 0.05% bromophenol blue). Equal amounts of protein extracts (20 mg/lane) were subjected to SDS-PAGE on a 5% stacking gel and a 10% separating gel, followed by transfer of the proteins onto nitrocellulose membranes (20 min at 10 V). After blocking in PBS containing 0.05% Triton X-100 (TBS) and 5% FCS for 1 h, the blots were incubated overnight with primary antibodies (rabbit anti-rat nesprin-1) at 4˚C. After washing, the membranes were incubated with the secondary antibody (HRP-conjugated goat anti rabbit IgG) for 1 h; the bound antibody was detected by ECL. β-actin was used as an internal control.

Design and cloning of small interfering RNA (siRNA) cassettes. The nesprin-1 DNA and protein sequence was according to the GenBank accession no. NM_001029909.1. The nesprin-1 gene siRNA sequence and the corresponding miRNA oligonucleotide sequence were then designed and synthesized using Ambion design software and the sequences were verified using BLAST software: (5'-CGGGAGTTGTTG ACTATGAAA-3'); the corresponding miRNA oligonucleotide sequence was as follows: nesprin-1 forward, TGCTGTTT CATAGTCAACAACTCCCGGTTTTGGCCACTGACTGA CCGGGAGTTTGACTATGAAA; and reverse, CCTGTTT CATAGTCAAACTCCCGGTCAGTCAGTGGCCAAAACC GGGAGTTGTTGACTATGAAAC. The Vector Cloning kit was then used for restructuring; the double-stranded miRNA oligonucleotide was inserted into the miRNA expression vector (Invitrogen), and the cells were transfected with miRNA plasmids infected with *E. coli* DH5. The oligonucleotides were annealed and cloned into the *Bgl*II-*Hin*dIII site. The pDONR221 vector was processed with BP recombination reaction, in order to obtain the entry vector containing the siRNA. The sequence

of the entry vector with siRNA and the lentiviral expression vector pLenti6/V5-DEST were processed with LR recombination reaction in order to obtain the lentiviral expression vector expressing siRNA targeting nesprin-1 (LV-siNesprin-1).

Transfection. The 293T cells were co-transfected with a plasmid expressing GFP together with a plasmid expressing siRNA specific for GFP (siGFP) at a ratio of 1:5 using FuGENE® transfection reagent (Roche, Indianapolis, IN, USA).

Lentiviral vector production. Recombinant lentiviruses were produced by the transient transfection of 293T cells using the calcium-phosphate method as previously described (13-15). Infectious lentiviruses were harvested at 48 and 72 h post-transfection and filtered through 0.22-μm-pore cellulose acetate filters as previously described (13-15). Recombinant lentiviruses were concentrated by ultracentrifugation (2 h at 50,000 x g) and subsequently purified on a sucrose 20% gradient (2 h at 46,000 x g) as previously described (16). Vector concentrations were analyzed using an immunocapture p24-gag ELISA (Alliance; DuPont/NEN, Boston, MA, USA) as previously described (16); the concentrated suspension of the activity of the viral titer was measured ($1x10^6$ TU/ml).

Protein levels were analyzed by western blot analysis (concrete steps as the former) and immunoblotting was carried out according to standard methods with rabbit moclonal antibody against GFP (Abcam) or β-actin (Sigma-Aldrich Chemical Co.). Fluorescence-activated cell-sorter analysis was carried out as previously described (17-19).

MTT cell proliferation assay. The cells were plated on 24-well plates ($2x10^4$ cells/well) in the growth medium for the assays. The protocols for MTT assays were as previously described (20). In brief, growth medium containing 0.25 mg/ml MTT was added to each well and the cells were further incubated at 37˚C for 20 min, following which the medium was replaced by 0.2 ml DMSO/well. MTT dye conversion was determined by measuring the $OD_{540\ nm}$ of the DMSO extracts using DMSO as the blank control.

Detection of cell cycle of MSCs by low cytometry. The cells ($5x10^5$) were centrifugated for 5 min at 800 rpm, the supernatant was collected, and they were then washed with cold PBS twice and fixed with 700 ml/l cold ethanol at 4˚C overnight. The ethanol was removed by centrifugation prior to detection. The cells were washed with PBS twice, then stained with 1 ml PI (bromide tablets) at 4˚C for 30 min in the dark.

Detection of MSC apoptosis by flow cytometry. The cells ($5x10^6$) were centrifugated for 5 min at 1,000 rpm, then the culture medium was discarded. They were then washed once with PBS, centrifuged and the supernatant was removed; they were then fixed with 70% cold ethanol at 4˚C for 1-2 h. The ethanol was then removed by centrifugation. The cells were resuspended with 3 ml PBS for 5 min, filtered once and centrifuged at 1,500 rpm for 5 min. The PBS was discarded prior to detection. The cells were stained with 1 ml PI and FITC-Annexin V in 4˚C for 30 min in the dark.

Morphology of the nucleus following staining of MSCs with 4,6-diamidino-2-phenylindole (DAPI) for 72 h and transfection with LV-siNesprin-1. The cells were passaged when they became nearly confluent. Sterile DAPI solution was added to the culture medium. The MSCs were rinsed 6 times in PBS solution to remove all excess unbound DAPI. The MSCs were cultured in culture medium [DMEM, supplemented with 20% FBS and penicillin (100 U/ml)/streptomycin (100 μg/ml)] at 37˚C in a humidified atmosphere with 5% CO_2 for 72 h; they were then examined under a microscope.

Statistical analysis. Image programmer software was used to analyze the images. Data are presented as the means ± standard deviation (SD). Statistical analyses were performed using paired t-tests where applicable. Statistical analysis was performed using SPSS and GraphPad Prism 5 Demo software. A p-value <0.05, based on a two-tailed test, was considered to indicate a statistically significant difference.

Results

Characterization of MSCs. After discarding the non-adherent cells by the first medium change and washing with PBS 3 times at 24 h of primary culture, approximately 80% of the MSCs had adhered to the culture dishes; the medium was then changed to remove the suspension of hematopoietic stem cells. After 3 days in primary culture, the MSCs adhered to the plastic surface, presenting a small population of single cells. The cells were spindle-shaped with a single nucleus (Fig. 1A). Seven to 10 days after initial plating, the cells resembled long spindle-shaped fibroblast cells and began to form colonies (Fig. 1B and C). After replating, almost 100% of the cells had adhered to the culture dishes, and were polygonal or spindle-shaped, with long processes.

The rat MSC surface antigen profiles obtained by flow cytometry (Fig. 2), were positive for CD90, CD29 and negative for CD45. The percentage of CD90 and CD29 was 99.96 and 99.75%, respectively; however, the percentage of CD45 was 1.12%.

Detection of protein expression of nesprin-1 in MSCs by immunofluorescence and western blot analysis. Immunofluorescent staining for nesprin-1 protein verified the presence of rat MSCs (Fig. 3A), with granular green fluorescence distributed around the nuclear membrane of the MSCs. The protein expression of nesprin-1 was detected by western blot analysis (Fig. 3B).

Transfection of MSCs with LV-siNesprin-1, LV-GFP and the detection of protein expression of nesprin-1 by western blot analysis. The MSCs were successfully transfected with LV-siNesprin-1 or LV-GFP, as shown by green fluorescence (Fig. 4A-C). After the MSCs were transfected with LV-siNesprin-1, the protein expression of nesprin-1 in the LV-siNesprin-1 group was lower than that in the LV-GFP and normal group (Fig. 4D). The protein expression of nesprin-1 in the LV-GFP group was the same as that in the normal group, but was significanlty different from that in the LV-siNesprin-1 group ($P=0.03$ and $P=0.028$, respectively; $P<0.05$) (Fig. 4E). The expression of β-actin did not differ between the 3 groups ($P=0.10$ and $P=0.12$, respectively; $P>0.05$).

Detection of cell proliferation by MTT assay. The 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were

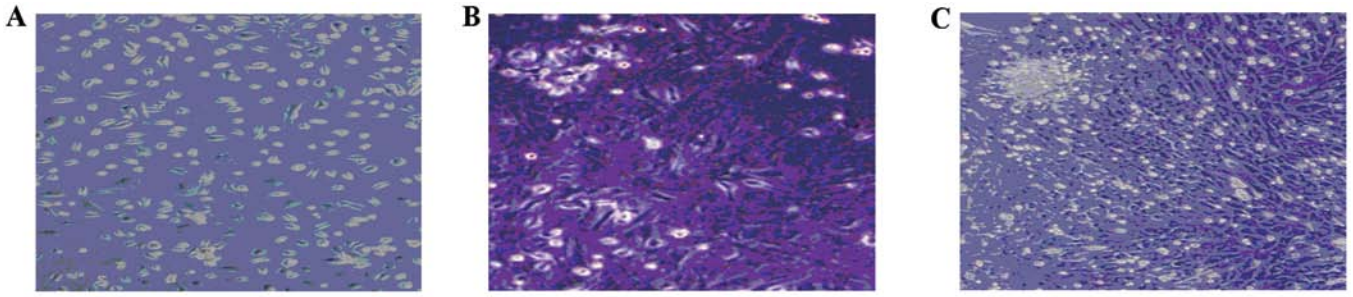

Figure 1. Characterization of mesenchymal stem cells (MSCs) and differentiated cardiomyocyte-like cells *in vitro*. (A) Morphological observation of rat MSCs after cell culture for 3 days (x100). The cells were spindle-shaped with a single nucleus. (B) Morphological observation of rat MSCs after cell culture for 7 days (x100). (C) Morphological observation of rat MSCs after cell culture for 10 days (x100). The cells had long spindle-shaped fibroblasts and began to form colonies.

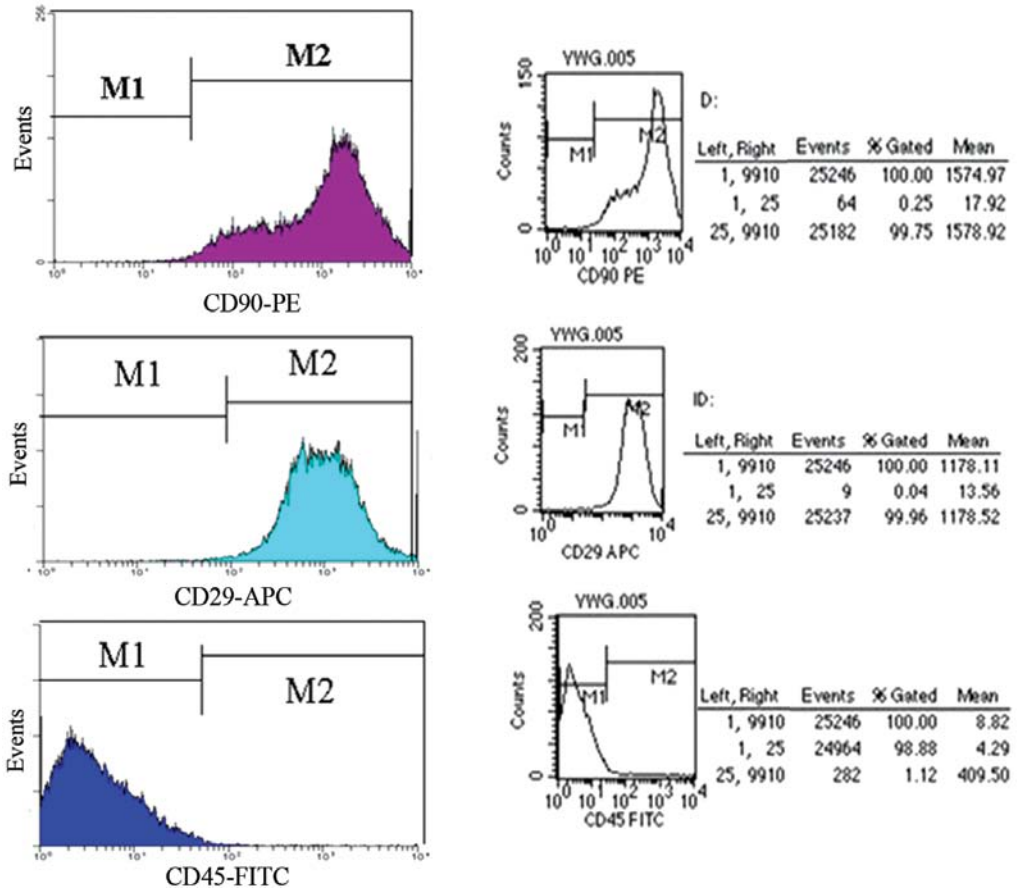

Figure 2. Surface-associated antigens (CD29, CD90, CD45) of MSCs were detected by flow cytometry. MSCs were positive for CD90, CD29 and negative for CD45. The percentage of CD90 and CD29 was approximately 99%; the percentage of CD45 was approximately 1%.

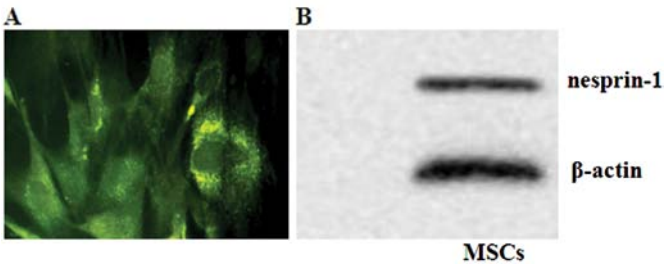

Figure 3. Immunofluorescence and western blot analysis for the identification of the protein expression of nesprin-1. (A) MSCs were found to be positive for nesprin-1 by immunofluorescence (x400). (B) Western blot analysis revealed the protein expression of nesprin-1 in the MSCs.

cultured for 24, 48, 72 and 96 h under the same conditions. The proliferation of the cells was then detected by MTT assay at 24, 48, 72 and 96 h. As shown in Fig. 5, the knockdown of nesprin-1 expression led to a decrease in the proliferation of MSCs; however, the proliferation rate of the cells in the LV-GFP group was not altered and was similar to the normal group.

Cell cycle of MSCs in the 3 groups (LV-siNesprin-1, LV-GFP and normal group). The 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were cultured for 72 h under the same conditions. The cell cycle was detected by flow cytom-

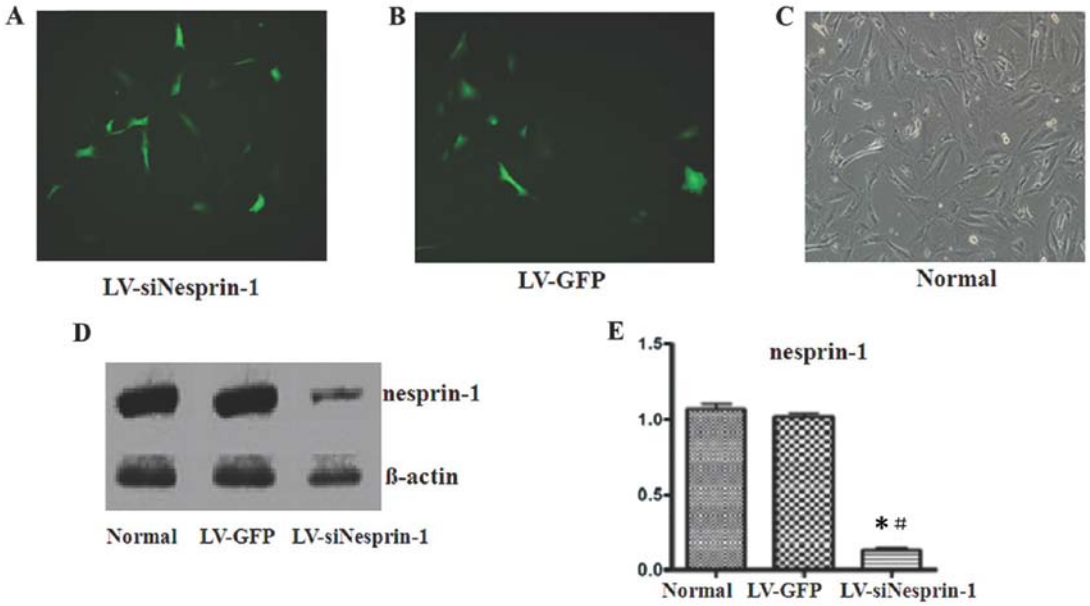

Figure 4. Mesenchymal stem cells (MSCs) were transfected with LV-siNesprin-1 or LV-GFP; the protein expression of nesprin-1 was detected by western blot analysis. (A-C) The MSCS were successfully transfected with LV-siNesprin-1 and LV-GFP (green fluorescence); x200. (D and E) The protein expression of nesprin-1 in the MSCs in the LV-siNesprin-1 group was lower than that in the MSCS in the LV-GFP and normal group. The protein expression of nesprin-1 in the LV-GFP group was the same as that in the normal group. The difference between the LV-siNesprin-1 group and the LV-GFP/normal group was significant (*P=0.03, $^{\#}$P=0.028, respectively; P<0.05).

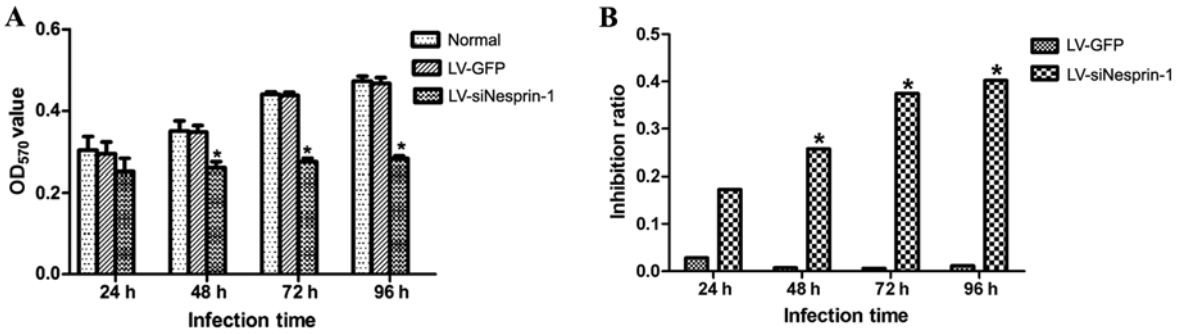

Figure 5. Cell proliferation was detected by MTT assay. (A) Cell proliferation was detected by MTT assay at 24, 48, 72 and 96 h when the 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were cultured for 24, 48, 72 and 96 h under the same conditions. The proliferation of the cells in the LV-siNesprin-1 group was reduced compared with the other 2 groups; this difference was statistically significant (*P=0.025; P<0.05). However, the proliferation of the cells in the LV-GFP group was the same as that in the normal group. (B) Transfection with LV-siNesprin-1 inhibited cell proliferation; however, transfection with LV-GFP did not affect cell proliferation.

etry. In the LV-siNesprin-1 group, the cells were mainly in the G0/G1 phase of the cell cycle (Fig. 6).

Apoptosis of MSCs in the 3 groups (LV-siNesprin-1, LV-GFP and normal group). The 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were cultured for 72 h under the same conditions. The apoptosis of the cells was detected by flow cytometry. In the LV-siNesprin-1 group, the apoptotic rate of the cells was higher than that of the cells in the LV-GFP and normal group (Fig. 7).

Changes in the morphology of the nucleus following transfection of MSCs with LV-siNesprin-1. As shown in Fig. 8, the morphology of the nucleus in the LV-siNesprin-1 group was altered; morphological changes such as fusion and fragmentation were observed.

Discussion

Nesprins have been reported to bind to the nuclear membrane. Through interaction with both emerin and lamin A/C, nesprin is localized to the nuclear membrane (2,4,5,21). The structure of the various nesprin isoforms suggests that they also form a protein scaffold that links the nuclear membrane with the nucleus, cytoplasmic organelles and the cell membrane via the actin cytoskeleton. Nesprin (*C. elegans* ANC-1) mutations may disrupt the positioning of the mitochondria in mononuclear cells (22). These studies suggest a structural role for nesprins in nuclear migration and the positioning of major organelles (23). Nesprin (*Drosophila* MSP-300) has been shown to be localized to regions of the cell margin involved in exoskeletal attachment and to the Z-lines in sarcomeres. These studies suggest its role in the structural organization of the sarcomere

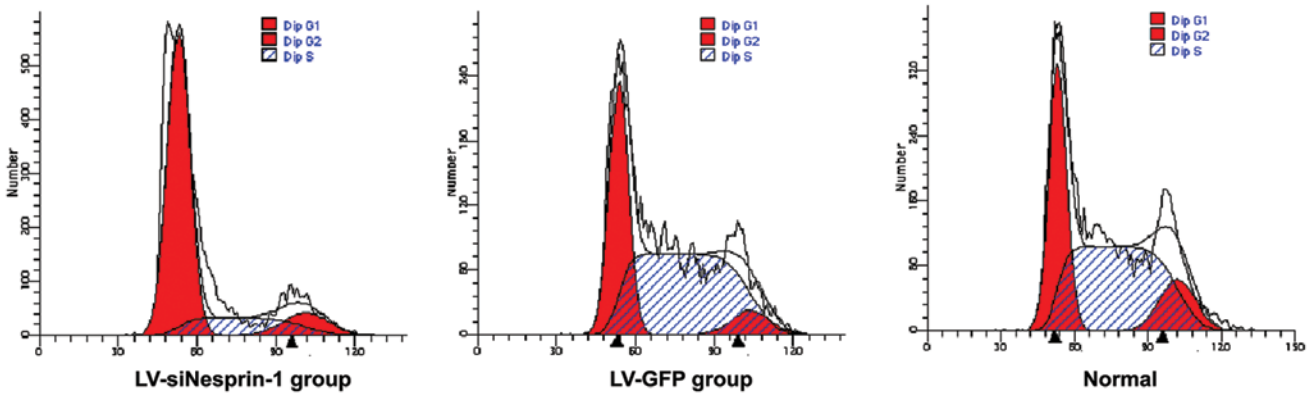

Figure 6. Cell cycle of mesenchymal stem cell (MSC) in the 3 groups (LV-siNesprin-1, LV-GFP and normal group) analyzed by flow cytometry. The 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were cultured for 72 h under the same conditions. Cell cycle was then analyzed by flow cytometry. The differentiation of cells in the LV-siNesprin-1 group was slower than that of cells in the LV-GFP and normal group; the cells were mainly in the G0/G1 phase of the cell cycle, (approximately 68.45±3.75%). In the LV-GFP and normal group, the percentage of cells in this phase was approximately 40.57±2.45 and 42.17±2.05%, respectively. The difference between the LV-siNesprin-1 group and the normal group was statistically significant (P=0.035; P<0.05).

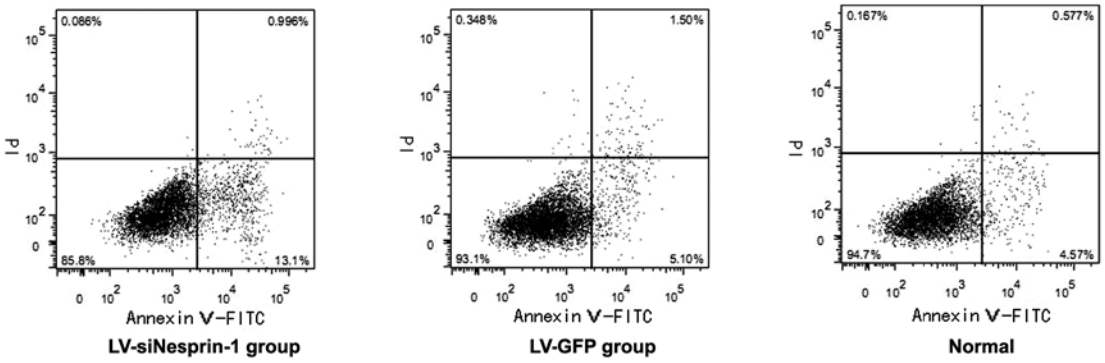

Figure 7. Cell apoptosis of mesenchymal stem cells (MSCs) in the 3 groups (LV-siNesprin-1, LV-GFP and normal group) detected by flow cytometry. The 3 groups of cells (LV-siNesprin-1, LV-GFP and normal group) were cultured for 72 h under the same conditions. The cell cycle was analyzed by flow cytometry. The apoptotic rate of the cells in the LV-siNesprin-1 group was higher than that of cells in the LV-GFP and normal group; the percentage of apoptotic cells in the LV-siNesprin-1 group was approximately 15.12±0.97% at 72 h. The percentage of apoptotic cells in the LV-GFP and normal group was approximately 6.86±1.26 and 5.04±0.80%, respectively. The difference between the LV-siNesprin-1 group and the LV-GFP/normal group was statistically significant (P=0.032, P=0.025, respectively; P<0.05).

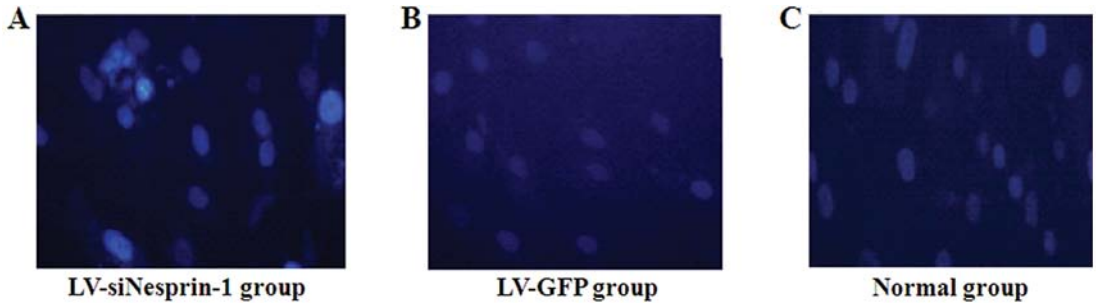

Figure 8. The morphology of the nucleus in the mesenchymal stem cells (MSCs) was observed 72 h after transfection and staining with DAPI. (A) The morphology of the nucleus in the LV-siNesprin-1 group was altered; morphological changes such as fusion and fragmentation were observed (x200). (B) The morphology of the nucleus in the LV-GFP group was not altered (x200). (C) The morphology of the nucleus in the normal group (x200).

and in signalling from the extracellular environment to the nucleus. Gough *et al* (24) concluded that the nesprin-1 gene is expressed in a variety of forms that are multifunctional and are capable of functioning at both the Golgi and the nuclear envelope, with the linking of the 2 organelles taking place during muscle cell differentiation.

Nesprins are widely expressed in a variety of tissues; the high expression of both nesprin-1 and -2 has been observed in skeletal, cardiac and vascular smooth muscls. Zhang *et al* (2) suggested that nesprins have a specific function in muscle cell differentiation. However, the high expression of nesprin-1 has also been found in peripheral blood leukocytes and spleen,

and nesprin-2 in the pancreas and testes. Nesprins are highly expressed in muscles with both nesprin-1 and -2 muscle-specific isoforms (2,21,25). *In vitro*, during the differentiation of C2C12 myoblasts into myotubes, nesprin changes its localization from the nuclear membrane to the cytoplasm/sarcomere, indicating its specific roles during muscle differentiation (2,4). In the sarcomere of skeletal muscle cells, different nesprin-1 and -2 epitopes are associated with the Z-line, the A/I junction, the sarcoplasmic reticulum and the mitochondrial membrane, indicating that nesprins may play the role of maintaining sarcomeric structure (4,25). In addition, sarcomeric proteins have been identified as potential interacting partners for nesprins, including the ryanodine receptor and mAKAP (25,26). mAKAP is targeted to the nuclear membrane by nesprin-1 and they interact through their closely related spectrin repeats (26). Potentially, nesprins may be involved in maintaining and/or targeting protein complexes common to both the nuclear membrane and the sarcoplasmic reticulum (25). Cardiomyocyte nuclei have been found to be elongated in Δ/Δ KASH mouse hearts. These findings reflect what has been reported on lamin A/C gene mutations and reinforce the importance of an intact nuclear membrane complex for normal heart function (6). It has been shown that Δ/Δ KASH mice (lacking the carboxy-terminus of nesprin-1) develop cardiomyopathy with associated cardiac conduction system disease. Older mutant animals have been shown to have elongated P wave duration, elevated atrial and ventricular effective refractory periods, indicating conduction system defects in the myocardium. It has been found that cardiomyocyte nuclei are elongated with reduced heterochromatin in Δ/Δ KASH mouse hearts (6).

The abovementioned data indicate that nesprin seems to play a key role in adult cell mitosis, RNA transport and the stability of the nuclear membrane. Yet, little is known about the role of the nesprin-1 protein, particularly nesprin-1 in stem cells (MSCs). In the present study, we aimed to elucidate the function of nesprin-1 protein in BMSCs by designing a nesprin-1 siRNA lentiviral vector. Following the transfection of the MSCs with LV-siNesprin-1, we found that the protein expression of nesprin-1 in the LV-siNesprin-1 group was lower than that in the LV-GFP and normal group; the proliferation of the MSCs in the LV-siNesprin-1 group was also reduced. However, the apoptotic rate was increased in the LV-siNesprin-1 group compared with the LV-GFP and normal group. As shown by morphological analysis using a microscope, in the LV-siNesprin-1 group, morphological changes were observed in the nucleus, such as fusion and fragmentation. Thus, nesprin-1 mediates cell differentiation and regulates the proliferation and apoptosis of MSCs.

MSCs were first described in 1968 by Friedenstein *et al* (27). These cells can be expanded and induced, either *in vitro* or *in vivo*, and terminally differentiate into osteoblasts, chondrocytes, adipocytes, tenocytes, myotubes, neuronal cells and hematopoietic cells with strong self-renewal ability and genetic stability *in vitro*. Several research groups have reported that MSCs are able to proliferate and potentially differentiate *in vitro* (28-30). However, the ratio of MSCs in bone marrow cells is very low, approximately 0.001-0.01%; hence, the separation and amplification of MSCs is of vital significance. Wakitani *et al* (31) described a method of isolating MSCs from rat bone marrow with Ficoll density gradient separation and adherent culture. Currently, the International Society for Cellular Therapy proposed 3 minimal criteria for defining MSCs (32): i) MSCs must be plastic-adherent if maintained in standard culture conditions; ii) MSCs must express CD105, CD73 and CD90, but must not express haematopoietic markers, such as CD45, CD34, CD14 or CD11b; and iii) MSCs must be capable of differentiating into fibroblasts, osteoblasts, adipocytes and chondroblasts under the corresponding lineage, particularly in *in vitro* conditions. In this study, we detected the MSC surface antigens, CD90 and CD29, by flow cytometry, with the percentage of CD90 and CD29 as high as 98%, and the percentage of CD45 approximately 1%.

Xu *et al* (33) reported that the ability of human MSCs to proliferate remained strong between passages 2 and 6, then the apoptosis of MSCs began to accelerate. In the process of MSC proliferation and differentiation, the upregulation of the protein expression of nesprin-1 may maintain the stability of the MSC nuclear membrane and reduce the apoptosis of the MSCs; this may provide a theoretical basis and gain more seed cells for the improvement of therapeutic modalities for heart disease.

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