Materials and Methods

Rat and human MSCs isolation, culture and characterization

The detailed methodology for human and rat MSCs isolation has been described in our previous report[1, 2] . Rat MSCs were harvested from femurs and tibias of 8-week-old male SD rats (150g-200g, purchased from SLAC Laboratory). The human MSCs (hMSCs) were obtained from 3 old (age over 65) and 3 young patients who underwent orthopedic surgery. Passage 3 to 5 MSCs were determined by fluorescence-activated cell sorting (FACS) using fluorescein-isothiocyanate-conjugated antibodies against CD73, CD105, CD45, CD34 and CD90, and cultured cells were identified as $CD90^{+}$ /$CD73^{+}$ /$CD105^{+}$ /$CD34^{-}$ /$CD45^{-}$ cells[2, 3]. All antibodies were purchased from BD Biosciences (Franklin Lake, NJ, USA).

Hypoxic preconditioning

Using the same method as described in our previous report[1] , MSCs were plated at 1×10^{5} cells/cm^{2} in complete culture medium and incubated under normoxia (21% O_2, 5% CO_2) or hypoxia (0.5% O_2, 5% CO_2) using a finely controlled ProOx-C-chamber system (Biospherix, Redfield, NY, USA) for 24 hours.

RNA isolation and quantitative real time PCR

For quantitative real time PCR analysis of miR-211, total RNA was isolated with Trizol

Reagent (Invitrogen Inc., Carlsbad, CA, USA), and 1ug total RNA was reversely transcribed with Bulge-Loop miRNA-specific reverse transcription-primers (RiboBio, Guangzhou, China) and reverse transcriptase (Takara, Hangzhou, P. R. China). Quantitative real time PCR was performed with SYBR Green SuperMix (Takara, Hangzhou, P. R. China) and Bulge-Loop primers (RIBOBIO Co., Ltd., Guangzhou, China) on a 7500 Fast Real Time PCR system (Applied Biosystems, Foster City, CA, USA) with small nuclear RNA U6 as the normalization control. Primers for each target gene are listed in Supplementary Table. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method using RNA U6 as the reference gene.

Western blot analysis

The total proteins were extracted from the whole cells infected with different lentivirus, or from snap frozen and grounded heart tissue obtained from the border zone of.infarcted rat heart tissue, using the RIPA lysis buffer (Beyotime, Jiangsu, China). Proteins (60μg) were separated on a 12% SDS-polyacrylamide gel, and blotted to polyvinylidene fluoride membrane. The blots were blocked with 5% nonfat milk and probed with primary antibodies against total-STAT5A (ab31043; 1:500 dilution; Abcam, Cambridge, MA, USA), p-STAT3 (9145; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), total-STAT3 (9139; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), phospho-ERK (Thr202/Tyr204) (9145; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), total-ERK(9145; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), JNK(9145; 1:1000 dilution;

Cell Signaling Technology, Danvers, MA, USA), phospho-JNK(9145; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), phospho-p38MAPK (Thr180/Tyr182) (9215;1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA), total-p38MAPK(9212; 1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) and MMP9 (ab76003; 1:1000 dilution; Abcam, Cambridge, MA, USA) in 5%BSA in PBS-T and incubated at 4°C overnight. The membranes were then washed with PBS-T and incubated with HRP-conjugated specific secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at room temperature. Finally, the western blot bands were detected using an imaging system (Bio-Rad, Hercules, Californa, USA), quantified by densitometry and normalized to either α-tubulin or β-actin which served as internal protein loading control. Results were expressed as fold changes vs. controls.

Transwell assays

MSCs were infected with miR-211-over, miR-211-shRNA, STAT5A-over, STAT5A-shRNA or in combination (described in the text), respectively. Seventy-two hours after infection, MSCs were subjected to transwell assays. Briefly, 2 x10^4 infected cells were resuspended in DMEM with 1% FBS, and added to the upper chamber of the transwell system (8μm pore size, #3422, Corning Inc., Corning, NY, USA). Complete culture medium supplemented with 10% FBS was added to the lower chamber. Cells were cultured at 37°C in 5% CO_2 and allowed to migrate for 24 hours before the membranes were fixed. The non-migrated cells in the upper well were removed with a

cotton wrap, and migrated cells at the lower chamber were stained using Hoechst, and quantified by using Image Pro.

In vivo myocardial infarction (MI) model and cell transplantation

Sprague Dawley (SD) rats were purchased from SLAC Laboratory. Female SD rat (8 weeks old, 150g-200g) underwent general anesthesia with 4% chloral hydrate (300 mg/kg, administered intraperitoneally) and were then intubated. The effect of anesthesia was judged by the animal's response to moderate pressure exerted upon its tail and a dose of anesthetic was added if necessary. The chest was opened between the left 4th and 5th intercostals space and the heart was exposed. MI was induced by permanent ligation of the left anterior descending coronary artery proximal to the first branch. Rats in the sham-operation group underwent the same procedure without a tie-down of the suture. PBS-treated $5X10^6$ MSCs or $5X10^6$ MSCs infected with lectivirus that contained different plasmids or in combination as described in the text were intravenously delivered via the tail vein as a bolus at 1 day after MI. Each vector also contained GFP, as a reporter gene. Infection of MSCs was done 72 hours before transplantation. A minimum of 6 rats were enrolled in each experimental group.

MSCs cell retention assay by quantitative real time PCR of Y-Chromosomal DNA

The retention of transplanted MSCs in heart tissue was evaluated by quantifying the copies of SRY gene of male donor MSCs, using quantitative real time PCR. The heart

tissues at peri-infarct area were collected 3 days after cell delivery from the different groups. DNA of the cells or tissues was extracted using a Takara kit (Takara Bio, Otsu, Japan). The DNA was dissolved in 100 ul of water, and 5ul of DNA was used for quantitative real time PCR. The relative DNA copy was calculated based on the equation derived from the standard curve obtained from the serial dilution of MSCs, the retention rate was expressed as engrafted number of male MSCs (i.e. DNA copies) over the amount of heart tissue.

To make a standard sry gene expression curve, genomic DNA was extracted from $2X10^6$ MSCs that were obtained from male rats, and dissolved in 100 ul of water. A 5-ul sample corresponding to 10^5 copies of male Y-chromosomal DNA was obtained and further diluted so that DNA copy ranged from 10^1 to 10^5 copies to create the standard curve. MSCs obtained from another ten individual rats were tested for evaluation of reproducibility.

Cardiac echocardiographic and LV catheterization examination

At 28 days post-MI, All the rats were anesthetized using the same method as described above. Two-dimensional and M-mode echocardiographic imaging were obtained and recorded (Vevo 2100 system). Once a standard cross-section at the papillary muscle level was obtained, M-mode eachocardiography was acquired and images were stored. Fractional shortening (FS), left ventricular end-diastolic diameter (LVEDD) were then analyzed for at least five consecutive cardiac cycles and a mean valued was used. Hemodynamic parameters were also measured with LV pressure

recording by invasive catheterization examination. A 1.4 F pressure catheter retrogradely inserted into the aorta and LV via the right common carotid artery cut down approach. The transducer was connected to a recording system (Powerlab, AD Instruments, Castle Hill, Australia). The position of the catheter was confirmed with a sudden drop of diastolic pressure from aortic diastolic pressure to a low left ventricular diastolic chamber pressure. The left ventricular pressure were recorded for at least 30 seconds when a stable pressure morphology was detected and left ventricular end diastolic pressure (LVEDP), the maximum rate of increase or decrease in LV chamber pressure (+dP/dt_{max} and -dP/dt_{max}, respectively) were then obtained[4].

Immunofluorescence staining

At 3 and 28 days after cell transplantation therapy, each group rats were sacrificed by a sufficient dose of potassium chloride and heart tissues were collected and part of the sample were used for both Masson trichrome and immunofluorescence staining. Heart tissues were dehydrated in 30% sucrose solution and embedded in Tissue-Tek OCT compound and frozen on dry ice. Frozen tissue slices of 6.0 μm thick were then prepared and tissue slices were then fixed with 4% formaldehyde solution for ten minutes, permeabilized with 0.02% Triton X-100 PBS and blocked with PBS containing 5% goat serum. Each section was then incubated with primary antibody against GFP (1:200, SantaCruz) for tissue slices obtained at day 3 after infarction or CD31 (1:200, Abcam) for tissue slices obtained at day 28 after infarction at 4°C overnight, followed by incubation with specific secondary antibodies (Santa Cruz

Biotechnology, Santa Cruz, CA, USA). Staining without the primary antibodies was used as control for nonspecific fluorescence. Hochest staining was done to counterstain the nuclei. The fluorescence was examined and photographed using Leica fluorescence microscope. Positively stained cells were counted in three randomly selected sections per heart, five high-power fields (HPFs) per section.

Masson trichrome staining

At day 28 post-MI, the infarcted size was measured by Masson trichrome staining. Briefly, frozen tissue sections of heart tissues from different groups were fixed in 4% paraformaldehyde, rinsed with PBS, and stained with Masson's trichrome kit. For each heart, four cross-section heart tissue at basal level, papillary muscle level, sub-papillary muscle level, and apical level were analyzed to quantify the ratio of the perimeter of the scar area over the mean of inner and outer circumference of the LV cross-section, using the Image Pro software. The infarct size was expressed as mean value of the ratio for the four sections[5]

Statistical analysis

All data are presented as means ± SE. Statistical analyses were performed by one-way ANOVA using the SPSS 17.0 software. A P value of less than 0.05 was considered to be statistically significant.

Figure Legends

Supplementary Figure 1. HP iincrease miR-211 expression in MSCs. MiR-211 expression was measured for both HP- and normoxia-treated MSCs (HP-MSCs and N-MSCs, respectively), and expressed as fold changes in HP-MSCs relative to N-MSCs (n=3).

Supplementary Figure 2. Efficiency of lentiviral infection of MSCs with miR-211-shRNA. MiR-211 expression was confirmed by real time PCR for MSCs that were infected with miR-211-over (over) or miR-211-shRNA (shRNA) and their respective controls are shown in black bars.

Supplementary Figure 3. Transplantation of MSCs infected with miR-211-shRNA in a rat MI model. MSCs infected with miR-211-shRNA (shRNA) or miR-211 scramble (NC) were delivered for MI rats, respectively, and another PBS treated MI rats served as controls (n≥5/group). Echocardiography was performed for each group MI rats (A) and hence fractional shortening was quantified (B). LV pressure recordings were also obtained and LVEDP (C) and $\pm dP/dt_{max}$ (D&E) were measured. Masson trichrome staining was performed for each group rats (H) to quantify the infarct size (I)

Supplementary Figure 4. Role of STAT5A in miR-211-mediated MSC migration. The efficiency of STAT5A shRNA was tested by measuring STAT5A protein

expression after MSCs infection with three different designs of shRNA targeting STAT5A compared with scramble (NC) (A, repeated three times) and the one resulted in the lowest expression of STAT5A by Western Blot (α-Tubulin as a loading control) was selected for the following experiments. The effect of STAT5A over (abbreviated as over) was also tested after MSCs were infected with over compared with empty vector control (CV). Western Blot was used to measure the protein expression level, with β-ACTIN as a loading control (B, repeated three times). Using transwell assay, the migrated MSCs were counted under a fluorescence microscope (C) and quantified for MSCs that were treated with over (compared with CV shown in black bar in D) or STAT5A shRNA (shRNA, compared with scramble abbreviated as NC as controls shown in black bar in D, n≥3), scale bar=200μm.

Supplementary Figure 5. Western blot for quantification of STAT5A. MSCs that were infected with STAT5A-over, miR-211-over or double gene over-expression virus were tested for STAT5A expression. Similarly, MSCs that were infected with STAT5A-shRNA, miR-211-shRNA or double gene silence were also tested for STAT5A expression. The band detecting β-ACTIN was used as a loading control (n≥3).

Supplementary Figure 6. HP induced STAT3 activation. Phosphorylation levels of STAT3 in normoxia cultured MSCs (N-MSCs) or HP treated MSCs (HP-MSCs) were quantified by western blots (expressed as the ratio of p-STAT3 over total STAT3),

with β-ACTIN used as a loading control (n≥3).

Supplementary Figure 7. MSCs were infected with lentiviruses for STAT3 gene manipulation. MSCs were infected with lentivirus containing different design of shRNA targeting STAT3 and efficiency of infection was confirmed by their protein expression using Western Blot compared with scramble controls (A, n≥3). The efficiency of infection with STAT3 over for MSCs was also tested by western blot compared with empty vector as control (B, n≥3), β-ACTIN or α-Tubulin was used as a loading control.

Reference

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