MiR-122 modification enhances the therapeutic efficacy of adipose tissue-derived mesenchymal stem cells against liver fibrosis

Guohua Lou, Ying Yang, Feifei Liu, Bingjue Ye, Zhi Chen, Min Zheng *, Yanning Liu *

Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Received: December 14, 2016; Accepted: March 11, 2017

Abstract

Mesenchymal stem cell (MSC) transplantation alone may be insufficient for treatment of liver fibrosis because of complicated histopathological changes in the liver. Given that miR-122 plays an essential role in liver fibrosis by negatively regulating the proliferation and trans-activation of hepatic stellate cells (HSCs), this study investigated whether miR-122 modification can improve the therapeutic efficacy of adipose tissue-derived MSCs in treating liver fibrosis. MiR-122-modified AMSCs (AMSC-122) were constructed through lentivirus-mediated transfer of pre-miR-122. MiR-122-modified AMSCs expressed high level of miR-122, while they retained their phenotype and differentiation potential as naïve AMSCs. AMSC-122 more effectively suppressed the proliferation of and collagen maturation in HSCs than scramble miRNA-modified AMSCs. In addition, AMSC-derived exosomes mediated the miR-122 communication between AMSCs and HSCs, further affecting the expression levels of miR-122 target genes, such as insulin-like growth factor receptor 1 (IGF1R), Cyclin G(1) (CCNG1) and prolyl-4-hydroxylase α1 (P4HA1), which are involved in proliferation of and collagen maturation in HSCs. Moreover, miR-122 modification enhanced the therapeutic efficacy of AMSCs in the treatment of carbon tetrachloride (CCl4)-induced liver fibrosis by suppressing the activation of HSCs and alleviating collagen deposition. Results demonstrate that miR-122 modification improves the therapeutic efficacy of AMSCs through exosome-mediated miR-122 communication; thus, miR-122 modification is a new potential strategy for treatment of liver fibrosis.

Keywords: adipose tissue-derived mesenchymal stem cells ● miR-122 ● hepatic stellate cells ● exosome ● liver fibrosis

Introduction

Transplantation of bone marrow mesenchymal stem cells (MSCs) is an alternative treatment of liver cirrhosis [1], an irreversible disease that may eventually lead to liver cancer. As adipose tissue is an accessible source of cells, adipose tissue-derived MSCs (AMSCs) are currently being investigated [2]. A sufficient number of cells for stem cell-based therapy can be obtained non-invasively from this tissue without damaging a patient’s health. These cells present very promising applications in therapy of liver diseases. Studies have suggested that AMSCs display a special affinity to hepatocyte differentiation in vitro and liver regeneration in vivo [2, 3]. Thus, AMSCs are very attractive tool for future stem cell therapy of liver diseases.

However, some studies have shown that many MSCs recruited into a fibrotic liver have become myofibroblasts, and at least some MSCs likely contribute to wound-healing mechanisms and fibrogenesis after liver parenchymal cell death [4]. Thus, genetic manipulation that induces MSCs to locally produce therapeutic genes possibly resulted in their altered cell fates and in their enhanced therapeutic abilities.

Liver cirrhosis is the result of pathological response to initial liver injuries of various causes. Hepatic stellate cells (HSCs), which play a key role in liver fibrogenesis, are well documented [5]. However, the mechanisms of pathogenesis and progression of liver fibrosis are complicated and may vary depending on the aetiology of the disease.
Increasing amount of evidence suggests that certain miRNAs are critically involved in different developmental stages of liver fibrosis, including activation of HSCs and production of ECM proteins [6, 7]. Being the most abundant miRNA in a normal liver, miR-122 is reduced in advanced liver diseases, such as cirrhosis and hepatocellular carcinoma [8, 9]. Mice with hepatocytes wherein miR-122 was selectively deleted displayed altered lipid metabolism, hepatic inflammation, fibrosis and high incidence of hepatocellular carcinoma [10]. Recent studies have suggested that miR-122 negatively regulates collagen production in HSCs [11, 12]. Thus, targeted delivery of miR-122 to the liver, particularly to the HSCs, is a potential novel therapeutic approach for treatment of liver fibrosis.

In this study, we suggest that the combination of AMSC transplantation and miR-122 transfer is superior over AMSC transplantation for the treatment of liver fibrosis.

Materials and methods

Mice

C57BL/6 mice (6-week old) were purchased from Zhejiang Academy of Medical Science, Hangzhou, Zhejiang, China. The animals were reared under a specific pathogen-free condition, and all procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Zhejiang University.

HSC isolation

Primary mouse HSCs were isolated as previously described [13] and cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% FBS (Gibco, Carlsbad, CA, USA) and 1% antibiotic–antimycotic (Gibco) under a humidified atmosphere with 5% CO₂. The primary HSCs used 3 days after isolation were as quiescent cells. HSCs were cultured for 7 days to allow transactivation.

Cell culture

LX-2, an immortalized human HSC line, was maintained in DMEM containing 10% FBS overnight. AMSCs were subsequently transfected with 100 nM LV-miR-122 or LV-cel-miR-67 (Vigene Biosciences, Rockville, MD, USA) at a multiplicity of infection (MOI) of 10 in the presence of polybrene (8 μg/ml; Sigma-Aldrich, St. Louis, MO, USA) for 24 hrs. Stable transfectants were used in the subsequent experiments.

Isolation and detection of miRNA

Total RNA enriched with miRNAs was isolated from AMSCs by using a miRvana miRNA isolation kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Complementary DNA was synthesized from the isolated miRNAs by using TaqMan™ hsa-miR-122- or mmu-miR-122-specific primers (Applied Biosystems) and TaqMan™ MicroRNA Reverse Transcription Kit (Applied Biosystems). To examine the expression of miR-122, we performed qPCR by using ABI PRISM7900 (Life Technologies, Waltham, MA, USA) and TaqMan™ Universal Master Mix (Applied Biosystems) according to the manufacturer’s instructions. The relative quantity of miR-122 expression (fold change) was determined using the 2⁻ΔΔCt method (ΔCt = CtmiRNA-modified AMSC−Ctnaive AMSC); U6 was used as endogenous control.

Flow cytometric analysis of cell immunophenotype

MiRNA-modified AMSCs were incubated with phycoerythrin (PE)-labelled primary antibodies raised against CD31, CD45, HLA-DR, CD29, CD44, CD73, CD90 and CD105 (Becton-Dickinson, San Jose, CA, USA) at 4°C for 30 min. PE-labelled IgG1 was used as isotype control. After washing and resuspension in PBS, the samples were analysed by a BD Accuri™ C6 flow cytometer (Becton-Dickinson).

Cell cycle analysis

Cells were stained by using a cell cycle staining kit (MultiSciences, Hangzhou, China) according to the manufacturer’s instructions. Distribution of cell cycle phases among cells with different DNA contents was determined by using a BD Accuri™ C6 flow cytometer.

Differentiation of AMSCs

Differentiation of miRNA-modified AMSCs into adipocytes and chondrocytes was determined using a StemPro™ adipogenesis and chondrogenesis differentiation kit (Gibco). Oil red O or Alcian Blue staining was subsequently performed to detect adipocytes and chondrocytes, respectively.

Cell viability

LX-2 cells (2 × 10⁵) were seeded on 96-well plates overnight, and then, the culture medium was replaced with the supernatant of AMSCs. Cell proliferation was determined 72 hrs after culture using an MTT assay (Sigma-Aldrich).

Journal of Cellular and Molecular Medicine published by John Wiley & Sons Ltd and Foundation for Cellular and Molecular Medicine.
Cell co-culture

AMSCs (2 × 10^5 cells/well) were inoculated in a semi-permeable membrane (Transwell insert) in the upper part of 6-well culture plates, and LX2 cells or primary mouse HSCs (2 × 10^5 cells/well) were inoculated in the lower part to establish a double-cell co-culture system. LX2 cells or primary HSCs cultured alone were used as control (untreated, U.T.). After co-culture for 48 hrs, the LX2 cells or primary HSCs were harvested for qPCR and Western blot assay.

RNA isolation and qPCR

Total RNA was isolated from LX2 cells or mice liver samples by using TRIzol. First-strand cDNA was subsequently synthesized followed by qPCR analysis by using ABI Prism 7900 (Applied Biosystems) to examine the expression levels of collagen type I alpha 1 (Col1A1), prolyl-4-hydroxylase α1 (P4HA1), Cyclin G(1) (CCNG1), insulin-like growth factor receptor 1 (IGF1R), TGF-β1 and α-SMA; GAPDH and β-actin were used as internal controls. The 2-ΔΔCT method was used to determine the relative mRNA expression levels of these genes.

Western blot analysis

To determine the protein expression levels, we prepared whole-cell or tissue extracts, which were fractionated using SDS-PAGE. After electrophoresis, the proteins were electro-transferred onto nitrocellulose membranes, blotted with COL1A1 (1:200), Santa Cruz Biotechnology, Dallas, TX, USA), P4HA1 (1:2000; Abcam, Cambridge, MA, USA), CCNG1 (1:1000; Abcam), IGF1R (1:1000; Abcam), TGF-β1 (1:1000; Cell Signaling Technology, Danvers, MA, USA), α-SMA (1:2000; Abcam), β-actin (1:10000, Huabio) and GAPDH (1:10000; Abcam) antibodies. The proteins were finally detected using an enhanced chemiluminescence reagent (Amersham, Stavanger, Norway). Blots against β-actin or GAPDH served as loading controls.

Exosome preparation and harvest

AMSC-derived exosomes were isolated and identified as previously described [14]. Exosomes were isolated from the supernatant of AMSCs by using an ExoQuick-TC Kit (System Biosciences, Johnstown, PA, USA) according to the manufacturer’s instructions. These exosomes were subsequently characterized through Western blot analysis of exosome surface markers, such as CD9 (1:2000; Abcam) and CD63 (1:2000; Abcam). Precipitates from AMSC supernatant were used as negative control. The protein content of exosomes was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Exosome pellets were subsequently resuspended in sterile PBS at a total protein concentration of 5 μg/μL.

Confocal microscopic studies

AMSCs were labelled with the phospholipid membrane dye lipophilic carbocyanine DilC18 (3) (1.25 μM) [15]. After 10 min. of incubation at 37°C, the cells were washed and then resuspended in fresh media for 48 hrs. Fluorescent exosomes were collected and added into recipient LX2 cells. Afterwards, the cells were fixed with methanol, mounted on slides, and imaged via confocal microscopy (Olympus, Center Valley, PA, USA). Background fluorescence was subtracted using unstained cells.

AMSC transplantation

Carbon tetrachloride (CCl4) (diluted 1:1 in olive oil; Sigma-Aldrich) was administered through intra-peritoneal (IP) injection at 1 ml/kg twice per week to induce liver cirrhosis. One day after the fourth injection of CCl4, 1 × 10^5 mAMSC-122 or mAMSC-67 or similar volume of saline as control was injected into the tail vein. The mice were further treated with CCl4. After 4 weeks, the mice were killed, and blood serum and liver samples were collected to assess the extent of liver fibrosis. Blood serum was evaluated for biochemical parameters. The liver samples were evaluated through histochemical analysis, qPCR and Western blot assay.

Histological examination

Four weeks after AMSC transplantation, liver samples were harvested 72 hrs after the last injection of CCl4, fixed with 10% formalin and embedded with paraffin. Histological analyses of liver tissues were conducted by Sirius red staining to determine the extent of the development of liver fibrosis.

Evaluation of serum parameters for liver fibrosis and function

Alanine aminotransferase aspartate (ALT) and aspartate aminotransferase (AST) were measured using FUJI DRI-CHEM Slide GFP/ALT-PIII and GOT/AST-PIII, respectively, according to the manufacturer’s instructions for DRI-CHEM 4000ie (FUJIFILM). Serum hyaluronic acid (HA) and procollagen III-N-peptide (P-III-P) were measured by using radio-immunoassay.

Evaluation of hepatic hydroxyproline

Hepatic hydroxyproline content was measured using a hydroxyproline kit (Nanjing Jian Cheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer’s instructions [16]. The hydroxyproline content of the liver was expressed in micrograms per gram of wet weight (μg/g).

Statistical analysis

Differences between groups were analysed using the conventional Student’s t-test or ANOVA. Each experiment was performed at least three times, and data are presented as mean ± S.D. P < 0.05 indicated statistical significance.

Results

Construction of miR-122-modified AMSCs

Mouse or human AMSCs were transfected with LV-miR-122 or LV-cell-miR-67, which has no known mRNA binding targets in mouse and...
human, to serve as control. After the stable transfection, miR-122 expression in miR-122-modified AMSC (AMSC-122) was assayed by qPCR and compared that in cel-miR-67-transfected AMSCs. MiR-122 expression was significantly higher in AMSC-122 than in scramble miRNA-modified AMSC (AMSC-67) (Fig. 1A and B). No differences in phenotype were observed between miRNA-modified mAMSCs and naive mAMSCs. They were both negative for CD31, CD45 and I-A/I-E and positive for CD29, CD44, CD90.2 and CD105. Moreover, the phenotype of miR-122-modified hAMSC was similar to naive hAMSCs; they were both negative for CD31, CD45 and HLA-DR and positive for CD29, CD44, CD73, CD90 and CD105 (Fig. 1C).

To further test the differentiation property of miR-122-modified AMSCs in vitro, multi-lineage differentiation into adipocytes and chondrocytes were induced, and differentiated cells were detected through Oil red O staining and Alcian blue staining, respectively. As shown in Figure 1E and F, miR-122-modified AMSCs can still differentiate into multi-lineage cells similar to naive AMSCs.

### MiR-122-modified AMSCs effectively suppress the proliferation of and collagen maturation in HSCs

Considering that HSCs play a key role in liver fibrogenesis, we evaluated the effect of miR-122-modified AMSC on HSCs. As shown in Figure 2A, cell proliferation of LX2 cells treated with the supernatant of hAMSC-67 was slightly inhibited compared with that of untreated (U.T.) LX2 cells. However, cell growth was significantly inhibited after treatment with the supernatant of hAMSC-122. Moreover, treatment of LX2 cells with hAMSC-122 supernatant for 24 hrs increased the percentage of G0/G1 population from 50.6% ± 1.3% to 71.2% ± 1.1%. Treatment of LX2 cells with hAMSC-67 supernatant also increased the G0/G1 population from 50.6% ± 1.3% to 66.8% ± 1.5% (Fig. 2B–E). We then investigated the effect of miR-122-modified AMSCs on Col1A1 expression in LX2 cells. As shown in Figure 2F, the protein expression of mature Col1A1 significantly decreased in hAMSC-122 co-cultured LX2 cells compared with that in the hAMSC-67 co-cultured group or U.T. LX2 cells. By contrast, co-culture with hAMSC-122 exerted no effect on the mRNA level of Col1A1 (Fig. 2G). These results indicated that miR-122 modification in AMSC inhibited collagen synthesis and maturation.

We further analysed the effect of miR-122-modified mAMSCs on primary mouse HSCs. As shown in Figure 3A, miR-122 was constitutively expressed in quiescent mouse HSCs. However, miR-122 expression was significantly down-regulated in mouse HSCs upon culture-induced activation. Treatment of the culture-activated mouse HSCs with mAMSC-122 supernatant increased the miR-122 level in the cells (Fig. 3B), also inducing significant cell growth inhibition (Fig. 3D) and G0/G1 arrest (Fig. 3E) in HSCs. Moreover, the protein level of Col1A1 significantly decreased in mAMSC-122 co-cultured HSCs similar to that in the hAMSC-122 co-cultured LX2 cells (Fig. 3C).

### Exosomes mediate miR-122 communication between AMSCs and HSCs

MiRNAs are abundant in extracellular exosomes and can be transferred from cell to cell via exosome release and uptake, resulting in cross-cellular gene regulation [17]. Increasing lines of evidence suggest that MSC-derived exosomes play a role in the therapeutic effect of MSCs through paracrine mechanism. As shown in Figure 4A, MSC-derived exosomes positively express of exosomal markers, such as CD9 and CD63. To assess the role of AMSC-derived exosomes on miR-122 communication, we labelled the miR-122-modified hAMSCs with lipophilic carboxylic DiIC(16)(3). After additional culture for 48 hrs, fluorescent exosomes were collected and added into the recipient LX2 cells. Confocal imaging revealed the delivery of labelled exosomes as indicated by the presence of fluorescent membrane dyes in the unlabelled recipient LX2 cells (Fig. 4B). Another proof is that the expression level of miR-122 was 2.95 × 10^5 and 7.24 × 10^4-fold higher in hAMSC-122 exosomes (122-Exo) and 122-Exo-treated LX2 cells than those in hAMSC-67 exosomes (67-Exo) and 67-Exo-treated LX2 cells, respectively (Fig. 4C and E). We also determined the miR-122 levels in hAMSC supernatant and its supernatant-treated LX2 cells; miR-122 expression was significantly up-regulated in both types of cells compared with that in hAMSC-67 supernatant and its supernatant-treated controls, respectively (Fig. 4D and F). These data indicated that AMSCs packaged miR-122 into secreted exosomes, and these exosomes delivered miR-122 into LX2 cells.

To further define the effects of exosome-mediated miR-122 communication on HSCs, we evaluated the expression levels of miR-122 target genes, such as P4HA1, IGF1R and CCNG1 which are involved in the proliferation and collagen maturation of HSCs [11, 18, 19], in 122-Exo-treated LX2 cells. As shown in Figure 4G and I, treatment of LX2 cells with 122-Exo significantly down-regulated the mRNA and protein levels of these genes. We also investigated these genes in hAMSC supernatant-treated LX2 cells, and the expression levels of these target genes were also significantly down-regulated in hAMSC-122 supernatant-treated LX2 cells, compared with those in hAMSC-67 supernatant-treated controls (Fig. 4H and J). These data showed that miR-122 modification improves the therapeutic potential of AMSCs against HSCs through exosome-mediated miR-122 communication.
MiR-122 modification improves the therapeutic potential of AMSCs against liver fibrosis

To further determine whether miR-122-modified AMSCs display better therapeutic abilities to reverse liver fibrosis, we administrated mAMSC-122 or mAMSC-67 into mice with chronic liver failure (2 weeks after CCl4 injection) via i.v. injection. Sirius red staining revealed that 6 weeks of exposure to CCl4 resulted in marked development of liver fibrosis. After administration of AMSCs for 4 weeks, the livers from the vehicle group showed formation of bridging fibrosis, whereas mAMSCs-122 administration significantly suppressed fibrosis development. Moreover, the degree of liver fibrosis in mAMSC-67 group slightly differed from that in the vehicle group (Fig. 5A).

The serum levels of HA, P-III-P, ALT, AST and liver hydroxyproline content were significantly suppressed in mAMSC-122 administration group compared with those in the vehicle group (Table 1). The expression levels of TGF-β1 and α-SMA in the liver were also significantly down-regulated in mAMSC-122 group. However, these markers were only slightly suppressed in mAMSC-67 group (Fig. 5B–D). Additionally, down-regulation of miR-122 in the mice liver as a result of repeated CCl4 treatment was ameliorated in mAMSC-122 group compared with that in vehicle group. The level of mature Col1A1 protein was also significantly reduced in mAMSC-122 group relative to that in the vehicle group. The levels of miR-122 and mature Col1A1 proteins slightly differed between mAMSC-67 group and vehicle group (Fig. 5E and F). These results indicated that miR-122 modification enhanced the therapeutic effect of AMSCs on liver fibrosis.

Discussion

Studies have shown that treatment of liver fibrosis with naïve MSC transplantation alone may be insufficient. Genetic manipulation, which aims to produce therapeutic genes, enhances the therapeutic abilities of MSCs [20, 21]. MiR-122 is down-regulated in the livers of patients with primary biliary cirrhosis [22] and chronic HCV-induced fibrosis [23, 24] and in the livers of mice treated with CCl4 [11, 12]; moreover, miR-122 plays a critical role in liver fibrosis by negatively regulating the proliferation and transactivation of HSCs [11]. Thus, targeted delivery of miR-122 into liver, particularly in HSCs, is a potential novel strategy for enhancing the therapeutic effect of MSCs on liver fibrosis. This study showed that miR-122 modification in AMSCs improved the therapeutic potential of MSCs against liver fibrosis. In addition, AMSC-derived exosomes may mediate the miR-122 communication between AMSCs and HSCs, affecting the expression levels of...
miR-122 target genes, which play a role in proliferation of and collagen maturation in HSCs.

Exosomes are small nanometer-sized vesicles of endocytic origin; they essentially function as intercellular shuttle loaded with a cargo of protein and RNA by effector cells for off-loading in target cells [17, 25, 26]. Increasing studies indicate that MSCs demonstrate a scalable capacity to mass produce exosomes, which may play a role in therapeutic effect of MSCs through paracrine mechanism [27–29]. Follow-up studies have confirmed that MSC-derived exosomes can be used as a vehicle for delivery of therapeutic miRNA [30–32]. Our study revealed that miR-122-modified AMSC effectively packaged miR-122 into secreted exosomes, which delivered their miR-122 content into HSCs, resulting in altered miR-122-target gene expression in HSCs.

To understand the mechanism of miR-122-modified AMSCs in rescuing liver fibrosis, we examined the expression levels of miR-122 target genes, which are involved in HSC proliferation and activation. Our results showed that IGF1R expression was significantly reduced by 122-Exo treatment. Given that enhanced IGFR signalling is implicated in rapid proliferation of activated HSCs [33], 122-Exo-mediated down-regulation of this gene may contribute to the inhibition of HSC proliferation by miR-122-modified AMSCs. A similar growth inhibitory effect of miR-122 by regulation of several tumorigenesis-related proteins, including ADAM10, TFDP2 and E2F1, was previously demonstrated in hepatoma cells [34, 35].

Cell cycle arrest contributes to growth inhibition. Reports have shown that MSCs exert a growth inhibitory effect on HSCs by inducing G0/G1 arrest via secretion of the soluble factors TGF-beta3 and HGF, resulting in up-regulation of p21 expression and down-regulation of cyclin D1 [36]. Other studies have shown that ectopic expression of miR-122 inhibits the growth of hepatoma cells. This finding is correlated with an increase in population of cells at the G1 phase and a decrease in that at the G2/M phase by modulating the expression of cyclin G1, which is involved in G2/M arrest in response to DNA damage [37]. Our results showed that miR-122-modified AMSCs more effectively induced G0/G1 arrest of HSCs than scramble miRNA-modified AMSCs. This finding was observed possibly because down-regulation of 122-Exo-mediated CCNG1 reduced the cell population in G2/M phase, thereby enhancing the G0/G1 arrest of HSCs by miR-122-modified AMSCs.

In addition to its ability to inhibit proliferation, miR-122 blocks collagen maturation in HSCs. P4HA1, which encodes a component of prolyl 4-hydroxylase, a key enzyme in collagen post-translational modification and maturation, is another target gene of miR-122 [11]. We found that 122-Exo treatment reduced P4HA1 expression level, and co-culture with miR-122-modified AMSCs significantly inhibited the production of mature Col1A1 in LX2 cells. These data suggested that 122-Exo-mediated down-regulation of P4HA1 contributed to the inhibitory effect of miR-122-modified AMSCs on maturation of collagen.

Systemically administered MSCs in animal models can engraft into the liver and ameliorate liver fibrosis caused by CCl4 [38, 39]. However, some studies have shown that timing of MSC administration is an important determinant of the therapeutic effects of MSCs [40]. Enhanced liver function recovery was observed when MSCs
were delivered at the onset of CCl₄ injury and not under delayed administration. This phenomenon is possibly caused by the irreversible course of CCl₄-induced injury; wherein once injury occurred, it can no longer be rescued by MSC administration. Our study showed that administration of naive AMSCs (data not shown) or scramble miRNA-modified AMSCs 2 weeks after the first CCl₄ injection did not...
effectively ameliorate liver fibrosis. While administration of miR-122-modified AMSCs successfully down-regulated the serum fibrotic markers HA and P-III-P, reduced the serum ALT and AST levels and reduced collagen deposition in liver fibrosis.

To further investigate the underlying mechanism of miR-122-modified AMSCs in rescuing CCl₄-damaged liver, we examined the expression levels of TGF-β1 and α-SMA, which are key cytokines involved in the development of liver fibrosis and in HSC activation [41]. We found that miR-122-modified AMSCs more effectively prevented the up-regulation of TGF-β1 and α-SMA in fibrotic mouse livers than the scramble miRNA-modified AMSCs. Moreover, the protein level of mature Col1A1 in AMSC-122 administration group decreased, which is correlated with the increase in miR-122 expression in the liver. These data suggest that AMSC-122 treatment may ameliorate liver fibrosis by suppressing HSC activation. Studies have shown that miR-122 expression decreases in advanced fibrosis and is negatively correlated with fibrosis stage and liver stiffness values in hepatic fibrosis with various aetiologies [12, 24, 42, 43]; thus, the lower degree of reduction in miR-122 level in the liver is possibly due to the amelioration of hepatic fibrosis by AMSC-122 administration and not solely because of the delivery of miR-122 into the liver. These results suggest that transplantation of miR-122-modified AMSCs presents several advantages over the transplantation of naive AMSCs for treatment of liver fibrosis; these advantages include inhibition of HSC activation and blockade of collagen maturation. Thus, miR-122 modification can broaden the time window for MSC transplantation.

In summary, miR-122 modification can improve the therapeutic efficacy of AMSCs for the treatment of liver fibrosis. Administration of miR-122-modified AMSC not only repairs damaged liver; rather, AMSC-derived exosomes mediate miR-122 communication of donor cells to host HSCs and inhibit the proliferation and collagen maturation of HSCs, thereby reducing fibrotic changes in liver.

### Author’s contributions

LG carried out the construction of miR-122-modified adipose tissue-derived MSCs (AMSCs), performed the in vivo studies and drafted the manuscript. YY, LF, YB, and CZ participated in the in vitro and in vivo experiments and performed the statistical analysis. ZM and LY conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript.

### Funding source

The work was supported by the National Natural Science Fund (81000730 & 81470851).

### Conflict of interest

The authors confirm that there is no conflict of interests.

### References

1. Terai S, Takami T, Yamamoto N, et al. Status and prospects of liver cirrhosis treatment by using bone marrow-derived cells and mesenchymal cells. Tissue Eng Part B Rev. 2014; 20: 206–10.
2. Ishikawa T, Banas A, Hagiwara K, et al. Stem cells for hepatic regeneration: the role of adipose tissue derived mesenchymal stem cells. Curr Stem Cell Res Ther. 2010; 5: 182–9.
3. Liu WH, Song FQ, Ren LN, et al. The multiple functional roles of mesenchymal stem cells in participating in treating liver diseases. J Cell Mol Med. 2015; 19: 511–20.
4. di Bonzo LV, Ferrero I, Cravanzola C, et al. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. Gut. 2008; 57: 223–31.
5. Hellerbrand C. Hepatic stellate cells—the pericytes in the liver. Pflugers Arch. 2013; 465: 775–8.
6. Hyun J, Wang S, Kim J, et al. MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression. Nat Commun. 2016; 7: 10993.
7. Bala S, Csak T, Saha B, et al. The pro-inflammatory effects of miR-155 promote liver fibrosis and alcohol-induced steatohepatitis. J Hepatol. 2016; 64: 1378–87.
8. Bandiera S, Pfeffer S, Baumert TF, et al. miR-122—a key factor and therapeutic target in liver disease. J Hepatol. 2015; 62: 448–57.

### Table 1 MiR-122-modified mAMSC suppress serum fibrosis markers in CCl₄-induced liver fibrosis

|                | HA (ng/ml)       | P-III-P (ng/ml) | ALT (U/ml)  | AST (U/ml)  | Hydroxyproline (µg/g) |
|----------------|------------------|----------------|-------------|-------------|-----------------------|
| N.C           | 26.2 ± 5.9*      | 8.2 ± 2.7†     | 32.4 ± 4.9‡ | 81.8 ± 6.3†  | 205.7 ± 22.8‡         |
| Vehicle       | 196.6 ± 24.7*    | 40.5 ± 4.1*    | 189.8 ± 35.5* | 232.3 ± 34.7* | 567.3 ± 74.7†        |
| mAMSC-67      | 141.8 ± 29.8*    | 31.9 ± 5.3*    | 131.5 ± 26.1* | 154.8 ± 22.9*    | 460.4 ± 56.3†        |
| mAMSC-122     | 41.2 ± 12.5†     | 16.3 ± 3.9*‡   | 47.4 ± 12.2‡ | 97.6 ± 21.5†     | 295.48 ± 48.5‡*       |

Data are presented as the means ± S.D. HA, hyaluronic acid; P-III-P, procollagen III-N-peptide; ALT, alanine aminotransferase; AST, aspartate aminotransferase. *As compared with vehicle group, P < 0.05. † as compared with vehicle group, P < 0.01; ‡ as compared with N.C group, P < 0.05; † as compared with N.C group, P < 0.01.
model of liver fibrosis. Mol Imaging Biol. 2016; 18: 43–51.
21. Huang CK, Lee SO, Lai KP, et al. Targeting androgen receptor in bone marrow mesenchymal stem cells leads to better transplantation therapy efficacy in liver cirrhosis. Hepatology. 2013; 57: 1550–63.
22. Pagdett KA, Lan RY, Leung PC, et al. Primary biliary cirrhosis is associated with altered hepatic microRNA expression. J Autoimmun. 2009; 32: 246–53.
23. Butt AM, Raja AJ, Siddique S, et al. Parallel expression profiling of hepatic and serum microRNA-122 associated with clinical features and treatment responses in chronic hepatitis C patients. Sci Rep. 2016; 6: 21510.
24. Trebicka J, Anadol E, Ellimova N, et al. Hepatic and serum levels of miR-122 after chronic HCV-induced fibrosis. J Hepatol. 2015; 58: 234–9.
25. Pitt JM, Kroemer G, Zitvogel L. Extracellular vesicles: masters of intercellular communication and potential clinical interventions. J Clin Invest. 2016; 126: 1139–43.
26. Sato K, Meng F, Glaser S, et al. Exosomes in liver pathology. J Hepatol. 2016; 65: 213–21.
27. Yeow RW, Lai RC, Zhang B, et al. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. Adv Drug Deliv Rev. 2013; 65: 336–41.
28. Xin H, Li Y, Buller B, et al. Exosome-mediated transfer of miR-133b from multi-potent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Stem Cells. 2012; 30: 1556–64.
29. Anderson JD, Johansson HJ, Graham CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-kappaB signaling. Stem Cells. 2016; 34: 601–13.
30. Phinney DG, Di Giuseppe M, Nahj J, et al. Mesenchymal stem cells use extracellular vesicles to orchestrate myophagy and shuttle microRNAs. Nat Commun. 2015; 6: 8457.
31. Katakowski M, Buller B, Zheng X, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett. 2013; 35: 201–4.
32. Shimbo K, Mlyaki S, Ishihi H, et al. Exosome-formed synthetic microRNA-143 is transferred to osteosarcoma cells and inhibits their migration. Biochem Biophys Res Commun. 2014; 445: 381–7.
33. Yasuda Y, Shimizu M, Sakai H, et al. (−)Epigallocatechin gallate prevents carbon tetrachloride-induced rat hepatic fibrosis by inhibiting the expression of the PDGFRβ and IGF-1R. Chem Biol Interact. 2009; 182: 159–64.
34. Wang B, Hsu SH, Wang X, et al. Reciprocal regulation of miR-122 and c-Myc in hepatocellular cancer: role of E2F2 and TGFβ2. Hepatology. 2014; 59: 555–66.
35. Chen CL, Wu JC, Chen GY, et al. Baculovirus-mediated miRNA regulation to suppress hepatocellular carcinoma tumorigenesis and metastasis. Mol Ther. 2015; 23: 79–88.
36. Wang J, Bian C, Liao L, et al. Inhibition of hepatic stellate cells proliferation by mesenchymal stem cells and the possible mechanisms. Hepatol Res. 2009; 39: 1219–28.
37. Kimura SH, Iikawa M, Itou A, et al. Cyclin G1 is involved in G2/M arrest in response to DNA damage and in growth control after damage recovery. Oncogene. 2001; 20: 3290–300.
38. Sakaida I, Terai S, Yamamoto N, et al. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. Hepatology. 2004; 40: 1304–11.
39. Volarevic V, Nurkovic J, Arsenijevic N, et al. Concise review: Therapeutic potential of mesenchymal stem cells for the treatment of acute liver failure and cirrhosis. Stem Cells. 2014; 32: 2181–23.
40. Fang B, Shi M, Liao L, et al. Systemic infusion of FLK1(−) mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice. Transplantation. 2004; 78: 83–8.
41. Batailler R, Brenner DA. Liver fibrosis. J Clin Invest. 2005; 115: 209–18.
42. Halicz T, Horváth G, Pága G, et al. miR-122 negatively correlates with liver fibrosis as detected by histology and FibroScan. World J Gastroenterol. 2015; 21: 7814–23.
43. Van Keuren-Jensen KR, Maletiča I, Courtright AL, et al. microRNA changes in liver tissue associated with fibrosis progression in patients with hepatitis C. Liver Int. 2016; 36: 334–43.