Comparison of the effects of different mesenchymal stem cells on attenuating phosgene-induced lung injury in rats

Yubei Qu
Fudan University
Lin Zhang
Jinshan Hospital of Fudan University
Daikun He
Jinshan Hospital of Fudan University
Ning Xu
Jinshn Hospital of Fudan University
Yuedong Tang
Jinshan Hospital of Fudan University
Jianwen Ding
Jinshan Hospital of Fudan University
Jie Shen (professorjieshen@163.com)
Jinshan Hospital of Fudan University

Research

Keywords: BMSCs, LRMSCs, hCMSCs, p-GSK-3β, β-catenin

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-27240/v1

Abstract

Background

Phosgene-induced lung injury is an important type of acute lung injury (ALI), which mainly leads to acute pulmonary edema. Currently, no effective clinical treatment has been developed yet. In the present study, the effects of lung-resident mesenchymal stem cells (LRMSCs), bone marrow mesenchymal stem cells (BMSCs) and human chorionic villi-derived MSCs (hCMSCs) were compared in phosgene-induced lung injury of male SD rats.

Methods

The changes in body weight, PaO_2 and respiratory indexes were recorded. Rats were sacrificed at 6 h, 24 h, and 48 h. Expressions of TNF-α, IL-1β, IL-6, HGF and IL-10 were tested by ELISA. SP-C mRNA expression was assessed by RT-PCR. The MSCs migration was assessed using Transwell migration assay and Wound-healing assay. Hepatocyte growth factor (HGF) was added to the MSCs culture medium for 48 h. Expressions of p-GSK-3β and β-catenin were determined by Western blot.

Results

Significant improvements in body weight, PaO_2 and respiratory indexes were observed after treatment with MSCs. BMSCs produced the highest effects followed by hCMSCs and LRMSCs. Compared with the phosgene group, MSCs decreased the levels of TNF-α, IL-1β, and IL-6 and increased the levels of IL-10, HGF and SP-C. Different MSCs displayed no significant differences in proliferation. However, BMSCs showed the strongest migration ability. HGF increased the expressions of p-GSK-3β and β-catenin in MSCs. All MSCs exerted protective effects on phosgene-induced lung injury by reducing inflammation, immune regulation and restoring cell function.

Conclusions

BMSCs showed the best protective effects followed by hCMSCs and LRMSCs. HGF enhances the role of MSCs by activating the p-GSK-3β/β-catenin signaling pathway.

Introduction

Phosgene, as a highly toxic gas, is widely used as an intermediate in the chemical synthesis. Lung injury caused by inhalation of phosgene mainly leads to acute toxic pulmonary edema. However, there is no clear consensus on the pathogenesis of poisoning caused by phosgene and there is no targeted treatment for phosgene induced lung injury. Currently, phosgene-induced lung injury is often treated with

clinical support, and new treatment methods are urgently necessary. In recent years, MSCs have become an important research hotspot in lung injury, bringing hope to the treatment of phosgene inhalation lung injury. BMSCs, LRMSCs, and hCMSCs are intensively studied. To date, BMSCs are the most extensively studied stem cells, due to their potential of self-renewal and multidirectional differentiation. BMSCs have a specific role in the treatment of multiple systemic diseases such as acute pancreatitis, ischemic stroke, fibrotic scar and so on [1–3]. Previous studies have confirmed that BMSCs had decreases the severity of lung injury caused by phosgene [4]. LRMSCs are a class of MSCs derived from the lungs. Studies have shown that LRMSCs co-cultured with type II epithelial cells (ATII) can differentiate into ATII cells [5]. LRMSCs are reported to treat hyperoxia-induced lung injury in mice [6]. Studies have also confirmed that LRMSCs can significantly improve inflammatory response and reduce pulmonary edema in endotoxin-induced lung injury [7]. HCMSCs also play a repair role in lung injury [8]. However, the specific effects of LRMSCs and hCMSCs in phosgene inhalation lung injury have not been reported. Although several studies have demonstrated the effects of MSCs in ARDS, very few studies have compared the effects of the different MSCs. Therefore, it is important to determine the most effective MSCs and cytokines for future clinical applications in phosgene-induced lung injury.

Recent studies in naphthalene-damaged mice have shown that BMSCs are distributed in the lungs following naphthalene exposure to help regenerate club cells. Besides, the migration of stem cells which is mediated by chemokines is an important differentiation point between BMSCs and hCMSCs [9]. This suggests that different migration ability of MSCs may be the key to the differences in the roles of different MSCs in repairing lung injury. HGF is a cytokine with functions such as mitogen, cytogen, morphogen and angiogenesis. In normal and tumor tissue, HGF can be produced by fibroblasts and other stromal cells and aggregated in the extracellular matrix and basement membrane to bind to glycosaminoglycan sulfate [10]. Studies have reported that HGF plays a major role in promoting migration in many tumors [11, 12]. Other studies have confirmed that HGF promotes the directional migration of MSCs [13, 14]. Roggia et al. confirmed HGF enhanced the differentiation of embryonic stem cells [15]. HGF was proved to be an effective differentiating factor in the comparison of embryonic stem cells, cardiac stem cells and adipose tissue stem cells. Therefore, HGF is associated with the differential mechanism of the anti-lung injury action of stem cells by affecting the activity and function of MSCs. HGF influences the role of stem cells and this may be an important factor affecting the differences in protective effects caused by the three MSCs in lung injury.

β-catenin plays an indispensable role as an adhesion adapter and transcriptional synergistic regulator. It also regulates and determines the fate of cells during embryogenesis and also involved in the induction of embryonic mesoderm [16, 17]. Activated β-catenin can improve the migration of MSCs in mice and humans [18, 19]. The migration of human LRMSCs has been closely related to the β-catenin signaling pathway [20]. Wnt, as the most famous activator, promotes β-catenin accumulation in the cytoplasm, which is transferred into the nucleus and binds to transcription factors of the LEF-1/TCF family to change the expression of the β-catenin gene [21]. Meanwhile, other ligands also affect the β-cateninn pathway [22]. GSK-3β is an important component involved in this signal pathway. GSK-3β phosphorylation affects β-catenin expression. Numerous studies have confirmed that GSK-3β plays an important role in the

protection of the viscera. Several pathways in the body converge on GSK-3β making it a key protein. Here, it is suggested that HGF triggers a signaling pathway in MSCs. This pathway may be that HGF induces phosphorylation of GSK-3β and futher leads to the accumulation and nuclear translocation of β-catenin in the cytoplasm. To verify this biological effect, it is hypothesized that HGF induces cell migration by activating β-catenin pathway. In this study, effects of BMSCs, LRMSCs, and hCMSCs in phosgene inhalation lung injury was compared and the mechanism of difference in the function of different MSCs was studied.

Materials And Methods

Phosgene inhalation lung injury

Clean grade Sprague-Dawley rats (Male, 180–220 g, 5–6 weeks old) were provided by the Medicine School of Fudan University and maintained in a clean animal house at 22 ~ 24℃ (dark/light cycle, 12 h). We obey the requirements of the Ethics Committee of Jinshan Hospital affiliated to Fudan University and complied with international guidelines for animal experimentation. The method of establishing the lung injury model by phosgene was developed as previously described [23]. Briefly, rats were poisoned with phosgene (8.33 mg/L) in a 24L sealed chamber for 5 minutes. Animals were randomly divided into several groups (n = 6 at each time point).

Isolation of LRMSCs and cell culture

LR-MSCs were isolated from BALF of rats treated by KGF-2 as previously described [24]. The lungs of each rat were added into rhKGF-2 (5 mg/kg, 200μL). After 72 h, LRMSCs were extracted from BALF. The cells precipitation was left and plant in T25 bottles. Cells were cultured in a incubator with 5% CO_2 at 37℃ for 14 days. Flow cytometry analysis demonstrated that CD73, CD105, CD90 and CD44 were positively expressed for cell surface markers while CD45 was negatively expressed for hematopoietic markers. Multi-lineage differentiation potential of LR-MSCs was confirmed by induction into other mesenchymal lineages such as osteocytes, adipocytes, and chondrocytes as previously demonstrated [24]. BMSCs and hCMSCs were provided by ScienCell Research Laboratory (Carlsbad, CA, USA) with primary cells (processing institutional identification certificate). Cells were cultured in high-glucose DMEM (Invitrogen, Carlsbad, CA) which was supplemented with 10% FBS, Penicillin/Streptomycin (100 U/ml) and fungizone (0.5%) in a humidified incubator with 5% CO_2 at 37℃. MSCs at P3 ~ P6 were used in the experiments.

CCK8 assay

When the numbers of MSCs reached 5×10^4 after being cultured in DMEM, MSCs were digested with 0.25% trypsin. The detached cells were transferred into the 96-well plates (10^3 cells/well). The medium (100μL) was added to each well and 8 repeated holes were set up in each group at each time. CCK8 (85 mg/mL, 10μL) was added. The cells were put back into the cabinet and incubated at 37℃ for

120 min. The OD450 values were determined every 24 hours until the ending of the experiment at the 96th hour.

Treatment of MSCs

The treatment groups were given with MSCs (1×10^6) in 100μL of PBS suspension by tracheal infusion immediately after being exposed in phosgene. The normal group and phosgene exposed group were given the same volume (100μL) of PBS as the control group through the trachea by using the same method. The rats were sacrificed in 6 h, 24 h and 48 h after being poisoned, respectively. Their lungs, BALF and serum were collected for subsequent analyses.

Histological study

The collected lungs were fixed in 10% formaldehyde. After that, lungs were embedded in paraffin and wad made to 5 μm sections. Then they were stained with hematoxylin and eosin (H&E). Lung injury scores of each animal were calculated according to previously described methods (Loughlin et al. 2007).

Lung wet to dry ratio

A small piece of tin foil paper (2cm × 2 cm) was weighed as W1. The right lower lung lobe was taken from every rat and wrapped in foil after its residual blood was wiped off the surface. The weight was measured as W2. Then it was placed in a 60 ℃ dryer, dried for 48 h and weighed as W3 then. According to the formula, W/D = (W2-W1)/(W3-W1), lung wet to dry ratio was recorded for evaluating the severity of pulmonary congestion and edema.

Bronchoalveolar lavage fluid (BALF) collection

The animal's neck skin was cut and opened to find the trachea at 6 h, 24 h and 48 h after phosgene inhalation. The trachea was cut 1/3 under the cricoid cartilage. The scalp needle connection tube with a length of about 3 cm was prepared and connected with a 5 mL needle tube. The connection was inserted from the incision along the direction of the rat trachea. Then, PBS (1 mL) was injected and lavage was performed for 3 times. The obtained BALF was transferred and centrifuged (1000 rpm × 10 min, at 4℃) to remove the precipitation and the supernatant was immediately detected.

Protein concentration and cell count

Protein concentration in BALF was assayed by bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL, USA) following the instructions. The cell pellet was obtained from BALF after centrifuge (1500 rpm × 10 min, at 4 °C). The total cell count was recorded by a hemocytometer.

ELISA

The protein expressions of IL-6, IL-1β, TNF-α, IL-10 and HGF in BALF and serum were detected with the corresponding kits (KANGLANG Technology Co., Ltd., Shanghai, China) according to the manufacturer's instruction. The OD450 value of each well was measured.

RT-PCR

The total RNA was isolated from each sample using Column Animal RNAout (Shanghai Zeye Biotechnology Co., Ltd., Shanghai, China) according to the instruction. An Easyscript first-strand cDNA synthesis super mix kit (Saiweier, Shanghai, China) was used to synthesize the first strand of cDNA by RT-PCR. The primers (Shenggong, Shanghai, China) were used in this experiment mainly for targeting SP-C (F:5'-GCCCACCGGATTACTCGAC-3', R:5'-TGACTCATGTGAAGGCCCAT-3'), HGF (F:5'-CTCCCCATCGCCATCCCCCC-3', R:5'-CACCATGGCCTCGGCTGG-3'), MMP-2 (F:5'-CCCCTATCTACACCTACACCA-3', R:5'-CACCACGGATCTGAGCAAT-3') and GAPDH (F:5'-GACATGCCGCCTGGAGAAAC-3', R:5'-AGCCCAGGATGCCCTTTAGT-3').

Analysis of target proteins via Western blot

The collected lung samples were firstly lysed in RIPA buffer and then centrifuged (13000rpm × 15 min, at 4^{o}C). The resulting supernatant was collected. The concentration of protein was assayed with BCA assay. Equal amounts of the protein were separated by sodium dodecyl sulfate-polyaramid gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PDVF) membrane. Then the membrane was washed with TBST three times after being blocked in TBST solution containing 5% skimmed milk power. The target proteins were tested with antibodies (abcam, Cambridge, UK) specific for VE-CAD (ab231227), HGF (ab83760), GSK-3β (12456S) and β-actin (ab179467, as control). Target proteins bands were scanned and their optical densities were acquired and calculated by a Gel Doc 2000. All the experiments conditions were set up in triplicates.

Wound-healing assay

MSCs (5×10^4/L) suspension (2 mL) was dropped to each hole of the test plate. The plate was shaken well and then was put into the 37°C cabinet. When MSCs grew to 80% of each hole, the end of the pipette was used to draw a vertical line in the center of the hole. Then the plate was put into the 37°C cabinet again for 48 h. MSCs were taken photos under the mirror.

Transwell assay

MSCs migration was evaluated using the transwell chamber (8 μm specification, provided by BD) was used. MSCs were seeded into the upper chamber (1×10^5/200μL) which were put into 24-well plates loaded with 600μL DMEM. In the first migration experiment, six visual fields counts were randomly selected after 24 h. 10%FBS, 30 ng/mL CXCL12 and 25 ng/mL HGF were added to the lower chamber in turn in the second migration experiment. After 4 hours, MSCs migrated through the chamber and were fixed and stained using Hema 3 staining kit (Fisher Scientific) was used and then the chamber was rinsed with water. The remaining cells in the upper chamber were swabbed and left to dry. HGF-induced migration of LRMSCs and hCMSCs were tested by transwell migration assays and stained with trypan blue. The cell counts were recorded from 5 random fields of per transwell chamber. The results were expressed as the mean number of cells.

Statistical analysis

All statistical analyses were performed using SPSS v19.0 software. Significance was determined by Nonparametric tests followed by Bonferroni correction. The results are presented as the median (quartile). $P < 0.05$ was considered statistically significant.

Results

Effects of BMSCs, hCMSCs and LRMSCs on histopathological changes and $PaCO_2$ in ALI

It could be seen from the pathological sections (H&E staining) that no inflammatory changes were detected in the normal rats (Fig. 1a) while the model group showed swollen lung interstitium and infiltration of many blood cells (Fig. 1a). Treatment with three types of MSCs restored damaged alveolar structure and significantly alleviated pathological damage (Fig. 1a). Compared to those of the normal group, lung injury scores, total protein content and cell count in BALF in the model group were all increased after 24h. However, while those in treatment groups were significantly decreased (Fig. 1b-e). $PaCO_2$ was significantly decreased at 6h, 24h and 48h after inhalation of phosgene (Fig. 1f-h). MSCs treatment resulted in the recovery of $PaCO_2$ (Fig. 2f-h). These changes were more prominent in the BMSCs group compared to the other groups.

Effects of BMSCs, hCMSCs and LRMSCs on reducing inflammatory response and immune regulation in ALI

The expressions of TNF-1α, IL-6 and IL-1β in BALF and serum were increased after phosgene inhalation while their expressions were decreased in three MSCs groups (Fig. 2a-c, e-g). Moreover, the changes of BMSCs were more significant than those of other MSCs groups (Fig. 2a-c, e-g). These results demonstrated that BMSCs could better resist inflammatory response both in the lungs and the entire body. IL-10 possess immunomodulatory properties [25]. After the rats were poisoned, the levels of IL-10 in both BALF and serum were increased and the increase was even higher after MSCs treatment (Fig. 2d, h). The effects of BMSCs were stronger than those of LRMSCs and hCMSCs, indicating that BMSCs had higher immune regulation ability in ALI than LRMSCs and hCMSCs.

Changes in HGF after the treatment of BMSCs, hCMSCs and LRMSCs

The expression of HGF in BALF and serum of each group were detected at 6h, 24h and 48h, respectively (Fig. 3a-f). Compared with healthy rats, the levels of HGF in BALF and serum in the phosgene group were increased. HGF expression was increased in all MSCs treatment groups. BMSCs showed the most significant effects followed by hCMSCs and LRMSCs.

Effects of BMSCs, hCMSCs and LRMSCs on repairing alveolar epithelia and vascular endothelia in ALI

Many red cells were shown to leak into the alveoli in the injured lungs (Fig. 4a). After MSCs treatment, fewer red cells were present in the alveoli indicating that vascular endothelial permeability had been significantly recovered (Fig. 4a). The expression of VE-CAD, a vascular endothelial cell marker, was

decreased after lung injury but was increased in rats treated with MSCs (Fig. 4e, f). BMSCs, hCMSCs and LRMSCs were found to have protective effects in repairing vascular endothelia. BMSCs were shown to have the best protective effects followed by hCMSCs and LRMSCs. SP-C expression reflects the secretory function of normal alveolar epithelia [26]. SP-C was significantly reduced due to alveolar epithelial damage caused by phosgene (Fig. 4b-d). The expression of SP-C mRNA in lung tissues in each group was detected by RT-PCR (Fig. 4b-d). SP-C mRNA expression was significantly decreased in the phosgene group. Meanwhile, MSCs treatment significantly increased SP-C mRNA expression, and the BMSCs group showed the highest increase.

Comparison of the proliferation potential of three types of MSCs by CCK8 assay

The proliferation of the three types of MSCs were found not to be significantly different (Fig. 5a). However, the proliferation of BMSCs was slightly higher compared to that of hCMSCs and LRMSCs at 72h. All MSCs entered the logarithmic growth from the second to the fourth day.

Comparison of the migration potential of BMSCs, hCMSCs and LRMSCs

The horizontal migration of MSCs was different based on the wound-healing test (Fig. 5b). The BMSCs group exhibited significantly higher cell migration with obvious cell aggregation at 48h, followed by the hCMSCs group and LRMSCs group. The vertical migration of MSCs was determined using the transwell migration assay (Fig. 5c-d). At 24h, the number of hCMSCs, BMSCs and LRMSCs that migrated to the lower compartment was 181.27±62, 708.75±20 and 106.31±17, respectively. The three types of MSCs exhibited different migration rates and BMSCs showed the highest migration. This finding was consistent with the change in HGF in each treatment group *in vivo*. It is speculated that the high migration potential of BMSCs may be related to HGF, which may also be important in defining the roles of different MSCs. According to the experimental result (Fig. 5e), the optimal concentration of HGF was 25ng/mL which was added to the MSCs medium for 48h. MSCs with HGF showed a higher migration ability compared to the group without HGF (Fig. 5e). The experimental result indicated that HGF promoted the migration of MSCs in a dose-dependent manner in a certain range. Previous studies have shown that CXCL12 is the most important factor affecting the homing ability of hBMSCs compared with hCMSCs which was determined by PCR array in all the chemokines and receptors [9]. The effect of HGF on BMSCs migration was compared with that of CXCL12. The HGF group was compared with the CXCL12 group (30ng/mL) [20] and the FBS (10%) group (Fig. 5f). HGF was shown to significantly induce migration compared to CXCL12. HGF increased MSCs' migration by about 2 fold when compared with 10%FBS. These results indicated that HGF was a strong inducer of BMSCs' migration. Different concentrations of HGF were co-cultured with hCMSCs and LRMSCs for 48 hours (Fig. 6a). The migration of LRMSCs and hCMSCs were increased with different doses of HGF (Fig. 6b, c). The migration ability in HGF (25ng/mL) treatment group was highest, which verified that the optimal concentration of HGF was 25ng/mL. The migration potential of the three types of MSCs after HGF treatment was different (Fig. 6d). BMSCs showed significantly increased migration potential when compared to LRMSCs and hCMSCs. This indicated that BMSCs had the highest HGF sensitivity among the three types of MSCs.

The effect of HGF on the p-GSK-3β/β-catenin signaling pathway.

The expressions of proteins in MSCs were tested by Western blot (Fig. 7a-d) to explore the mechanism of the effect of HGF on MSCs. Compared with the control group, the expressions of p-GSK-3β and β-catenin in MSCs after HGF treatment were significantly increased in a dose-dependent manner that indicated that HGF activated this signaling pathway. Studies have confirmed the MMP-2 gene is an important target gene for the β-catenin signal pathway and its expression is associated with the degree of cell migration [27]. Therefore, the changes in MMP-2 mRNA after treatment were detected to investigate the effect of HGF on the target gene. Compared with the control group, MMP-2 expression after HGF treatment increased significantly (Fig. 7e). MMP-2 transcription was inhibited by the addition of FH535 (20μM), a potent inhibitor of the β-catenin signal pathway [28]. This showed that HGF promoted the transcription of MMP-2 by activating the β-catenin signaling pathway which promoted the migration of MSCs.

Discussion

Phosgene-induced lung injury remains a common critical disease with high morbidity and mortality, however, its specific mechanism is not fully identified. In recent years, MSCs have become promising interventions for various diseases and are shown to be effective in both experimental and clinical practice. The main functions of MSCs are homing differentiation (limitation), immune regulation and secretion of cytokines. BMSCs, LRMSCs and hCMSCs have been widely studied in recent years. They are derived from the bone marrow, placental chorionic membrane and the lung respectively. Previous studies have shown that BMSCs, LRMSCs and hCMSCs play protective roles in lung injury caused by mechanical ventilation injury, endotoxin and plateau factors [6, 8]. Other studies have reported that BMSCs can improve the lung injury caused by phosgene [4]. In this study, the roles of LRMSCs and hCMSCs in phosgene poisoning were confirmed and the effects of BMSCs, LRMSCs and hCMSCs in the same disease were compared. The results revealed that the three types of MSCs improved PaO_2, lung injury score, leukocytes infiltration, and the expressions of various pro-inflammatory and anti-inflammatory factors in BALF and serum in rats. Besides, they promoted the recovery ability of functional cells to reduce the phosgene inhalation lung injury. BMSCs showed better activity and effect compared to LRMSCs and hCMSCs. Previously, BMSCs were reported to have better therapeutic potential compared with hCMSCs in naphthalene-injured mice [9]. These findings suggested that BMSCs may be the most suitable MSC in the clinical treatment of phosgene inhalation lung injury.

This study revealed that different MSCs played different roles in the same disease model. However, this mechanism needs to be studied further. There was no significant difference seen in the proliferation of MSCs. However, BMSCs showed the highest migration ability compared to LRMSCs and hCMSCs. These findings suggested that the differences in the role of the MSCs were partly related to their migration ability. *In vivo*, the expressions of HGF in each MSCs group differed and this was consistent with the trend of the effects of stem cells. Previous studies have reported that HGF can promote the directional migration of a variety of cells [13, 14]. Moreover, HGF is closely related to the vascular endothelium function [29]. Therefore, it was speculated that HGF was related to the mechanism of the differential

effects of the MSCs. Our results found that HGF affected the migration ability and the role of MSCs, and this was one of the important factors affecting the differences in the three types of MSCs. Besides, HGF was found to promote the migration ability of MSCs by activating the p-GSK-3β/β-catenin signal pathway. Previous studies have confirmed that activated β-catenin also plays an important role in the Wnt signaling pathway involved in phosgene inhalation lung injury. The activation of the β-catenin signaling pathway improves MSCs migration [18, 19]. GSK-3β phosphorylation is reported to affect the β-catenin signaling pathway. HGF activated GSK-3β/β-catenin signaling pathway has been found to promote cell migration and repair activity. Recent oncology studies have found that the activation and expression of the c-Met receptor of HGF are indispensable, and the HGF/ c-Met pathway is currently one of the research focus of tumor migration [30–32]. However, c-Met changes in MSCs needs to be explored further.

According to the collected literatures and previous studies, the therapeutic effects of BMSCs from rats and BMSCs from human sources in phosgene induced lung injury were compared. However, no significant differences in the therapeutic effects have been reported when the two types of MSCs are used in the same disease model. Other studies have shown that there is no significant difference in the repairing effect of dermal MSCs from mouse and human sources on skin fibroblast tissue. Since the mice are genetically similar to humans, it can be concluded that the species origin was not the key reason for the different effects of MSCs in this experiment. In addition to the influencing factors such as the source, differentiation state, passages and dosage, the effects of stem cells in diseases more depend on the key factors which include sensitivity, reactivity and genetic inheritance. The optimal treatment in animal models will need to undergo rigorous clinical trials in the future. This study payed attention to the migration of MSCs in the basic experiment.

Conclusions

In conclusion, this study suggested that BMSCs have the best protective effects in phosgene-induced lung injury, which providing a theoretical basis for the next clinical trial.

Abbreviations

ALI: Acute lung injury; LRMSCs: Lung-resident mesenchymal stem cells; BMSCs: Bone marrow mesenchymal stem cells; hCMSCs: Human chorionic villi-derived MSCs; HGF: Hepatocyte growth factor; ARDS: Acute respiratory distress syndrome; HGF: Hepatocyte growth factor; MSCs: Mesenchymal stem cells; LRMSCs: Lung resident mesenchymal stem cells; BMSCs: Bone marrow-derived mesenchymal stem cells; hCMSCs: Human chorionic villi-derived mesenchymal stem cells; SP-C: Surfactant protein C; mRNA: messenger RNA; ATII: Alveolar type II epithelial cell; IL-10: Interleukin-10; LPS: Lipopolysaccharide; IL-1β: Interleukin-1β; MMP: Membrane-type matrix metalloproteinase; TNF-α: Tumor necrosis factor-α; W/D: Wet/dry weight ratio; VE-CAD: VE-cadherin; HPMECs: Human pulmonary microvascular endothelial cells; GSK-3β: Glycogen synthase kinase-3β; IL-6: Interleukin-6

Declarations

Ackowledgements

The authors are grateful to Professor Liming Zhang for excellent technical support.

Authors' contributions

JS designed the experiments and revised the manuscript. YQ performed the experiments and wrote the manuscript. YT, NX and JD contributed to the data analysis and interpretation. LZ and DH revised the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81471850).

Availability of data and materials

The data used in supporting study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal protocol in this work was in accordance with guidelines for the care and use of laboratory animals sanctioned by the Ministry of Science and Technology of the People's Republic of China and approved by Jinshan Hospital of Fudan University.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Jung KH. Yi T, Son MK, et al Therapeutic effect of human clonal bone marrow-derived mesenchymal stem cells in severe acute pancreatitis. Arch Pharm Res. 2015;38:742–51.
2. Oshita J. Okazaki T, Mitsuhara T, et al *Early Transplantation of Human Cranial Bone-derived Mesenchymal Stem Cells Enhances Functional Recovery in Ischemic Stroke Model Rats. Neurologia medico-chirurgica*. 2020;*60*:*83–93*.
3. Kim M. Kim KH, Song SU, et al Transplantation of human bone marrow-derived clonal mesenchymal stem cells reduces fibrotic scar formation in a rat spinal cord injury model. J Tissue Eng Regen Med. 2018;12:e1034–45.
4. Chen J. Shao Y, Xu G, et al Bone marrow-derived mesenchymal stem cells attenuate phosgene-induced acute lung injury in rats. Inhalation toxicology. 2015; 27:254–61.
5. *Gong X*, *Sun Z*, *Cui D*, et al. *Isolation and characterization of lung resident mesenchymal stem cells capable of differentiating into alveolar epithelial type II cells*. *Cell biology international*. *2014*;*38*:*405–411*.
6. Mei Y. Chen C, Dong H, et al Treatment of Hyperoxia-Induced Lung Injury with Lung Mesenchymal Stem Cells in Mice. Stem cells international. 2018;2018:5976519.
7. Wang L. Shi M, Tong L, et al Lung-Resident Mesenchymal Stem Cells Promote Repair of LPS-Induced Acute Lung Injury via Regulating the Balance of Regulatory T cells and Th17 cells. Inflammation. 2019;42:199–210.
8. Feng Y. Wang L, Ma X, et al Effect of hCMSCs and liraglutide combination in ALI through cAMP/PKAc/β-catenin signaling pathway. Stem Cell Res Ther. 2020;11:2.
9. Ji S. Wu C, Tong L, et al Better therapeutic potential of bone marrow-derived mesenchymal stem cells compared with chorionic villi-derived mesenchymal stem cells in airway injury model. Regenerative medicine. 2019;14:165–77.
10. Matteucci E. Modora S, Simone M. Hepatocyte growth factor induces apoptosis through the extrinsic pathway in hepatoma cells: favouring role of hypoxia-inducible factor-1 deficiency. Oncogene. 2003;22:4062–73.
11. Boromand N. Hasanzadeh M, ShahidSales S, et al Clinical and prognostic value of the C-Met/HGF signaling pathway in cervical cancer. Journal of cellular physiology. 2018;233:4490–6.
12. Yu DD. Guo SW, Jing YY, et al A review on hepatocyte nuclear factor-1beta and tumor. Cell bioscience. 2015;5:58.
13. Yue Q. Zhang Y, Li X, et al MiR-124 suppresses the chemotactic migration of rat mesenchymal stem cells toward HGF by downregulating Wnt/β-catenin signaling. European journal of cell biology 2016;95:342–53.
14. Wang L, Li S, Wang HY, et al. In a Rat Model of Acute Liver Failure, Icaritin Improved the Therapeutic Effect of Mesenchymal Stem Cells by Activation of the Hepatocyte Growth Factor/c-Met Pathway. Evidence-based complementary and alternative medicine: eCAM. 2019;2019:4253846.

15. Roggia C, Ukena C, Böhm M. Hepatocyte growth factor (HGF) enhances cardiac commitment of differentiating embryonic stem cells by activating PI3 kinase. Experimental cell research. 2007;313:921–30.
16. Haegel H, Larue L, Ohsugi M, et al. Lack of β-catenin affects mouse development at gastrulation. Development. 1995;121:3529–37.
17. Huelsken J, Vogel R, Brinkmann V, et al. Requirement for β-catenin in anterior-posterior axis formation in mice. J Cell Biol. 2000;148:567–78.
18. Cheon SS, Cheah AY, Turley S, et al. β-catenin stabilization dysregulates mesenchymal cell proliferation, motility and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. Proc Natl Acad Sci USA. 2002;99:6973–8.
19. Lam AP, Flozak AS, Russell S, et al. Nuclear betacatenin is increased in systemic sclerosis pulmonary fibrosis and promotes lung fibroblast migration and proliferation. Am J Respir Cell Mol Biol. 2011;45:915–22.
20. Badri L. Lysophosphatidic acid induces migration of human lung-resident mesenchymal stem cells through the β-catenin pathway. Stem cells. 2012;30:2010–9.
21. Novak A, Dedhar S. Signaling through β-catenin and Lef/Tcf. Cell Mol Life Sci. 1999;56:523–37.
22. Shevtsov SP, Haq S, Force T. Activation of β-catenin signaling pathways by classical G-proteincoupled receptors: mechanisms and consequences in cycling and non-cycling cells. Cell Cycle. 2006;5:2295–300.
23. Xu N, He D, Shao Y, et al. Lung-derived exosomes in phosgene-induced acute lung injury regulate the functions of mesenchymal stem cells partially via miR-28-5p. Biomedicine Pharmacotherapy. 2020;121:109603.
24. Tong L, Zhou J, Rong L, et al. Fibroblast Growth Factor-10 (FGF-10) Mobilizes Lung-resident Mesenchymal Stem Cells and Protects Against Acute Lung Injury. Scientific reports. 2016;6:21642.
25. Chai YS, Chen YQ, Lin SH, et al. Curcumin regulates the differentiation of naïve CD4 + T cells and activates IL-10 immune modulation against acute lung injury in mice. Biomedicine pharmacotherapy. 2020;125:109946.
26. Lu H, Chen X, Lu Y, et al. Effects of C/EBP α overexpression on alveolar epithelial type II cell proliferation, apoptosis and surfactant protein-C expression after exposure to hyperoxia. BMC pulmonary medicine. 2019;19:142.
27. Sun YS, Thakur K, Hu F, et al. Icariside II suppresses cervical cancer cell migration through JNK modulated matrix metalloproteinase-2/9 inhibition in vitro and in vivo. Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie. 2020;125:110013.
28. Zheng B, Wang C, He L, et al. Neural differentiation of mesenchymal stem cells influences chemotactic responses to HGF. Journal of cellular physiology. 2013;228:149–62.
29. Wang H, Zheng R, Chen Q, et al. Mesenchymal stem cells microvesicles stabilize endothelial barrier function partly mediated by hepatocyte growth factor (HGF). Stem Cell Res Ther. 2017;8:211.

30. Yang J, Yang L, Li S. HGF/c-Met Promote Renal Carcinoma Cancer Stem Cells Enrichment Through Upregulation of Cir-CCDC66. Technology in cancer research treatment. 2020;19:1533033819901114.
31. Jung J, Yang K, Kim HJ, et al. RhoA-Dependent HGF and c-Met Mediate Gas6-Induced Inhibition of Epithelial-Mesenchymal Transition, Migration, and Invasion of Lung Alveolar Epithelial Cells. Biomolecules. 2019;9:1–17.
32. Yan B, Jiang Z, Cheng L, et al. Paracrine HGF/c-MET enhances the stem cell-like potential and glycolysis of pancreatic cancer cells via activation of YAP/HIF-1. Experimental cell research. 2018;371:63–71.

Figures

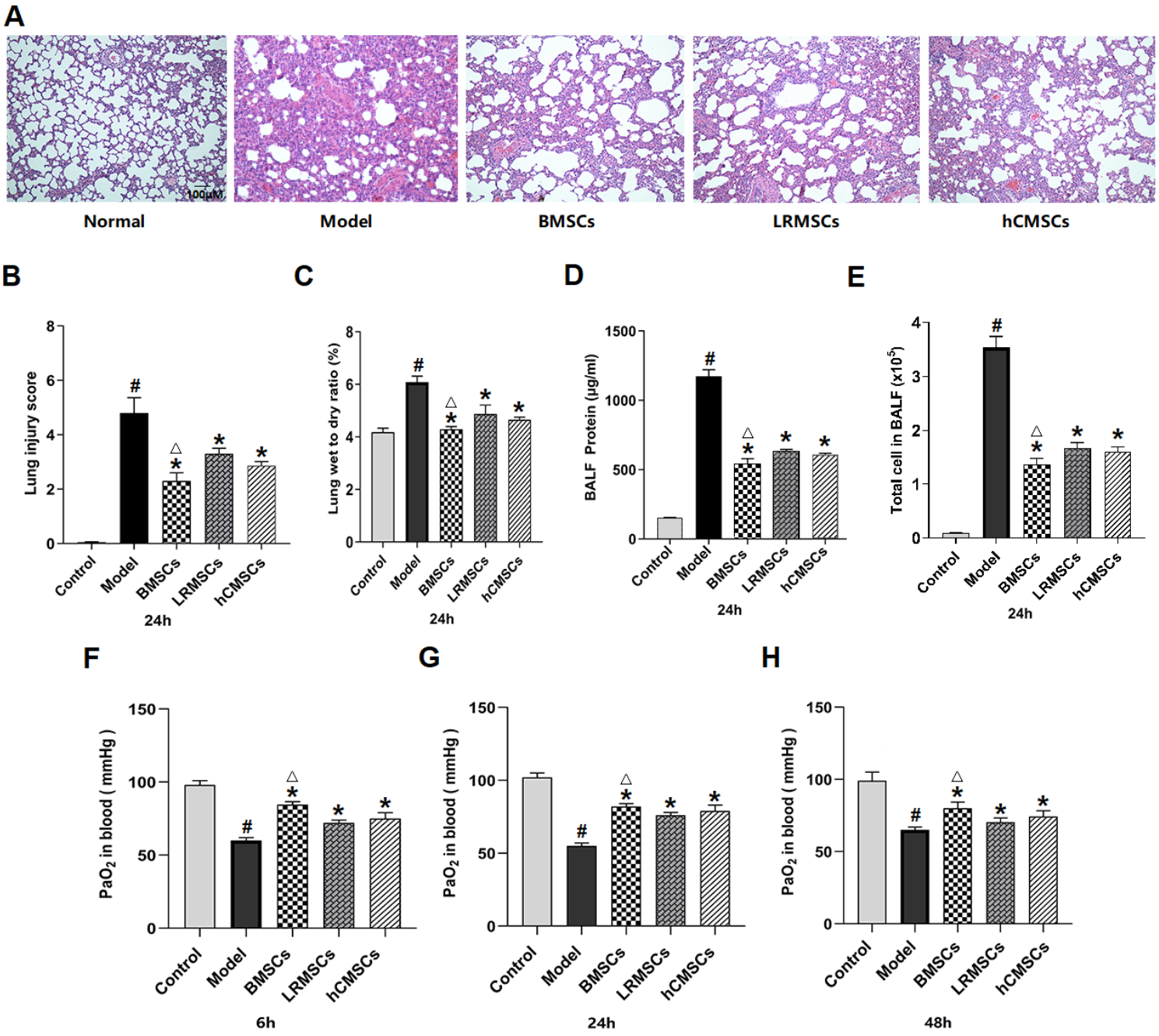

Figure 1

Histopathological change and PaCO2 in each group Representative images (a) of HE stained sections under the light microscopic at 24h for the normal, model, BMSCs, LRMSCs and hCMSCs groups. Lung injury score, lung wet to dry ratio, total protein content and total cell count in BALF (b-e) are shown. Comparing PaCO2 in each group at 6h, 24h and 48h (f-h). n=6, #P<0.01 versus. the normal group; *P<0.05, **P<0.01 versus. the model group; △P<0.05, versus. the LRMSC group; &P<0.05, versus. the hCMSC group.

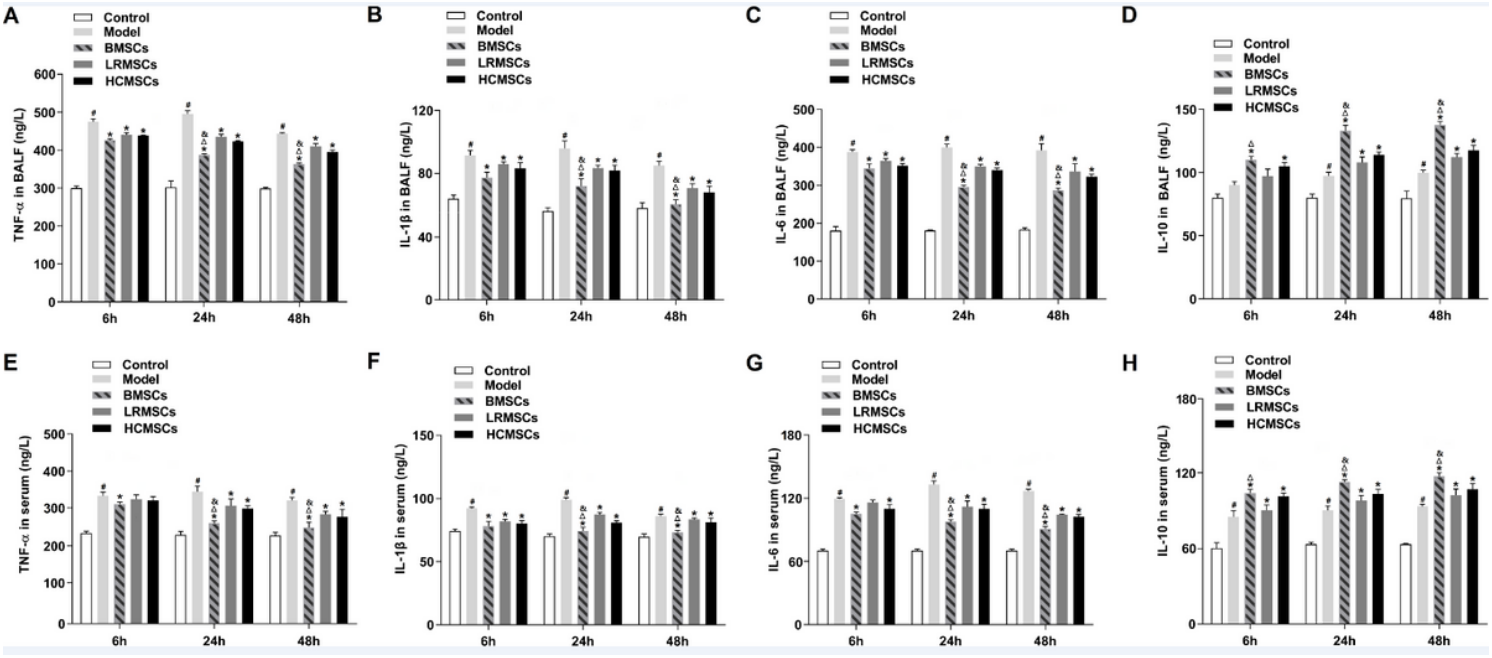

Figure 2

Comparing the reduction in the inflammatory response and immune regulation in each group TNF-α, IL-1β, IL-6 and IL-10 expressions in BALF (a-d) and serum (e-h) were measured by ELISA. n=6, #P<0.01 versus. the normal group; *P<0.05, **P<0.01 versus. the model group; △P<0.05, versus. the LRMSC group; &P<0.05, versus. the hCMSC group.

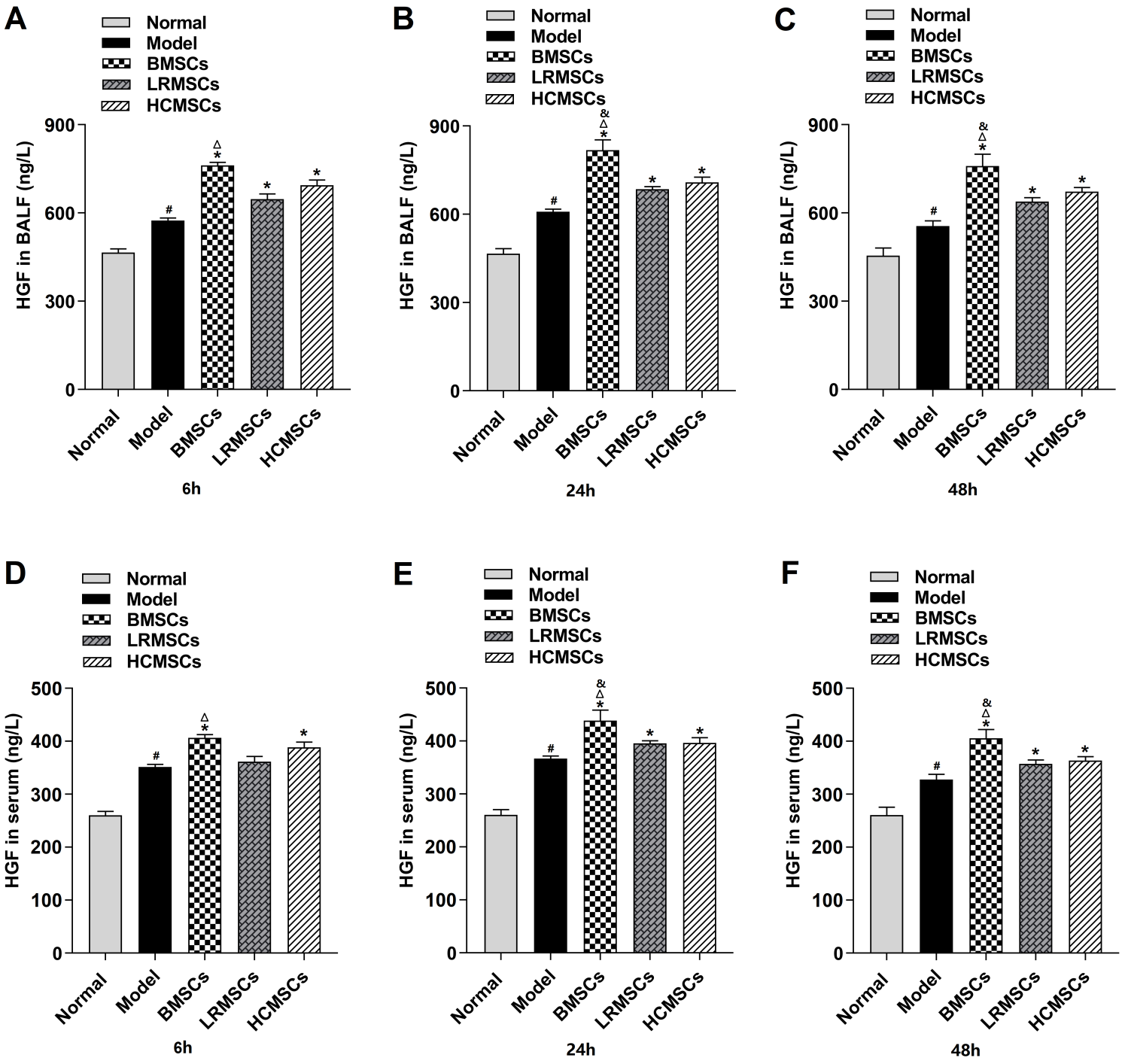

Figure 3

Changes in HGF at each time point in each group The expression of HGF in BALF (a-c) and serum (d-f) was detected by ELISA at 6h, 24h and 48h, respectively. n=6, #P<0.01 versus. the normal group; *P<0.05, **P<0.01 versus. the model group; △P<0.05, versus. the LRMSC group; &P<0.05, versus. the hCMSC group.

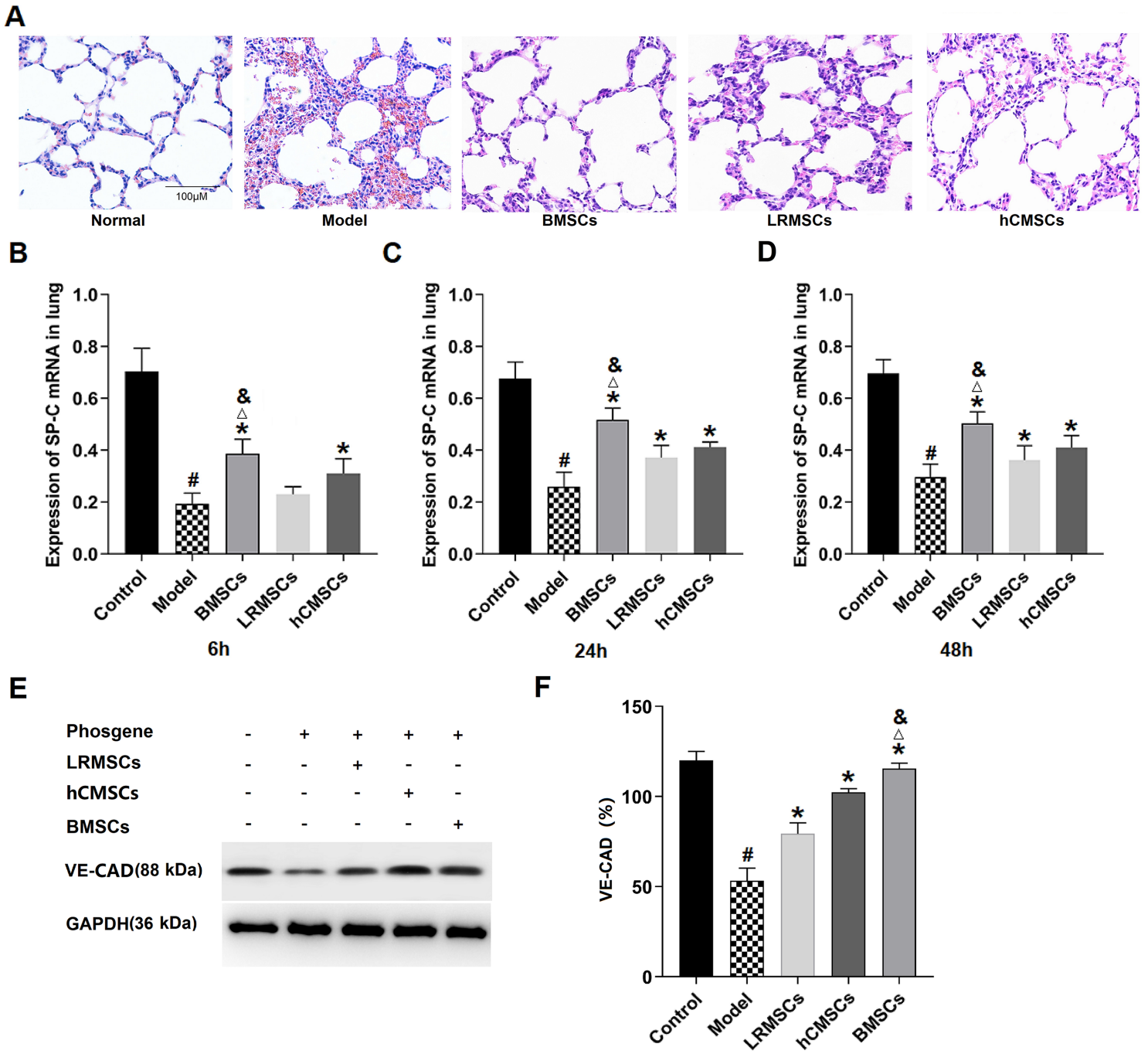

Figure 4

Comparison of repairing alveolar epithelium and vascular endothelium in each group. Lung histopathological sections were observed under microscope at 24h (a). Expression of SP-C mRNA in each group (b-d) at 6h, 24h and 48h is shown. VE-CAD expression in each group (e,f) is shown. n=6, #P<0.01 versus. the normal group; *P<0.05, **P<0.01 versus. the model group; △P<0.05, versus. the LRMSC group; &P<0.05, versus. the hCMSC group.

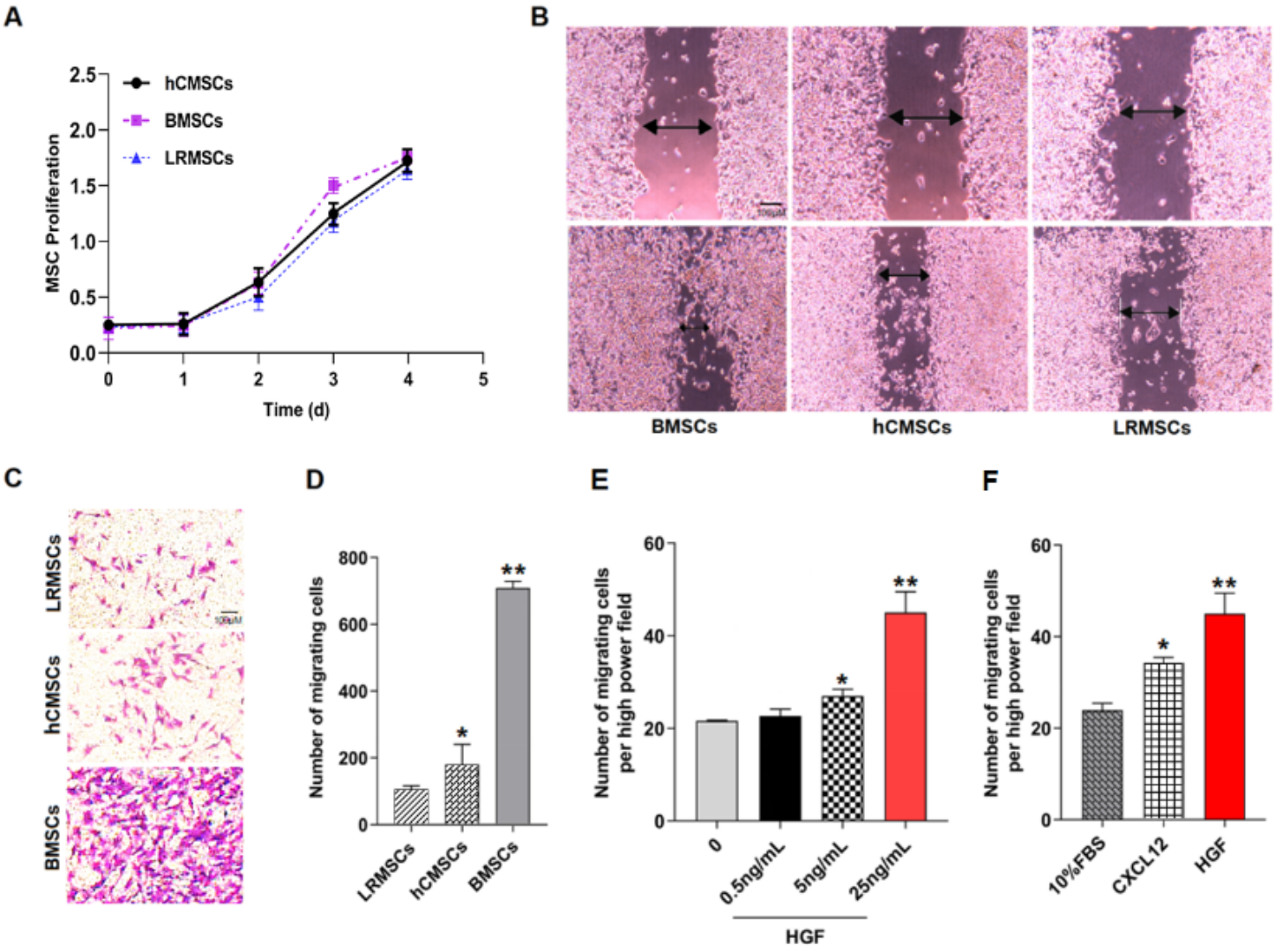

Figure 5

Comparing the proliferation and migration ability of MSCs The proliferation of different MSCs is shown in (a). Comparison of the migration of the three types of MSCs (b-d) is shown in transwell assays and wound-healing assays. The migration of BMSCs was effected by HGF with different concentrations (e). The migration of stem cells effected by HGF in comparison with other chemokines (f) is shown. n=3, *P<0.05, **P<0.01 versus. the control group.

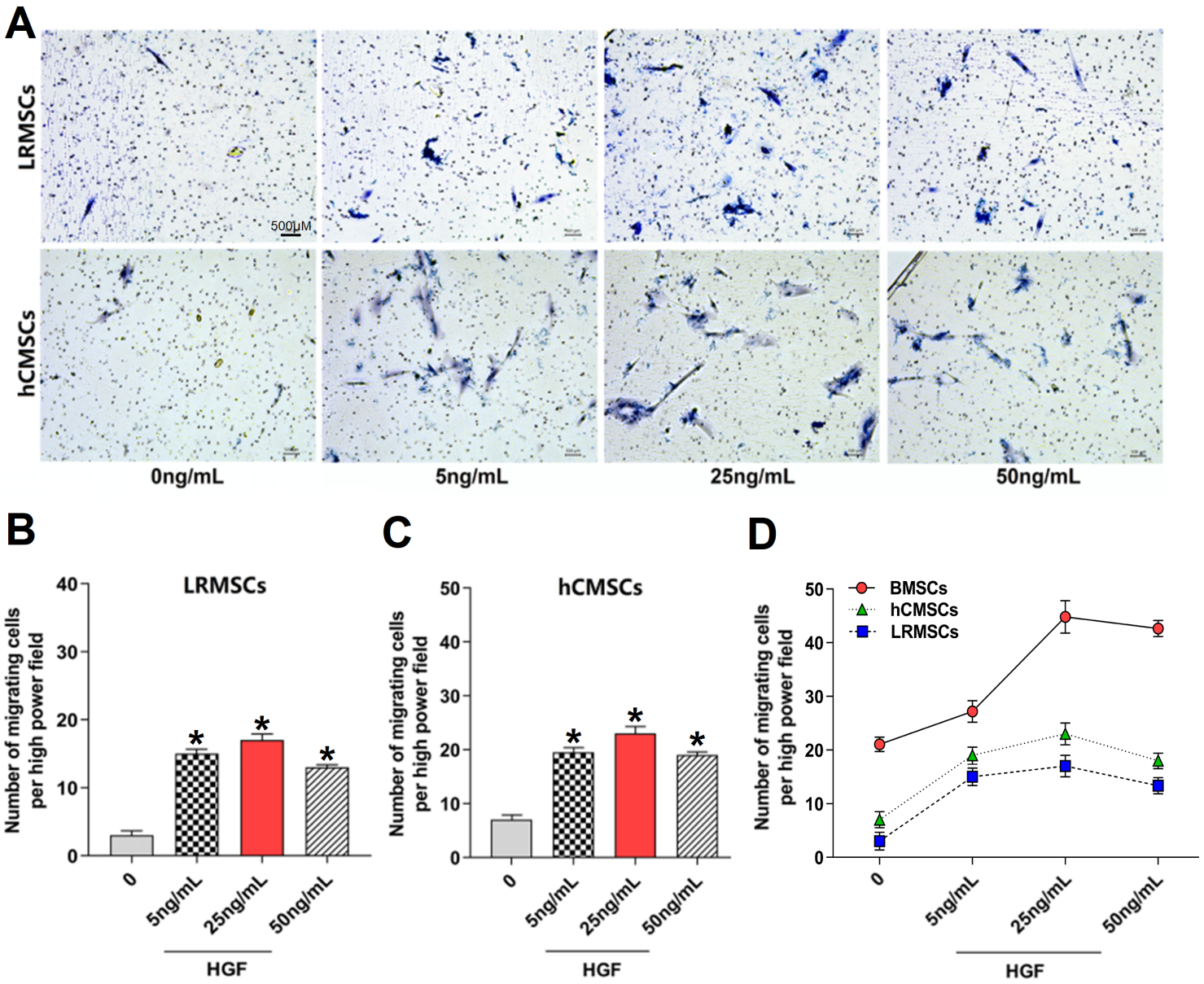

Figure 6

Comparing the migration ability of different MSCs The migration of stem cells effected by HGF (a-c) with different concentrations is shown in the Transwell assay. A comparison of the migration effected by HGF is shown in different MSCs (d). n=3, *P<0.05 versus. the control group.

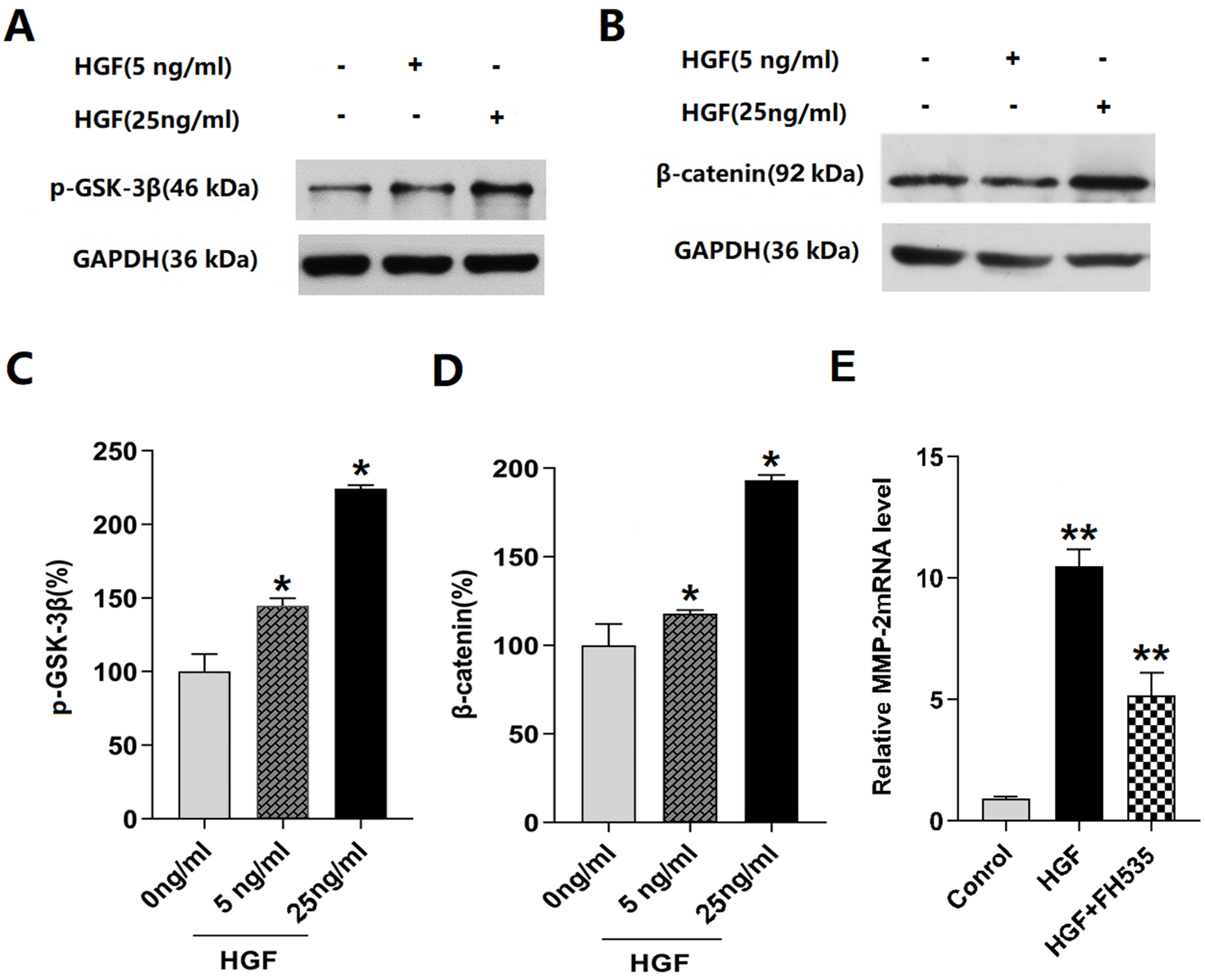

Figure 7

The effect of HGF on the p-GSK-3β/β-catenin signaling pathway The activation of p-GSK-3β/β-catenin signaling pathway by HGF is shown by Western blot (a-d). The effect of inhibitor FH535 on the expression of migratory proteins is shown in (e). n=3, *P<0.05, **P<0.01 versus. the control group.