International Journal of Chronic Obstructive Pulmonary Disease

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ORIGINAL RESEARCH

Wnt3a Enhances Mesenchymal Stem Cell Engraftment and Differentiation in a Chronic Obstructive Pulmonary Disease Rat Model

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Background: Bone marrow mesenchymal stem cell (BMSC) therapy is a novel approach for treating COPD. However, the difficulty in engraftment and easy clearance of BMSCs in vivo has hindered their clinical application. Hence, exploring effective methods to improve the engraftment and differentiation rates of BMSCs in vivo is urgent.

Methods: We constructed BMSCs overexpressing Wnt3a by lentivirus infection and transplanted them into a COPD rat model. The damage level of COPD rat lung tissue was assessed by pathology analysis and inflammatory cytokines analysis. The engraftment of BMSC was detected by immunofluorescence staining. Statistical analysis was performed using GraphPad Prism 7.

Results: We found that Wnt3a significantly enhanced the engraftment rate of BMSCs in the lungs of rats and further increased their differentiation rate into type II alveolar epithelial cells. We also assessed the expression of inflammatory factors in the lung tissues of COPD rats and discovered that Wnt3a reduced the levels of the inflammatory factors IL-6 and IL-1β while increasing the level of the anti-inflammatory factor IL-10. Our study demonstrates that Wnt3a can improve the engraftment and differentiation rates of BMSCs in the host and further alleviate COPD symptoms by regulating the secretion of inflammatory factors.

Conclusion: Constructing BMSCs overexpressing Wnt3a could serve as a new strategy for stem cell therapy in COPD.

Keywords: Wnt3a, BMSCs, COPD, inflammatory cytokines

Introduction

Chronic obstructive pulmonary disease (COPD) ranks among the top 10 causes of death globally.[1] It is characterized by inflammation and remodeling of the lower airways and lung parenchyma, along with activation of inflammatory and immune responses. Dyspnea, primarily due to airflow limitation, is the primary symptom. Clinical treatment of COPD primarily focuses on symptom relief (enhanced physical capacity and reduced dyspnea) and minimizing the risk of exacerbations and mortality.[2] Current therapies can temporarily alleviate symptoms and reduce the risk of exacerbations and mortality. However, effective methods for repairing and improving damaged airway structures underlying COPD remain elusive in clinical practice. The primary pathological feature of alveolar epithelial lesions, predominantly involving type I and II alveolar epithelial cells, is the structural damage to alveoli. Type I alveolar epithelial cells (AT I cells) are characterized as membranous flat cells, constituting 96% of the alveolar surface area, facilitating gas exchange. Type II alveolar epithelial cells (AT II cells), on the other hand, are large cuboidal cells situated at the alveolar corners, comprising 4% of the alveolar surface area. They produce surfactant, a substance that reduces surface

Received: 27 August 2024
Accepted: 28 December 2024
Published: 8 January 2025

tension and prevents alveolar collapse. AT II cells possess the capacity for self-renewal and transdifferentiation into AT I cells.[3] Hence, promoting the regeneration of damaged AT II cells holds significant therapeutic implications for COPD.

Bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent stem cells with immunosuppressive properties capable of differentiating into various cell types such as bone, cartilage, muscle, liver, and lung tissue. Several studies have demonstrated the potential of BMSCs in alleviating and repairing acute lung injuries.[4–7] This potential is achieved through BMSC differentiation into lung epithelial cells or Type II alveolar epithelial cells and the suppression of inflammation.[8–10] However, their limited implantation and differentiation rates within the lungs[7] have affected their potential therapeutic efficacy, necessitating further exploration to enhance their implantation and differentiation methods.

The Wnt/β-catenin signaling pathway and its downstream signaling molecules play crucial roles in the self-renewal and differentiation of BMSCs.[11] Studies have shown that activation of the canonical Wnt signaling pathway by Wnt3a or LiCl can promote the migration of BMSCs to damaged lung tissues.[9] Low doses of Wnt3a can enhance proliferation, self-renewal, and maintain the multipotent potential of BMSCs both in vitro and in vivo,[12] while high doses of Wnt3a inhibit BMSC differentiation into adipocytes.[13] Whether the expression of exogenous Wnt3a in BMSCs can enhance the therapeutic efficacy of BMSC transplantation for treating COPD is unknown.

In this study, we overexpressed Wnt3a in bone marrow-derived mesenchymal stem cells (BMSCs) and transplanted them into a rat model of chronic obstructive pulmonary disease (COPD). We observed in vivo that Wnt3a promoted the engraftment and differentiation of BMSCs in the lungs of rats, facilitated the differentiation of rat bone marrow-derived mesenchymal stem cells (mMSCs) into AT II cells, and alleviated the elevated inflammatory factors in COPD. Our findings provide a novel insight that the construction of Wnt3a-overexpressing BMSCs may serve as a potential strategy for the treatment of COPD.

Methods

Animal

Healthy male Sprague-Dawley (SD) rats aged 6 weeks, weighing 160–200g, were used for BMSCs extraction. Male SD rats aged 8 weeks, weighing 200–230g, were used to establish the COPD model. A total of 48 experimental animals were purchased from Jiangxi University of Traditional Chinese Medicine (License number SCXK (GAN) 2018-0003). This study fully complied with the ethical principles of animal experiments at the First Affiliated Hospital of Nanchang University and the guidelines of Nanchang University, PR China for the care and use of laboratory animals.

Reagents

DMEM low-glucose medium (Hyclone); fetal bovine serum (Gibco); Opti-MEM media (Gibco); live cell dye CM-Dil (Life Technologies); Wnt3a antibody (Abcam); rabbit anti-mouse anti-Prosurfactant Protein C antibody (Abcam). Flow cytometry antibodies: anti-CD34 (eBioscience), anti-CD29 (eBioscience), anti-CD45 (eBioscience), anti-CD90 (eBioscience), anti-CD44 (eBioscience); anti-rat monoclonal β-actin antibody and IgG/HRP secondary antibodies are purchased from Zhongshan Golden Bridge. Wnt3a lentivirus and negative control virus (constructed by Shanghai Nobio Biotechnology Co., Ltd.); Baisha brand cigarettes (tar content 14mg, nicotine content 1.1mg, produced by Hunan Zhongyan Industrial Co., Ltd.); Homemade smoking chamber (50 cm × 40 cm × 40 cm, with 2 smoke holes on the side walls).

Isolation and Culture of BMSCs

The femurs and tibias of SD rats were isolated and sterilized. Then cutted bones at both ends under aseptic conditions. The bone marrow cavity was flushed with DMEM culture medium, and the bone marrow cell suspension was collected and centrifuged at 1000rpm/min for 5 minutes at 4°C. The pellet was resuspended in DMEM culture medium supplemented with 10% FBS and cultured at 37°C, 5% CO2, and 100% humidity. After 24 hours, cell adhesion was observed, and the cells were rinsed with DPBS and the culture medium was changed. Culturing continued until the cell density reached approximately 80–90% confluence, and cells were passaged using 0.25% Trypsin-EDTA.

Identification of BMSCs by Flow Cytometer

We obtained the 3rd passage (P3) BMSCs from separated cultures for purity identification. Cells were counted at 2×10^5 cells/sample and incubated with CD29-PE, CD44-PE, CD34-PE, CD45-PE, and isotype control PE-IgG for 30 minutes, avoiding light. Unbound antibodies were discarded by centrifugation at 1000rpm for 5 minutes and the cells were washed once with DPBS. Subsequently, the cells were resuspended in 1mL DPBS and analyzed by flow cytometer.

BMSCs Wnt3a-Lentivirus Transfection

P3 generation BMSCs were seeded at a density of 1×10^5 cells/well in a 6-well plate. After cell adhesion, appropriate Wnt3a-Lentivirus (at an MOI of 50) and transfection enhancer Polybrene were added. Both a blank virus control group and a blank control group were set up. The cells were then cultured at 37°C, 5% CO2. After 4 hours of transfection, 1mL of fresh culture medium was added to each well to reduce the cytotoxicity of Polybrene. After 12 hours, the medium was changed again. GFP fluorescence expression was observed under a fluorescence microscope at 24h, 48h, and 72h to determine the infection efficiency. After determining the optimal conditions for viral transfection, to avoid interference from GFP green fluorescence in subsequent fluorescence staining, we purchased Wnt3a-lentivirus without GFP fluorescence for BMSC infection for subsequent experiments.

Protein Expression Level Was Detected by Western Blot

After infecting P3 BMSCs with Wnt3a-lentivirus for 72 hours, cells were collected. Cells or lung tissues were lysed with RIPA lysis buffer supplemented with proteinase inhibitors on ice, followed by pulse sonication for 30 seconds. The cell extracts were then boiled for 5 minutes at 95°C. Equal amounts of protein were resolved by SDS-PAGE and transferred onto PVDF membranes. After blocking with 5% non-fat milk in TBST (Tris-buffered saline and Tween 20) at room temperature for 1 hour, the membrane was incubated with primary antibodies overnight at 4°C. Western blot detection was performed using an enhanced chemiluminescence system and peroxidase-conjugated secondary antibodies. The primary antibodies used were as follows: anti-Wnt3a, anti-β-actin, anti-IL-1b, IL-6, IL-10, anti-GAPDH.

mRNA Level Was Detected by qPCR

After viral infection, the mRNA expression levels of Wnt3a in BMSCs and the mRNA expression levels of various inflammatory factors in rat lung tissues following BMSC transplantation were detected by qPCR. Cells or lung tissues were collected according to the experimental objectives, lysed with Trizol, and Rverse transcribed into cDNA. Real-time quantitative PCR reactions were performed according to the reaction system. The amplification conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes; 95°C for 30 seconds, 60°C for 30 seconds, for 40 cycles. After the reaction, the data were analyzed using the relative quantitative 2-ΔΔCt method. Primer sequences as following:

Wnt3a
Forward: 5′-GAGTCAGCCTTTGTCCATGC-3′;
Reverse: 5′-GCCTCATTGTTGTGACGGTT-3′;
IL-1β
Forward: 5′-AGGCTGACAGACCCCAAAAG-3′
Reverse: 5′-CTCCACGGGCAAGACATAGG-3′
IL-6
Forward: 5′-AGCGATGATGCACTGTCAGA-3′
Reverse: 5′- GGAACTCCAGAAGACCAGAGC-3′
IL-10
Forward: 5′-CCTCTGGATACAGCTGCGAC-3′
Reverse: 5′-TGAGTGTCACGTAGGCTTCT-3′

Establishment of COPD Rat Model

We utilized an intratracheal injection of lipopolysaccharide (LPS) with smoking method to construct a rat model of chronic obstructive pulmonary disease (COPD). The details of construction method are as: On day 1 and day 14, intratracheal injection of LPS was performed as follows: Rats were anesthetized with 0.3% pentobarbital (10mg/kg) by intraperitoneal injection, fixed on a rat fixator, exposed the glottis. Then, 0.3mL of LPS (1g/L) was slowly injected, followed by a small amount of air, and the rats were rotated left and right to evenly distribute the LPS in both lungs. From day 2 to day 30 (except day 14), the rats were placed in a homemade smoking chamber and exposed to cigarette smoke twice a day, with an interval of 8 hours between each session, each lasting 40 minutes, for a total of 30 days. At the end of the modeling period, selected 5 modeled rats randomly and euthanized them by intraperitoneal anesthesia. And lung tissues were extracted and fixed in formaldehyde.

Transplanting BMSCs into COPD Rats

According to the experimental purpose, rats were divided into four groups (30 rats/group): Normal Control Group (NT), COPD Group, BMSCs Transplantation Group, and Wnt3a-BMSCs Transplantation Group. The Normal Control Group consisted of healthy rats subjected to the same "sham surgery" procedure as the COPD model group. The BMSCs Transplantation Group served as the transplantation of virus-empty control group, while the Wnt3a-BMSCs Group involved the transplantation of BMSCs cells overexpressing Wnt3a.

Firstly, we used P3 BMSCs for Wnt3a-Lentivirus and virus-empty transfection. Cells were collected 48 hours after transfection. The collected cells were labeled with CM-Dil (3μg/mL). Subsequently, cells from each group were injected intravenously into rats at a concentration of 1×10^6 cells/mL, while the control group was injected with 1mL of sterile PBS.

After transplantation, blood samples were collected from the rat's heart at 7, 14, and 28 days post-transplantation, and serum was isolated by centrifugation and stored at −80°C. Ten rats from each group were euthanized at each time point, and lung tissues were collected and fixed in 10% formaldehyde.

Immunohistochemical Staining

Lung tissues fixed in formaldehyde were sent to the Pathology Department of the First Affiliated Hospital of Nanchang University for embedding, sectioning, and hematoxylin-eosin staining.

For immunofluorescence tissue staining, the tissue slides were de waxed and antigen retrieved, tissue sections were blocked with 3% BSA/0.2% Triton X-100 at room temperature for 30 minutes. Subsequently, they were incubated with primary antibodies (SPC 1:100) overnight at 4°C, washed with DPBS, and then incubated with fluorescence secondary antibodies for 1 hour at room temperature. The nuclei were counterstained with DAPI, and the slides were mounted with anti-fade mounting media. Images were captured using an EVOS5000 fluorescence microscope or Leica TCS SPE platform.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7. Descriptive statistics for continuous data were presented as mean ± standard deviation (x ± stdev). Independent sample *t*-tests were used to compare means between two groups, with a significance level set at $\alpha = 0.05$. One-way ANOVA was employed for comparisons of means among multiple groups, and a p-value < 0.05 was considered statistically significant.

Results

Constructed BMSCs That Overexpress Wnt3a

Isolation and Culture of BMSCs

After 24 hours of cell isolation and culture (Figure 1A), under microscopic observation, some cells adhered to the wall, scattered, mostly round, with some spindle-shaped or star-shaped, mixed with anucleate, flattened red blood cells. After 48 hours, most of the red blood cells underwent apoptosis, the number of adherent cells increased compared to before,

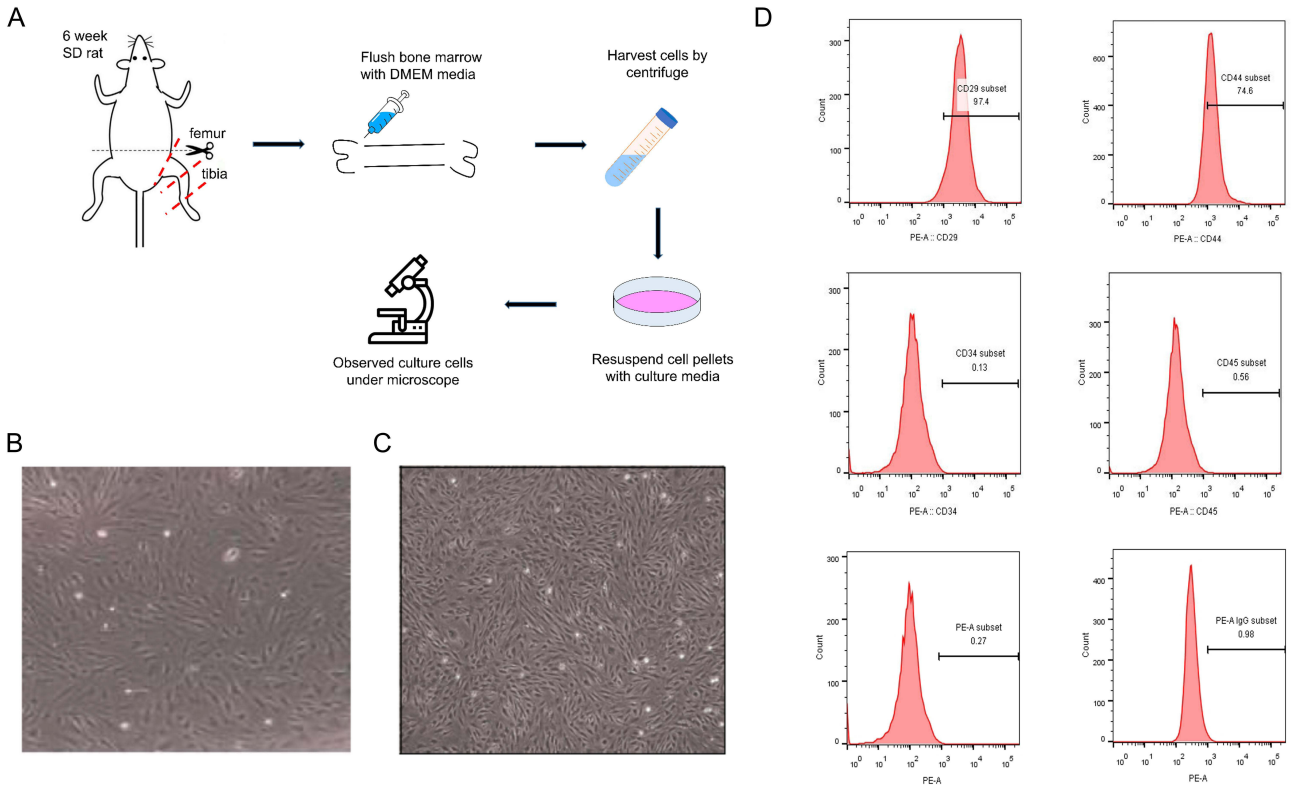

Figure 1 BMSCs isolation and identification. (**A**) Flowchart of BMSCs isolation from SD rat; (**B**) Cell morphology after 48h isolation; (**C**) Cell morphology after 1 weeks isolation; (**D**) Identification of BMSCs by flowcytometer.

and the cell volume increased, showing the formation of small cell clones (Figure 1B). One week later, cell proliferation significantly increased, cell clones gradually merged into patches, and cells converged into vortex or radial patterns. The cells were spindle-shaped or spindle-shaped, with translucent cytoplasm, fewer nuclei but larger (Figure 1C). Flow cytometer analysis of surface molecular markers on P3 passage rat BMSCs showed that the expression rates of CD29 and CD44 on BMSCs were 97.4% and 74.6%, respectively, while the expression rate of CD34, a surface marker of hematopoietic stem cells, was 0.13%, and the expression rate of CD45, a surface marker of common leukocyte, was 0.56% (Figure 1D). These results indicate that we successfully isolated and cultured bone marrow mesenchymal stem cells.

Express Wnt3a in BMSCs

After infecting BMSCs with lentiviral particles carrying GFP-Wnt3a for 72 hours, fluorescent microscopy revealed that the GFP fluorescence signal reached up around 90% (Figure 2A). Cell samples infected for 72 hours were collected, and the expression of Wnt3a was detected at the transcriptional and protein levels. The results showed that compared to the control group, the expression of Wnt3a increased at both the transcriptional and protein levels (Figure 2B and C).

Generate COPD Rat Model

Rat lung tissue was obtained and subjected to pathological sectioning followed by hematoxylin and eosin (HE) staining (Figure 3A). The results revealed that in the normal control group, there were numerous alveoli with uniform and similar-sized alveolar spaces, without evidence of alveolar septal rupture or fusion. The vascular wall thickness in the lung interstitium was normal, with no infiltration of inflammatory cells, and the tracheal lumen appeared normal. Compared to the normal group, in the COPD group, there was a significant decrease in the number of alveoli, irregular enlargement of alveolar spaces (Figure 3B), and disruption of alveolar septa, forming bullae (Figure 3B). The vascular wall in the lung interstitium was thickened, accompanied by infiltration of inflammatory cells. The tracheal lumen was narrowed, and

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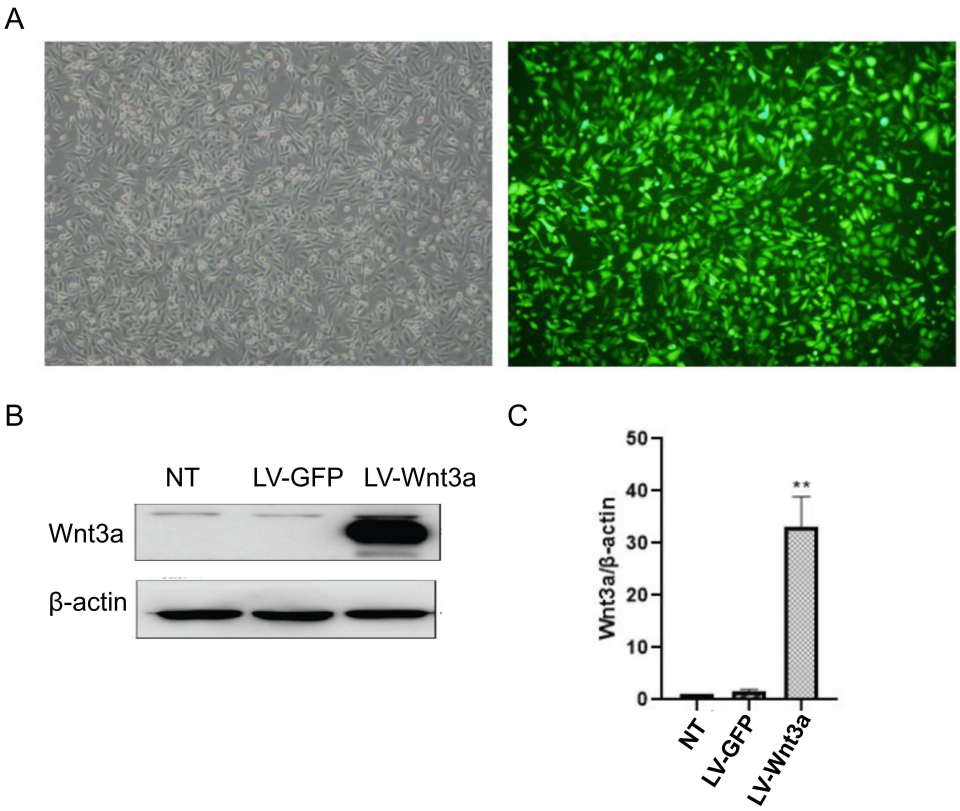

Figure 2 Expressing Wnt3a in BMSCs. (**A**) GFP signals after Wnt3a-Lentivirus infection 72h; (**B**) Protein level of Wnt3a was detected by Western blot; (**C**) mRNA level of Wnt3a was detected by qPCR. mRNA level of Wnt3a in LV-Wnt3a was significantly increased compare with NT and LV-GFP group. Data were from three independent experiments and were analyzed by Two-tailed unpaired *t*-test. Data were expressed as the mean±standard deviation. $^{**}p<0.01$.

desquamated tracheal mucosal epithelial cells were observed (Figure 3B). The HE staining of lung tissue indicated that the lung tissue structure in the COPD group exhibited pathological features consistent with COPD, confirming the successful establishment of the rat COPD model.

Wnt3a Promotes BMSCs Engraftment in Lung Tissue

In COPD rats, BMSCs and Wnt3a-BMSCs were transplanted, and lung tissue was collected, fixed, embedded, and sectioned on the 7th, 14th, and 28th days after BMSC transplantation (Figure 4A). Immunofluorescence staining was performed on tissue sections, revealing red fluorescence (CM-Dil-labeled BMSCs) in lung tissue sections from rats transplanted with both BMSCs and Wnt3a-BMSCs (Figure 4B). After DAPI staining of the sections, the number of CM-Dil-labeled BMSCs and total cells (blue fluorescence) in each slice was counted using ImageJ. The engraftment rate (%) was calculated using the formula: Engraftment rate (%) = number of BMSCs / total number of cells in the same field of view×100%. The results showed that at the same time points, the engraftment rate of BMSCs in the Wnt3a-BMSCs group was significantly higher than that in the BMSCs group (Figure 4C).

Wnt3a Promotes BMSCs Differentiation in Lung Tissue

We performed immunofluorescence staining for SPC (surfactant protein C), a specific marker for type II alveolar epithelial cells, on lung tissue sections. The results showed co-localization of CM-Dil (red fluorescence) and SPC (green fluorescence) in both the BMSCs and Wnt3a-BMSCs groups. Compared to the BMSCs group, the co-localization signal was significantly increased in the Wnt3a-BMSCs group (Figure 5A). We conducted statistical analysis on lung tissue collected on the 7th, 14th, and 28th days post-transplantation to calculate the proportion of BMSCs differentiated into type II alveolar epithelial cells. The differentiation rate was calculated using the following formula: Differentiation rate (%) = Number of double-positive cells / Total number of CM-Dil-labeled BMSCs. The results showed that at all

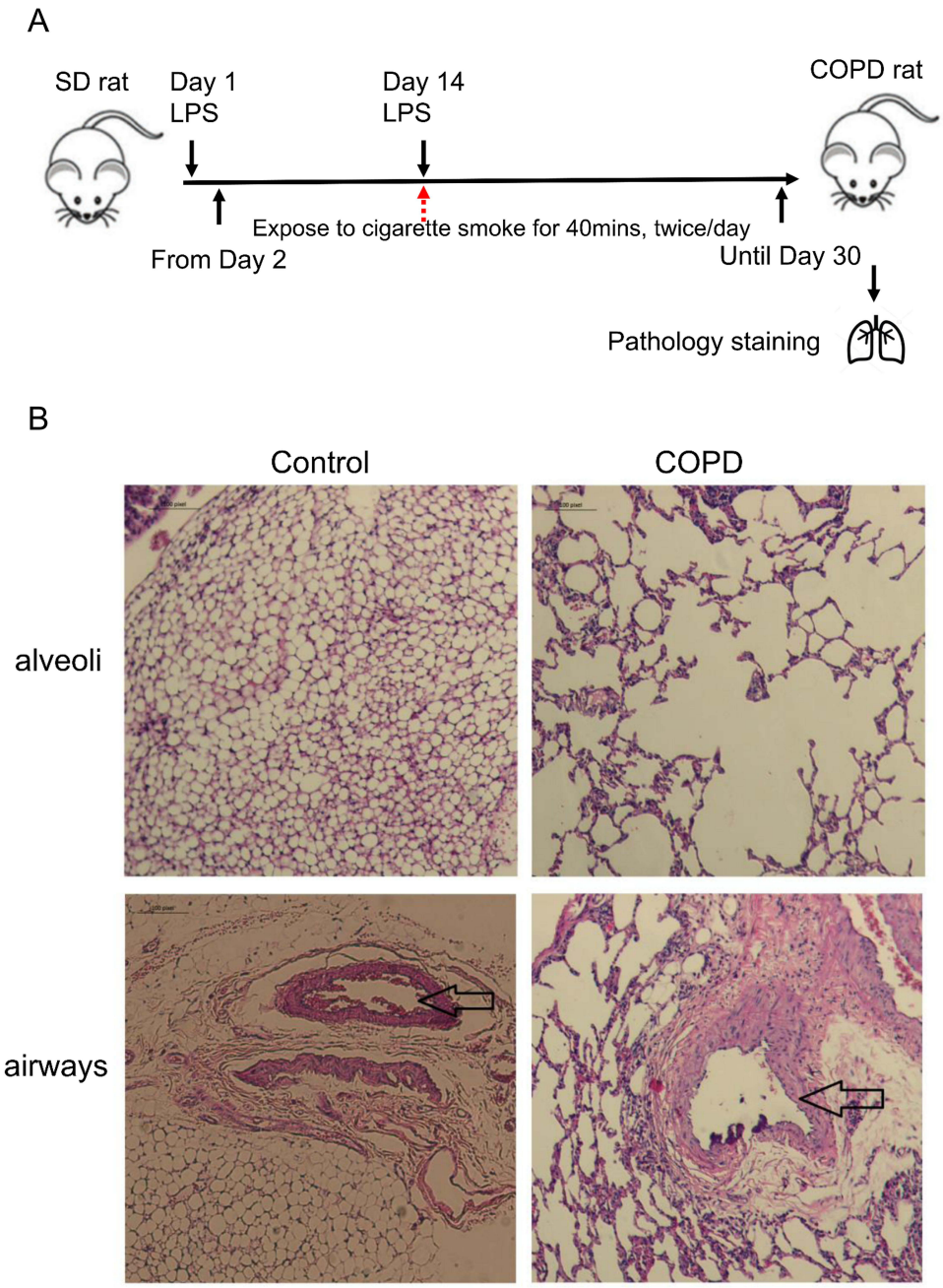

Figure 3 Generate COPD rat model. (**A**) Procedure of generating COPD rat; (**B**) HE staining of rat lung tissue.

three time points, the differentiation rate in the Wnt3a-BMSCs group was significantly higher than that in the BMSCs group (Figure 5B). This indicates that Wnt3a can promote the differentiation of BMSCs into type II alveolar epithelial cells.

Wnt3a Regulate Immunocytokines Level in Lung Tissue

IL-6 and IL-1β play a role in the inflammatory process of COPD, while IL-10 is an anti-inflammatory cytokine that can alleviate COPD inflammation. We measured the levels of IL-6, IL-1β, and IL-10 in lung tissues of different groups at different time points (D7, D14, D28). The results showed that at the transcriptional level, compared to the normal control group, the expression of the pro-inflammatory cytokines IL-6 and IL-1β in the COPD group increased over time, with

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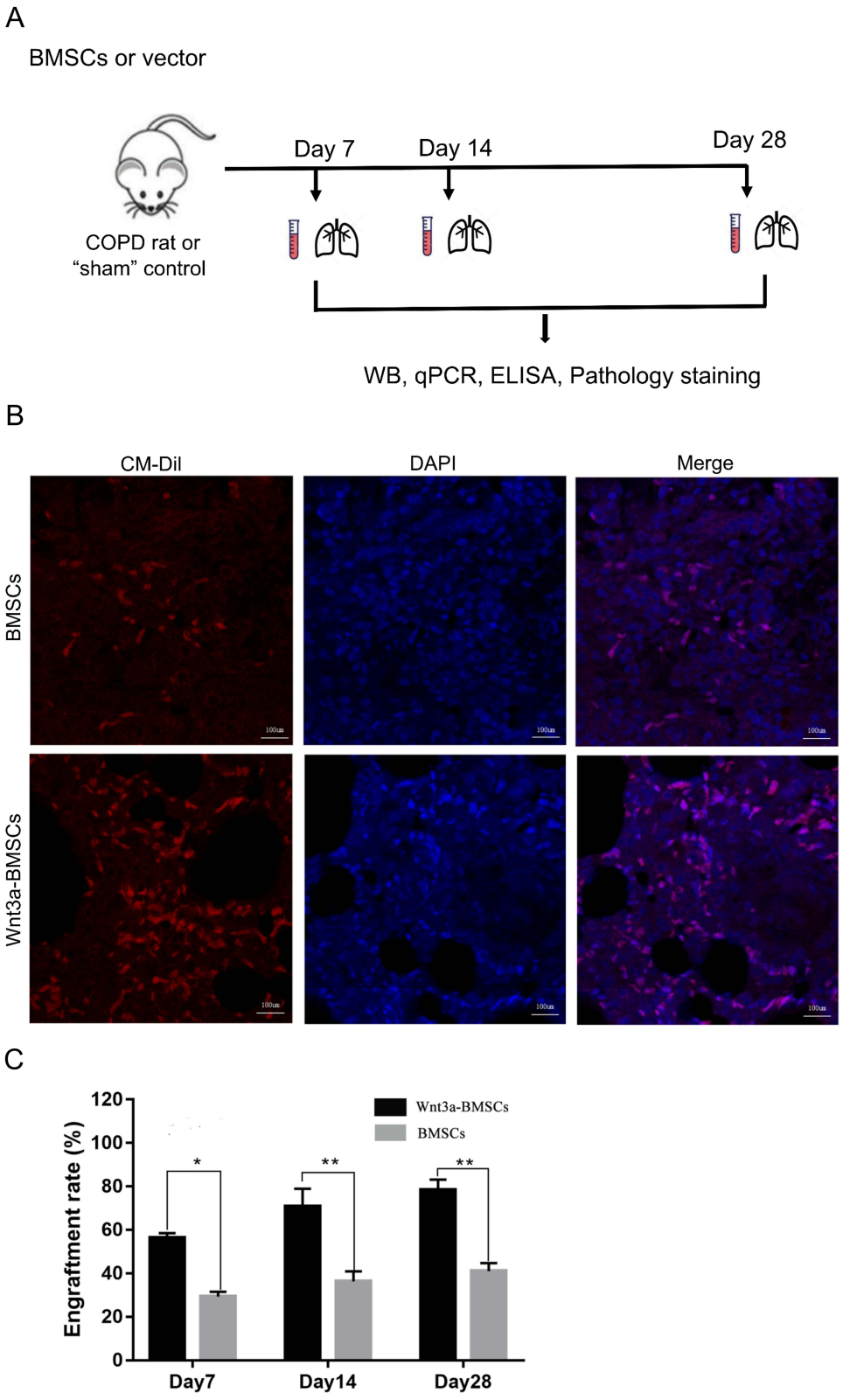

Figure 4 Wnt3a promotes BMSCs engraftment in rat lung tissue. (**A**) Procedure of BMSCs transplantation; (**B**) BMSCs engraftment in lung tissue. BMSCs were labeled with CM-Dli (red), cells were stained with DAPI (blue); (**C**) BMSCs engraftment rate between BMSCs and Wnt3a-BMSCs. Data were from three independent experiments and were analyzed by Two-tailed unpaired *t*-test. Data were expressed as the mean±standard deviation. *p<0.05, **p<0.01.

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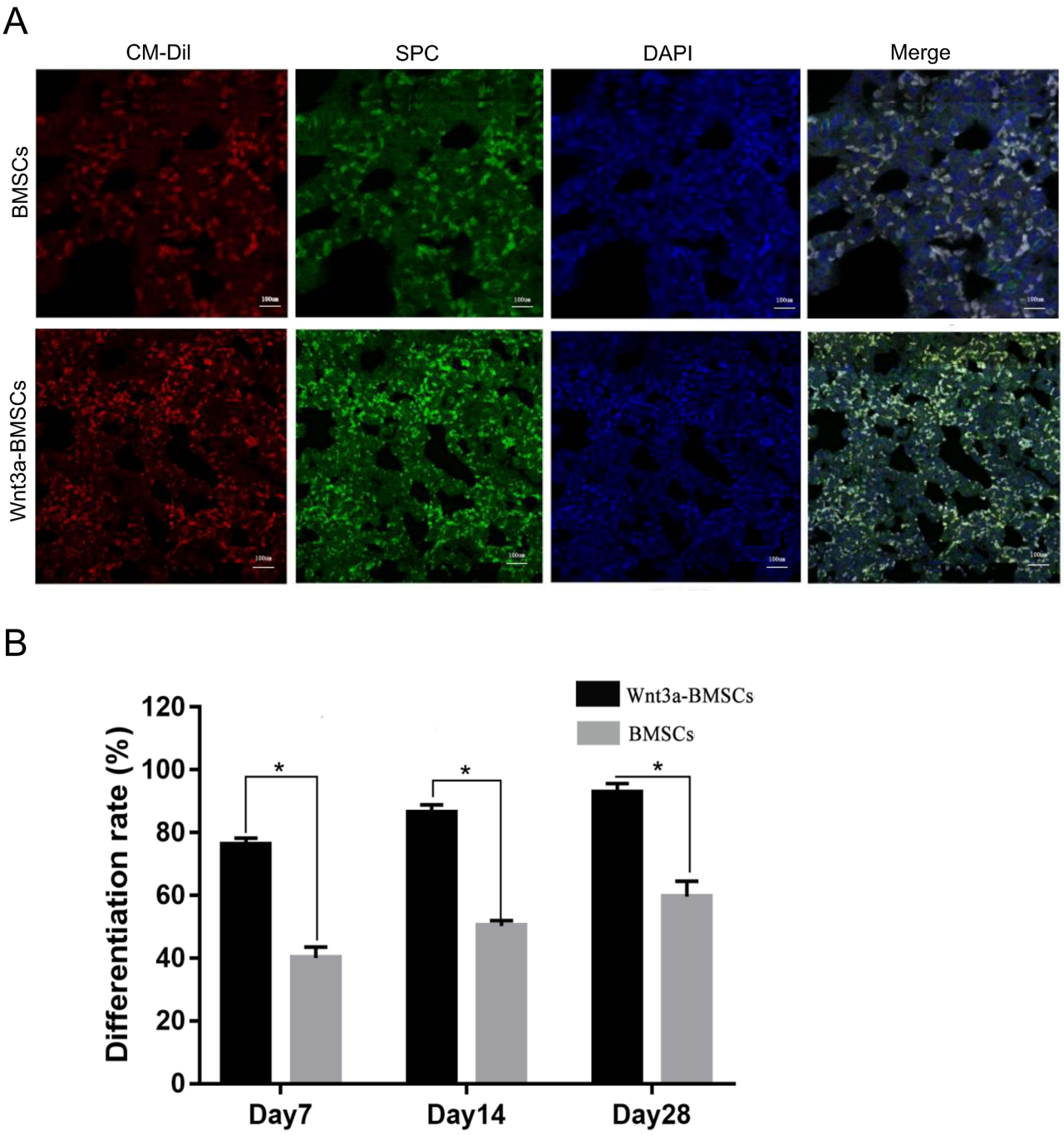

Figure 5 Wnt3a promotes BMSCs differentiation in rat lung tissue. (**A**) BMSCs differentiated to type II alveolar epithelial cells (green, SPC), BMSCs (red); (**B**) The differentiation rate of different time point between BMSCs group and Wnt3a-BMSCs. Data were from three independent experiments and were analyzed by Two-tailed unpaired *t*-test. Data were expressed as the mean±standard deviation. *$p<0.05$.

a significant elevation at D28, while the anti-inflammatory cytokine IL-10 showed a decreasing trend, with the most significant decrease at D28 (Figure 6A). This trend was also confirmed at the protein level (Figure 6B).

Furthermore, we compared the transcriptional levels of these three inflammatory factors at D28 in COPD rats transplanted with BMSCs and Wnt3a-BMSCs. The results showed that compared to the COPD group, both IL-6 and IL-1β decreased in the BMSCs transplantation group, while IL-10 remained at higher levels. Moreover, the Wnt3a-BMSCs group exhibited a more significant reduction than the BMSCs group (Figure 6C). This finding was further confirmed by Western blot analysis of protein expression of these factors (Figure 6D).

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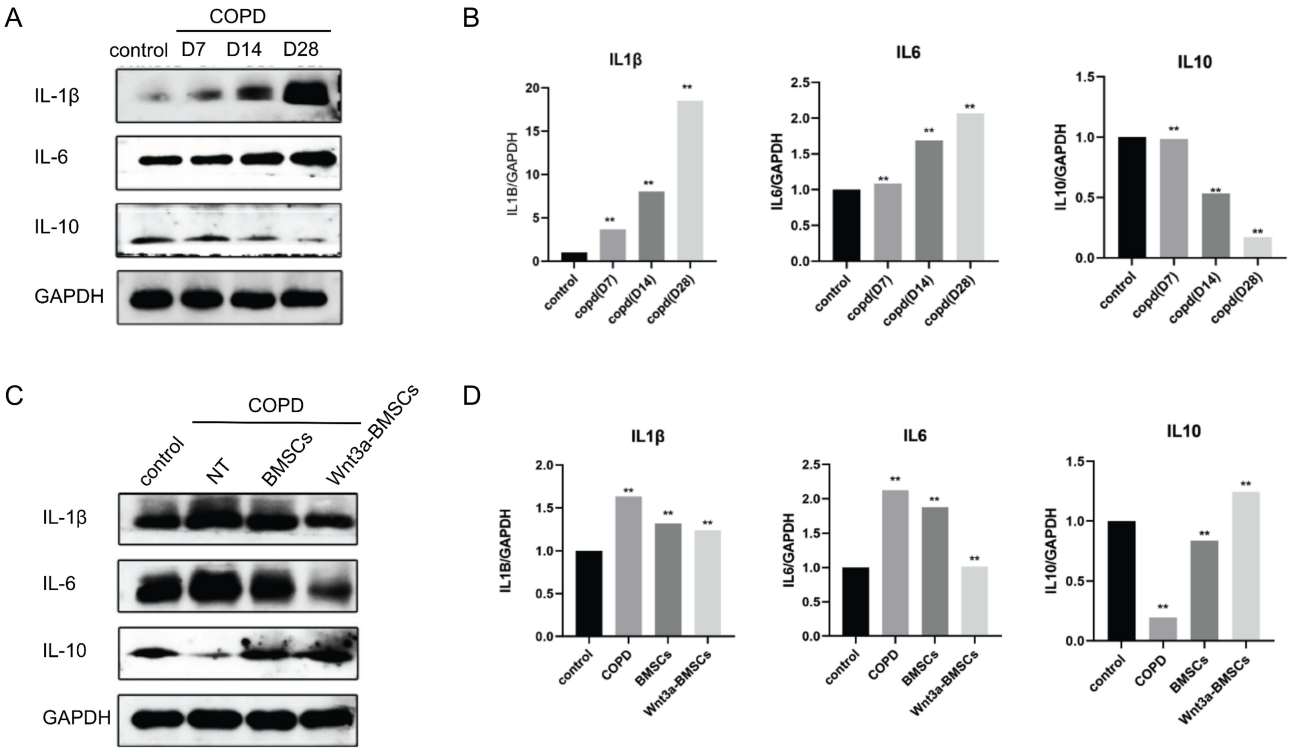

Figure 6 Wnt3a regulate COPD induced inflammation. (**A** and **C**) Protein level of IL-1β, IL-6 and IL-10 in rat lung tissue between health rat, COPD model with/without BMSCs transplantation, COPD model with Wnt3a-BMSCs transplantation; (**B** and **D**) mRNA level of IL-1β, IL-6 and IL-10 in rat lung tissue between health rat, COPD model with/without BMSCs transplantation, COPD model with Wnt3a-BMSCs transplantation. Data were from three independent experiments and were analyzed by Two-tailed unpaired *t*-test. Data were expressed as the mean±standard deviation. $^{**}p<0.01$.

These results suggest that BMSCs can reduce the levels of inflammatory factors in COPD, effectively alleviating COPD inflammation. Additionally, Wnt3a can enhance the anti-inflammatory function of BMSCs in COPD.

Discussion

Chronic obstructive pulmonary disease (COPD) is a common respiratory condition characterized by chronic and progressive dyspnea, chronic cough, sputum production, and chest tightness or fatigue.[14] It is caused by various factors such as environmental exposures, smoking, infections, and also has a genetic predisposition component. Acute exacerbations of COPD are the main cause of worsening symptoms and declining lung function, reflecting widespread inflammatory processes during the disease stage, and are a major contributor to the high mortality rate associated with COPD.[2,15] Currently, COPD treatment includes medications such as anti-inflammatory drugs and bronchodilators, as well as non-pharmacological treatments such as smoking cessation and pulmonary rehabilitation. While these measures can temporarily alleviate and improve respiratory symptoms, reduce the risk of acute exacerbations, and slow disease progression, there is still no effective method to reverse the sustained decline in lung function.

BMSC transplantation for the treatment of lung injury has become a recent research hotspot.[16,17] Studies have shown that the therapeutic effects of BMSCs on lung injury mainly manifest in two aspects:[18] (i) BMSCs can engraft into the lungs and differentiate into alveolar epithelial cells and endothelial cells, playing a role in repairing damaged tissues. And (ii) transplanted BMSCs have immunomodulatory functions, exerting anti-inflammatory effects, and protecting and maintaining lung tissue integrity.[19] We successfully prepared a model of chronic obstructive pulmonary disease (COPD) by intratracheal injection of lipopolysaccharide combined with cigarette smoke exposure (Figure 3A). Histological staining of lung tissue from the model rats revealed features such as alveolar wall rupture, irregular expansion of alveolar spaces, and infiltration of inflammatory cells, consistent with pathological changes in COPD lungs (Figure 3B). In the COPD model group, the inflammatory factors tumor necrosis factor-alpha, IL-6, and IL-1β were significantly elevated, while the anti-inflammatory factor IL-10 was decreased, reflecting the characteristic inflammatory

response of COPD disease models (Figure 6A and B). However, BMSC transplantation improved lung pathological damage and inhibited the production of inflammatory factors while promoting the generation of anti-inflammatory factors (Figure 6C and D). Our results indicate that the BMSCs we isolated and cultured can improve lung injury and inflammation in COPD. Although BMSCs have shown initial efficacy in disease models, their clinical application is limited by issues such as rapid clearance, low survival rate at the transplantation site, and low differentiation efficiency.[20,21] Therefore, it is necessary to study methods to improve the survival and differentiation of BMSCs after transplantation.

The canonical Wnt pathway regulates various physiological functions such as cell and organism development, differentiation, among others. Wnt/β-catenin signaling plays a crucial role in lung biology and pathobiology.[22] Wnt3a, a novel Wnt protein specifically promoting the Wnt/β-catenin signaling pathway, has been demonstrated to enhance the proliferation and migration of MSCs.[23] Additionally, activating Wnt/β-catenin signaling can promote the differentiation of BMSCs into alveolar AT II epithelial cells.[9] In our study, we utilized lentiviral vectors to construct stable BMSCs expressing Wnt3a[24] and transplanted them into a COPD rat model. We observed that overexpression of Wnt3a could enhance the engraftment of BMSCs in the lungs (Figure 4B and C) and promote the differentiation of engrafted cells into alveolar AT II epithelial cells (Figure 5). Through pathological tissue sections, we observed improvement in COPD pathological damage by BMSCs overexpressing Wnt3a. Based on these results, we concluded that we successfully transplanted BMSCs into rats, and Wnt3a increased their engraftment and differentiation rates in rats. However, the efficiency of engraftment and differentiation rates did not significantly increase with the prolongation of the transplantation time in vivo (Figure 5B), suggesting the need for further optimization of the transplantation conditions in our next steps. Studies have shown that BMSCs exert their effects through paracrine secretion, and their derived extracellular vesicles possess similar functions to their parent cells, such as Wnt3a-loaded extracellular vesicles promoting alveolar epithelial regeneration after lung injury by activating the Wnt signaling pathway.[25] In this study, we did not delve into the specific mechanisms by which Wnt3a exerts its effects, and further research is needed to refine this aspect in the future.

BMSCs participate in immune regulation and can alleviate inflammation in COPD models.[26–28] In ischemic brain injury models, Wnt3a has also been shown to reduce neuroinflammation after ischemia through its immunomodulatory function.[29] We further observed the regulatory effect of BMSCs overexpressing Wnt3a on inflammatory factors in the COPD model. Compared to the group receiving only BMSC transplantation, Wnt3a was found to enhance the anti-inflammatory response of BMSCs, further improving inflammation in the COPD model (Figure 6). Therefore, stable expression of Wnt3a in BMSCs is also beneficial for alleviating inflammation.

Although we have derived preliminary research findings, our study still have some limitations. Utilizing pathological techniques, we have ascertained the efficacy of the COPD rat model that we have constructed. However, due to the absence of requisite animal equipment, we were unable to quantify the pulmonary functional alterations in the COPD rats. Despite conducting pathological examinations, our study did not include Masson's Trichrome staining, a method crucial for evaluating alterations in collagen fiber content within lung tissue. Furthermore, the elucidation of underlying molecular mechanisms and the exploration of clinical translational applications require further exploration and research.

Conclusion

Our study demonstrates that express Wnt3a in BMSCs can improve the engraftment and differentiation of BMSCs in rat and further alleviate COPD symptoms by regulating inflammatory cytokines. Therefore, constructing BMSCs over-expressing Wnt3a could serve as a new strategy for stem cell therapy in COPD in future.

Abbreviations

BMSCs, Bone marrow mesenchymal stem cells; COPD, Chronic obstructive pulmonary disease; AT I cells, Type I alveolar epithelial cells; AT II cells, Type II alveolar epithelial cells; IL-6, Interleukin 6; IL-1β, Interleukin1β; IL-10, Interleukin10.

Data Sharing Statement

The data are available from the corresponding authors on reasonable request.

Ethics Approval and Consent to Participate

This study was approved by Ethics Committee of the First Affiliated Hospital of Nanchang University.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the China National Natural Science Foundation (No.81660009), Jiangxi Provincial Natural Science Foundation (Nos. 20161ACB20012, 20212BAB206034).

Disclosure

The authors declare no competing interests.

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