Stem Cells International

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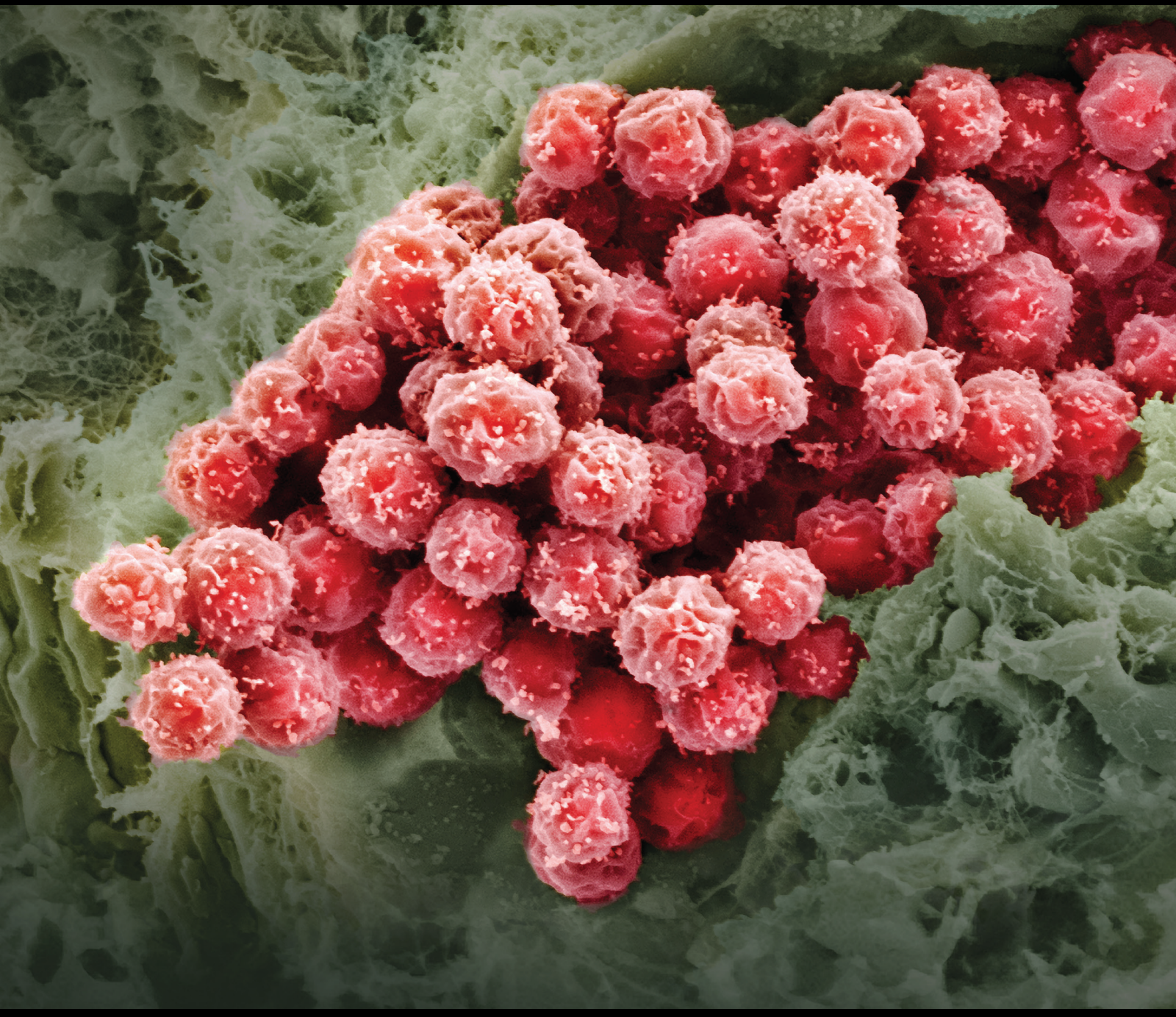

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Research Article

miR-370-3p as a Novel Biomarker Promotes Breast Cancer Progression by Targeting FBLN5

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miRNAs play a crucial part in multiple biological processes of cell proliferation, migration, apoptosis, and chemoresistance. In cancer, miRNAs can be divided into oncogenes or tumor suppressors on the basis of their functions in the carcinogenic process. The purpose of this study was to explore the roles and clinical diagnostic value of miR-370-3p in breast cancer. Our results demonstrated that miR-370-3p significantly promoted proliferation, metastasis, and stemness of breast cancer *in vitro* and *in vivo*. In particular, clinical data revealed that high expression of serum miR-370-3p and exosomal miR-370-3p from breast cancer patients was remarkably correlated with lymphatic metastasis and tumor node metastasis (TNM) stages. Mechanistically, miR-370-3p inhibited FBLN5 expression and activated the NF-κB signaling pathway to promote breast cancer cell proliferation, migration, and stemness. FBLN5 expression was significantly decreased in breast cancer cells and tumor tissues of breast cancer patients. Our research identified that miR-370-3p promoted breast cancer progression by inhibiting FBLN5 expression and activating the NF-κB signaling pathway. Serum exosomal miR-370-3p would provide a potential biomarker for the diagnosis of breast cancer.

1. Introduction

Breast cancer is the most common form of cancer among women. Its incidence rate ranks first in malignant tumors of women [1], and it is also the second leading cause of cancer-related death in women [2]. Therefore, it is necessary to find out molecules that affect the progress of breast cancer, then study their functions and mechanisms, and explore their values as novel targets for early detection and treatment of breast cancer.

MicroRNAs (miRNAs) have emerged as important roles in the carcinogenesis and progression of breast cancer by regulating target gene expression *via* posttranscriptional processing [3, 4]. It has been previously reported that miR-128 can target Bcl-2-related x (Bax) gene to promote the proliferation of MCF-7 cells [5]. Metastatic breast cancer cells (MBC) could secrete and transport miR-1246 to mammary epithelial cells and nonmetastatic breast cancer cells to suppress the expression of cyclin-g2, thereby enhancing survival rates and mobility of breast cancer cells [6].

Except for affecting the progression of breast cancer, miRNAs can also be identified as good biomarkers to increase diagnostic accuracy of breast cancer. The combination of miR-21 and miR-1246 is beneficial to the early diagnosis of breast cancer [7, 8]. In addition, serum miR-301 is also considered an early diagnostic marker of breast cancer [9]. miR-101, miR-372, or miR-373 overexpression in serum is also helpful for the diagnosis of breast cancer [10].

In our previous research, we found that bacterial lipopolysaccharide (LPS) promoted the metastasis of breast cancer cells *in vitro* and *in vivo* by activating the TLR4/MyD88/NF-κB signaling pathway. To further determine whether miRNA is involved in this process, we used microarray to screen and found that the breast cancer cells

with higher metastasis ability significantly overexpressed miR-370-3p. However, the biological characteristics and molecular mechanisms of miR-370-3p in breast cancer are not clear. This study will clarify the effects of miR-370-3p on the progression of breast cancer and its clinical diagnosis value serving as a novel biomarker.

2. Materials and Methods

2.1. Cell Culture. Human breast epithelial cell line MCF-10A and human breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from the cell bank of Chinese Academy of Sciences (Shanghai, China). MCF-10A was cultured in RPMI-1640 containing 20% FBS (GIBCO). MCF-7 and MDA-MB-231 were propagated with high-glucose DMEM (GIBCO) containing 10% FBS. 293T cells were preserved in high-glucose DMEM (GIBCO) supplemented with 10% FBS. All cell lines were propagated at 37°C in an incubator containing 5% carbon dioxide.

2.2. Human Clinical Specimens. The ethics committee from the Second Affiliated Hospital of Soochow University has approved the study (Project No.: jd-lk-2019-065-01), and the patients involved have known and agreed. The specimens were taken from the cancer tissue and the surrounding normal tissue of female patients with breast cancer in our hospital. The serum was collected from female breast cancer patients in the hospital, and the serum of healthy female physical examinees with similar age was collected. The intravenous blood samples were centrifuged at 4°C at 3000 g for 10 minutes. The supernatant was then put into a new centrifuge tube and centrifuged for 10 minutes at 12000 g at 4°C. Finally, the remaining serum was stored at -80° C. All specimens were preserved in liquid nitrogen. Meanwhile, the pathological data of breast cancer patients were collected.

2.3. Exosomes Isolation and Identification. Exosomes in serum were separated according to the operating instructions in the ExoQuick precipitation solution (System Biosciences, Mountain View, CA, USA). In short, 63 μl ExoQuick solution was added to 250 μl serum and stored in a 4°C refrigerator overnight. The supernatant was centrifuged at 1500 g at 4°C for 30 minutes. The supernatant was removed and resuspended with 60 μl PBS.

2.4. Transfection. The transient transfection of miR-370-3p was achieved by transfection of miRNA oligonucleotides (miR-370-3p mimics, inhibitor, and corresponding negative control were synthesized by GenePharma, China). According to the instructions, miR-370-3p mimics and inhibitors were, respectively, transfected at 100 nM and 250 nM in breast cancer cells by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The target sequences of the oligonucleotides are provided in Supplementary Table S1.

2.5. RNA Extraction and Real-Time RT-PCR. TRIzol Reagent (Invitrogen, USA) can be used for total RNA extraction from tissues and cells. Total RNA in serum and exosomes was extracted with QIAzol Lysis Reagent (Invitrogen, USA). The miScript II RT Kit (Qiagen, Germany) can be used to reverse transcribe mRNAs, and the miScript SYBR Green PCR Kit (Qiagen, Germany) can be used to detect them. Similarity, the miScript II RT Kit (Qiagen, Germany) is used for miRNA reverse transcription, and the SYBR Green PCR Kit (Qiagen, Germany) can be used to detect reverse transcriptional mRNA. Quantitative analyses can use CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). All experiments were repeated three times. The internal reference of mRNAs is GAPDH. The internal parameter of miRNAs is U6. Sangon (Shanghai, China) (Supplementary Table S2) designed and synthesized primers for mRNAs. The primers for miRNA detection were purchased from Qiagen.

2.6. Double Luciferase Reporter Gene Experiment. The 3′-UTR region of the target gene was constructed and linked to the pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI). Mutation sites were constructed using the Quick Change II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA). In the overexpression system, the reporter vector was transfected into 293T cells together with MNC and 370-mimics. A Dual-Glo luciferase assay system (Promega, Madison, WI) can detect firefly luciferase activity, and the activity was standardized to that of Renilla luciferase. All experiments were repeated three times.

2.7. Western Blot. The samples were cleaved with RIPA buffer containing protease inhibitor. 10% SDS-PAGE gel was used to separate the protein extracted from the samples. After electrophoresis, the proteins on the gel were transferred onto 0.22 μm polyvinylidene fluoride membrane and blocked in 5% (w/v) skimmed milk powder, then incubated with primary antibodies against E-cadherin, N-cadherin, NF-κB-p65 (Cell Signaling Technology, Beverly, MA, USA), vimentin, PCNA (Bioworld Technology, Louis Park, MN, USA), FBLN5 (Santa Cruz Biotechnology, USA); OCT4(Cell Signaling Technology, Beverly, MA, USA), SOX2(Cell Signaling Technology, Beverly, MA, USA), and GAPDH; then, the membrane was fully washed, and the HRP-linked secondary antibodies were used (Kangcheng, Shanghai, China). Finally, the enhanced chemiluminescence system (ImageQuant LAS 4000 mini, GE, Japan) detected the signals. All experiments were repeated three times.

2.8. Colony Formation Assay. Transfected cells were plated into 35 mm plates (1000 cells/well) and propagated for 2 weeks. The medium was changed once every three days. Finally, the colonies were fixed with 4% paraformaldehyde and stained with crystal violet. 4% paraformaldehyde fixed the cells, and crystal violet stained them for 20 minutes. PBS was used to wash the dye, and photos were taken under microscope to analyze the results. All experiments were repeated three times.

2.9. Transwell Migration Assay. Transfected cells were seeded with appropriate density (1×10^5/well) into the upper chamber of 24-well Transwell plate, and serum-free medium was used. 600 μl medium containing 10% serum was added into the chamber under the culture plate. After 8 hours, the cells

transferred to the lower chamber were fixed with 4% paraformaldehyde and stained with crystal violet for 20 min. Photographs were taken under a microscope, and the number of cells migrating in each region was calculated. All experiments were repeated three times.

2.10. Subcutaneous Xenograft Model in BALB/c Nude Mice. The research on experimental animals in this paper has been approved by the Ethics Committee of the Second Affiliated Hospital of Soochow University (Project No.: jd-lk-2019-065-01). Female BALB/C nude mice aged 4-6 weeks were harvested from Suzhou Zhaoyan New Drug Research Center Co., Ltd. MDA-MB-231 successfully transfected with MNC, 370-mimics, INC, and 370-inhibitor, respectively, and were injected into the axillary subcutaneous of each group of nude mice (5×10^6 cells, $n = 6$). After injection for 18 days, the mice were sacrificed, and the breast tumors were photographed and weighted. The tumor volume was measured according to the formula $0.5 \times L \times W^2$. L represents the longitudinal diameter, and W represents the latitudinal diameter.

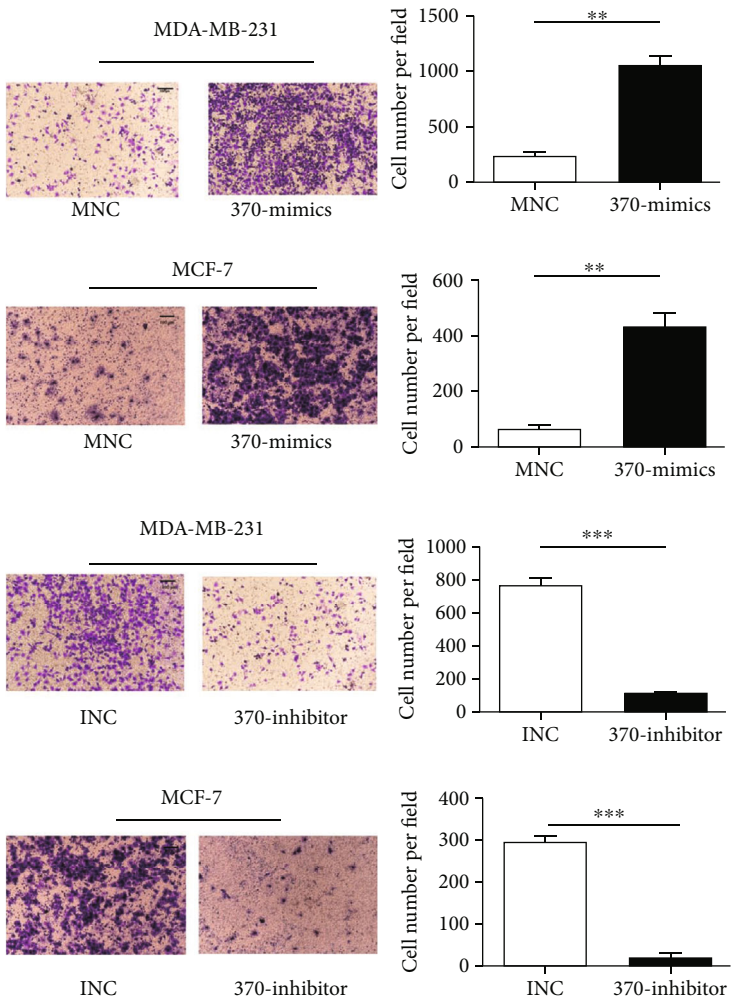
2.11. Statistical Analysis. Statistical analysis was carried out by the software GraphPad Prism5. Unpaired-sample t-test can be used to analyze the potential expression differences between the two groups after different treatments. The differences of miRNA expression between breast cancer patients and healthy volunteers were analyzed by the Mann–Whitney U test. The Wilcoxon signed-rank test was used to analyze the mRNA levels of miR-370-3p and FBLN5 in paired breast cancer tissues and adjacent normal tissues. $P < 0.05$ was considered significant.

3. Results

3.1. miR-370-3p Stimulates Proliferation and Migration of Breast Cancer Cells. In previous research, we have found that TLR4 was linked to the metastasis of breast cancer *in vitro* and *in vivo* by activating the TLR4/MyD88/NF-κB signaling pathway. The miR-370-3p level was dramatically increased in TLR4-activated breast cancer cells. In the current research, we aimed to study the effect of miR-370-3p in breast cancer. It was found that compared with mammary epithelial cells MCF-10A, miR-370-3p was markedly upregulated in breast cancer cells MCF-7 and MDA-MB-231. Additionally, in MDA-MB-231 cells, the expression of miR-370-3p was higher than that in MCF-7 (S1A), which implied that miR-370-3p might play as an oncomiRNA in the development of breast cancer. For further analysis, we transfected MNC and 370-mimics in MCF-7 and MDA-MB-231. The result of quantitative PCR indicated that the expression levels of miR-370-3p were significantly increased in the transfection group of 370-mimics than that in the control group (S1B). Transwell migration and colony formation assays revealed that miR-370-3p significantly enhanced breast cancer cell migration and proliferation compared to that in the MNC transfection group (Figures 1(a) and 1(b)). The results of western blot showed that the expression of mesenchymal markers N-cadherin, vimentin, and the proliferation-related marker PCNA was increased, whereas the protein levels of epithelial marker E-cadherin was decreased in the 370-mimics transfected cells than that in the control group (Figure 1(c)). Quantitative PCR also showed that the expression levels of stemness-related genes OCT4 and SOX2 were significantly overexpressed in 370-mimics transfection group than in the transfection group of MNC (Figure 2(a)). Western blotting showed that stemness-related proteins OCT4 and SOX2 were significantly higher with the transfection of 370-mimics (Figure 2(b)). Similarly, the expression levels of miR-370-3p were significantly decreased in the 370-inhibitor transfected cells than that in the control group (S1B). The migration and proliferation of breast cancer cells were reduced in 370-inhibitor transfected cells (Figures 1(a) and 1(b)). Moreover, the expression of N-cadherin, vimentin, and PCNA was decreased, whereas the expression of E-cadherin was increased in cells transfected with miR-370-3p inhibitor (Figure 1(c)). The expression levels of OCT4 and SOX2 were also significantly decreased in the transfection group of 370-inhibitor than in the INC transfection group (Figure 2(a)). Western blot results showed that with the transfection of miR-370-3p inhibitor, OCT4 and SOX2 levels were decreased (Figure 2(b)).

We also researched the significance of miR-370-3p in subcutaneous xenograft models in nude mice. Nude mice were injected into the armpit with breast cancer cell line MDA-MB-231 overexpressed or knocked-down miR-370-3p. The tumor size and weight showed that miR-370-3p mimics treatment remarkably increased tumor growth, and miR-370-3p inhibitor treatment remarkably decreased tumor growth (Figures 3(a)–3(c)). The expression of miR-370-3p was higher in overexpressed miR-370-3p group and lower in knocked-down miR-370-3p group than in the control group (Figure 3(d)). Western blotting showed that the expression of N-cadherin and vimentin related to epithelial-mesenchymal transition (EMT) was significantly higher, but the protein expression of E-cadherin was reduced in the 370-mimics transfection group than in the control group. On the contrary, the results were the opposite (Figure 3(e)). Collectively, the above results demonstrated that miR-370-3p could promote breast cancer growth and metastasis *in vivo*.

3.2. MiR-370-3p Promotes Breast Cancer through the Regulation of FBLN5 and the NF-κB Signaling Pathway. Bioinformatics software miRDB and TargetScan have predicted multiple target genes. The measurement of the expression of predicted target genes in MCF-10A, MCF-7, and MDA-MB-231 and transfected MCF-7 and MDA-MB-231 cells showed a negative correlation between the expression levels of FBLN5 and miR-370-3p, which suggested that FBLN5 might be the downstream gene of miR-370-3p (Figure 4(a)). Wild- and mutant-type reporter gene vectors of the mRNA $3'$-UTR binding sites in the target gene FBLN5 were constructed and used to transfect 293T cells together with miR-370-3p mimics. We observed that the luciferase activity of the wild-type reporter gene vector was obviously inhibited by miR-370-3p mimics rather than the mutant reporter gene vector (Figures 4(b) and 4(c)). Furthermore,



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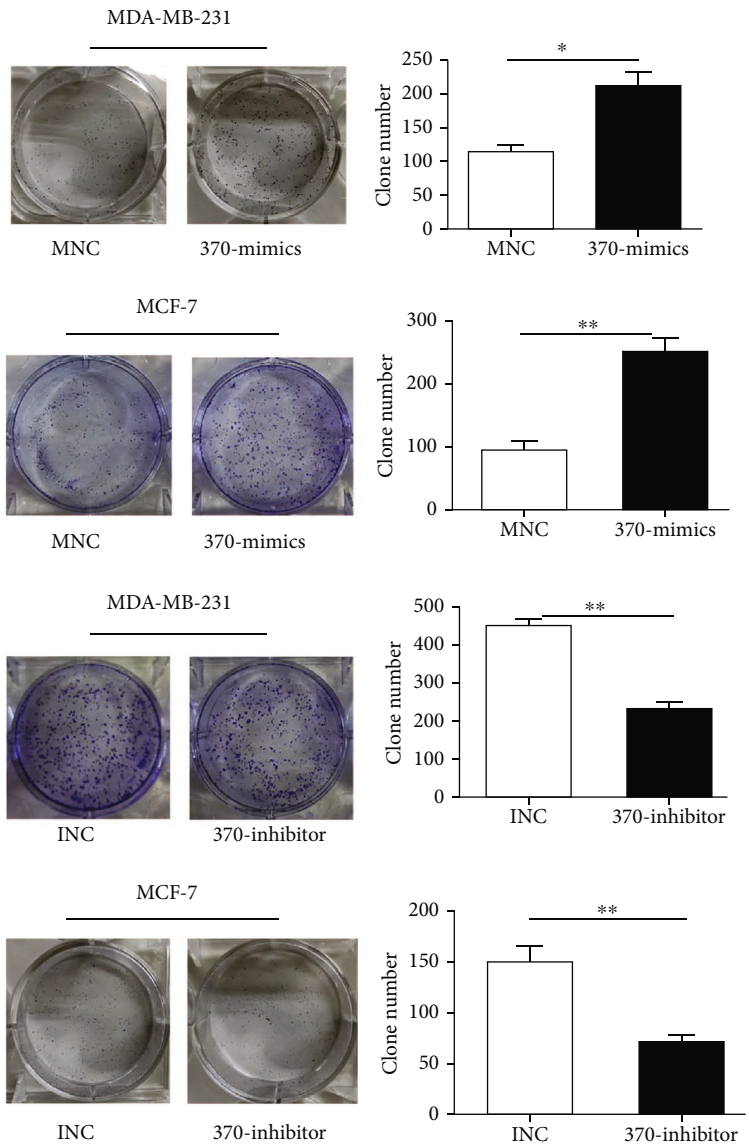

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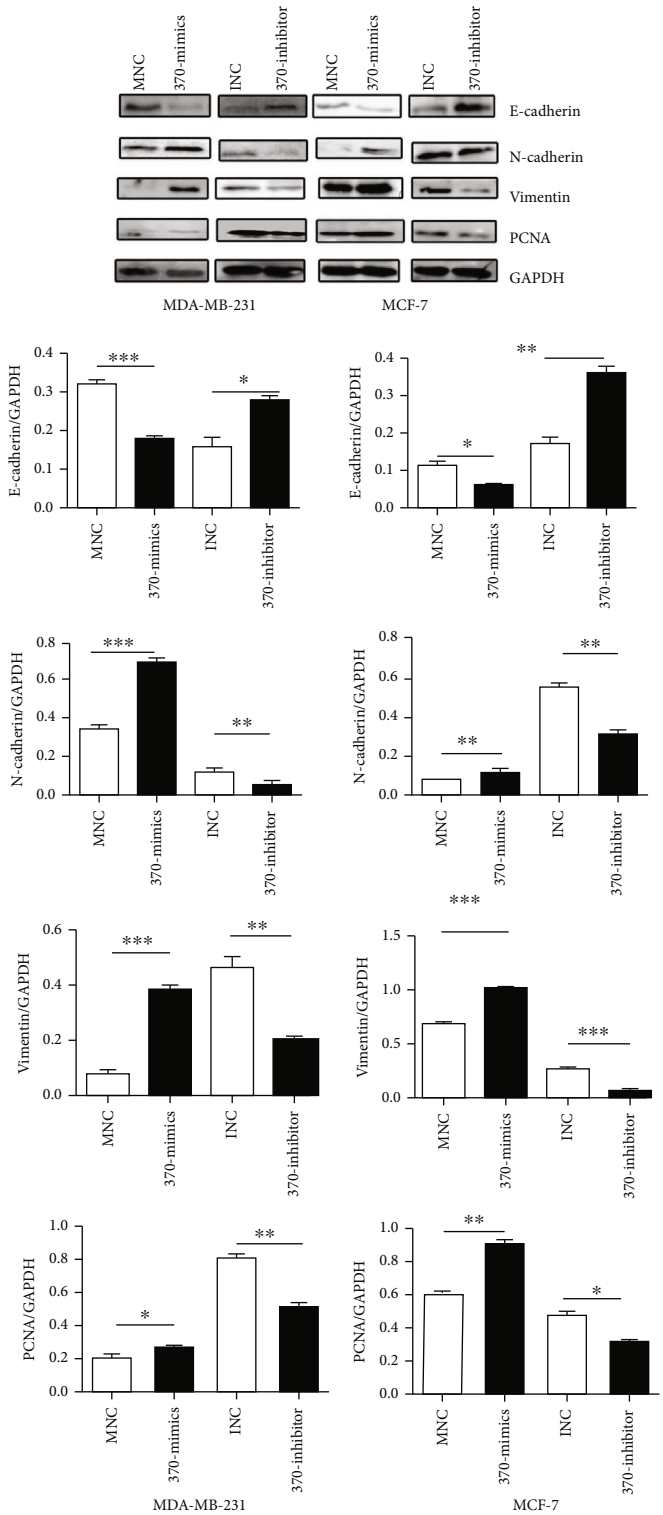


(c)

Figure 1: miR-370-3p can promote migration and proliferation of breast cancer cells *in vitro*. (a) Transwell migration assay was used to evaluate the migration of miR-370-3p in breast cancer cells (MDA-MB-231 and MCF-7). $^{***}P < 0.001$. The scale bar is in the upper right of the first picture in each row. (b) Evaluation of miR-370-3p on proliferation of breast cancer cells (MDA-MB-231 and MCF-7) by colony forming assay. $^{**}P < 0.01$, $^{*}P < 0.05$. (c) Western blot was used to detect the expression of EMT-related proteins and PCNA protein of miR-370-3p in breast cancer cells (MDA-MB-231 and MCF-7). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

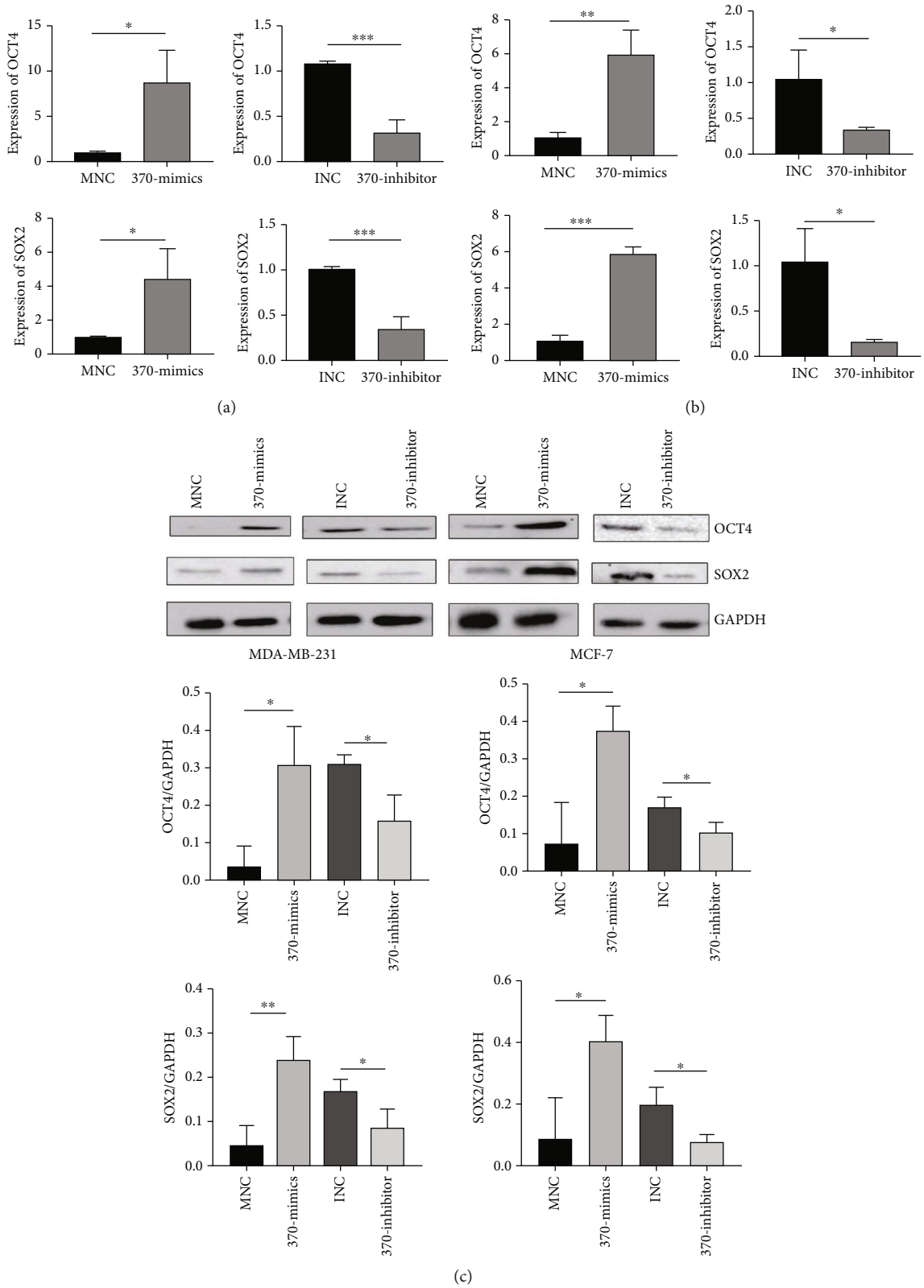
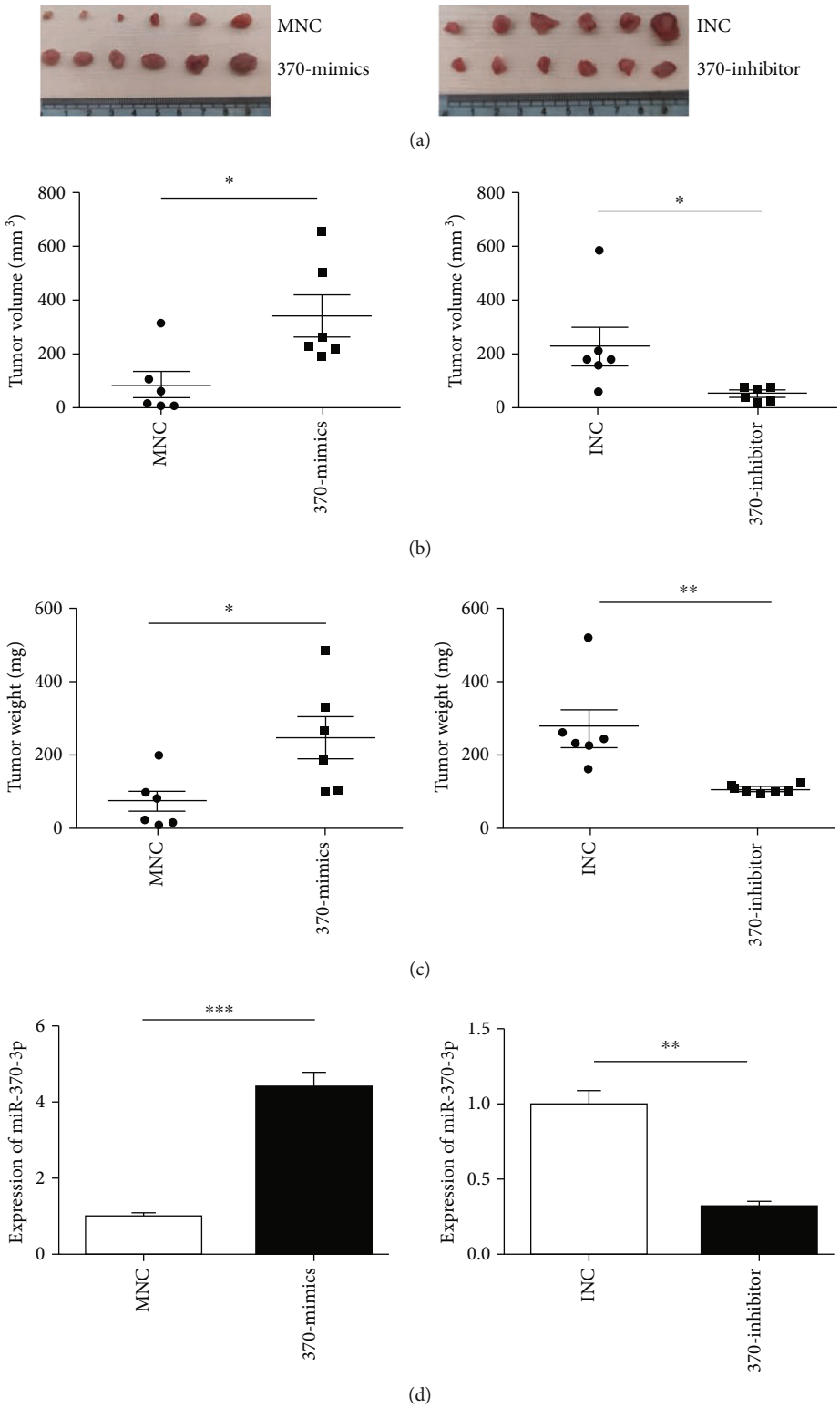


Figure 2: miR-370-3p promotes stemness of breast cancer cells *in vitro*. (a) The expression of OCT4 and SOX2 with MNC, 370-mimics, INC, and 370-inhibitor transfected breast cancer cells were examined by using quantitative RT-PCR. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. (b) The expression of stemness-related proteins in control and miR-370-3p-overexpressing cells or miR-370-3p knockdown cells was examined by using western blot. $^{**}P < 0.01$, $^{*}P < 0.05$.



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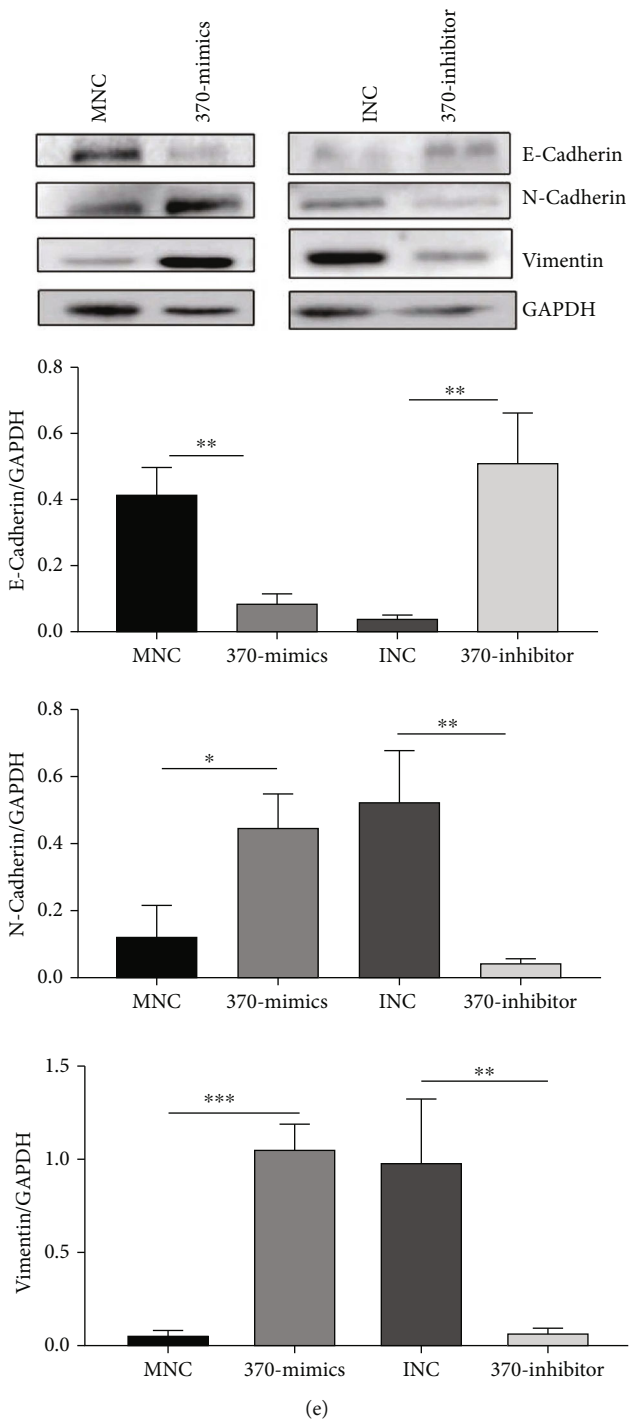
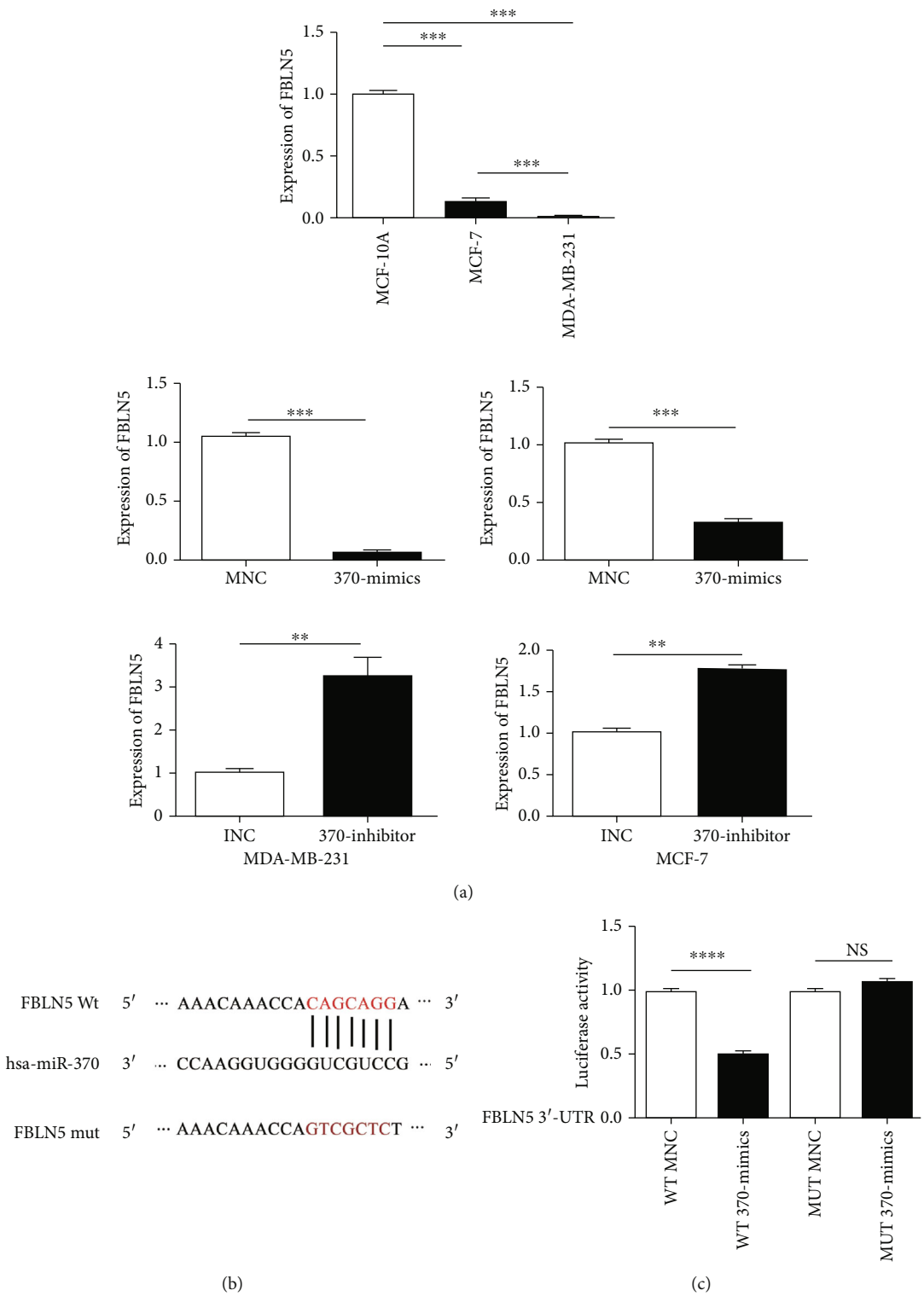


Figure 3: miR-370-3p can promote migration and proliferation of breast cancer cells *in vivo*. (a–c) Observe the appearance, weight, and volume of breast tumor in nude mice subcutaneously formed by MDA-MB-231 cells transfected with MNC, 370-mimics, INC, and 370-inhibitor. $^{**}P < 0.01$, $^{*}P < 0.05$. (d) The expression of miR-370-3p in the breast tumor tissues formed by MDA-MB-231 cells transfected with MNC, 370-mimics, INC, and 370-inhibitors which were injected into subcutaneous of nude mice was detected by quantitative RT-PCR. $^{**}P < 0.01$, $^{***}P < 0.001$. (e) The expression of EMT-related proteins in control and miR-370-3p-overexpressing cells or miR-370-3p knockdown cells were examined by using western blot. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

we found that the expression of FBLN5 was lower in the 370-mimics transfection group and higher in the 370-inhibitor transfection group than in the control group (Figure 4(d)).

It has been reported that FBLN5 can inhibit the NF-κB signaling pathway and participate in fibroblast apoptosis [11]. Our data showed that NF-κB-p65 expression was increased in breast cancer cells overexpressing miR-370-3p while the expression was decreased in cells with knocked-down miR-370-3p (Figure 4(d)). These results suggested that miR-370-3p promoted the proliferation and metastasis of



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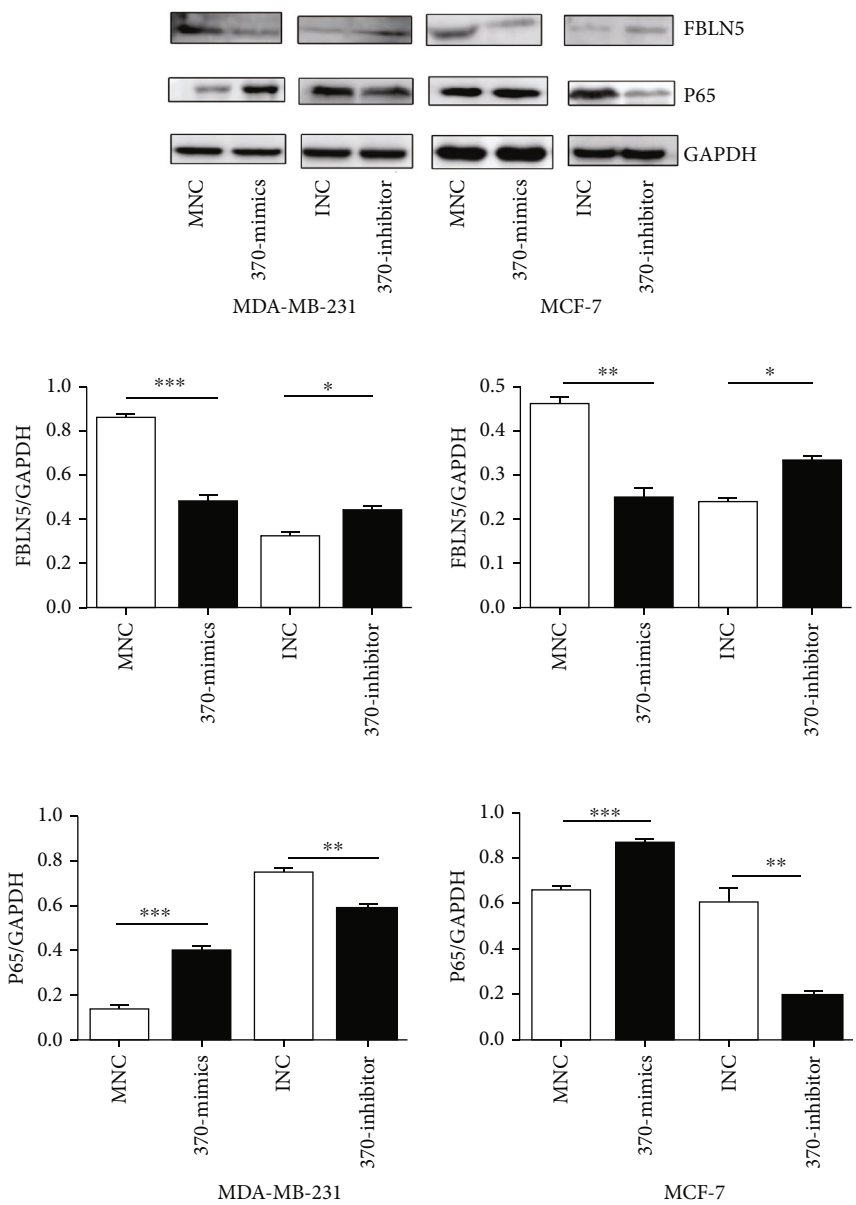

(d)

Figure 4: miR-370-3p targets and inhibits FBLN5 and activates the NF-κB signaling pathway. (a) Quantitative RT-PCR was used to detect the expression of FBLN5 in breast cancer cells and them transfected with MNC, 370-mimics, INC, and 370-inhibitor. $^{***}P < 0.001$, $^{**}P < 0.01$. (b) The binding sites of miR-370-3p seed region and 3′-UTR of FBLN5 mRNA were predicted. (c) The luciferase reporter gene analysis was used to detect the effect of miR-370-3p on the stability of FBLN5 mRNA. $^{***}P < 0.001$. (d) Western blot was used to detect the expression of FBLN5 and p-p65 in breast cancer cells transfected with MNC, 370-mimics, INC, and 370 -inhibitor. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

breast cancer cells through directly targeting FBLN5 and regulating the NF-κB signaling pathway.

3.2.1. Clinical Tests and Analysis of FBLN5 in Breast Tissues. We detected the expression levels of FBLN5 in clinical tissue specimens of patients with breast cancer. The level of miR-370-3p was significantly downregulated in 20 breast cancer tissue samples compared with matched adjacent normal tissues (Figure 5(a)). The diagnostic sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve for FBLLN5 in tissues were 80%, 95%, and 0.8650, respectively (Figure 5(b)). FBLN5 may serve as a novel molecular marker in tissue detection for breast cancer.

Combined with the clinicopathological data, we found that FBLN5 was correlated with clinicopathological parameters in breast cancer tissues (Table 1). The expression levels of FBLN5 in breast cancer patients were negatively related with tumor diameter and TNM stage (Figure 5(c)). Taken together, these findings suggested that FBLN5 inhibited the development of breast cancer.

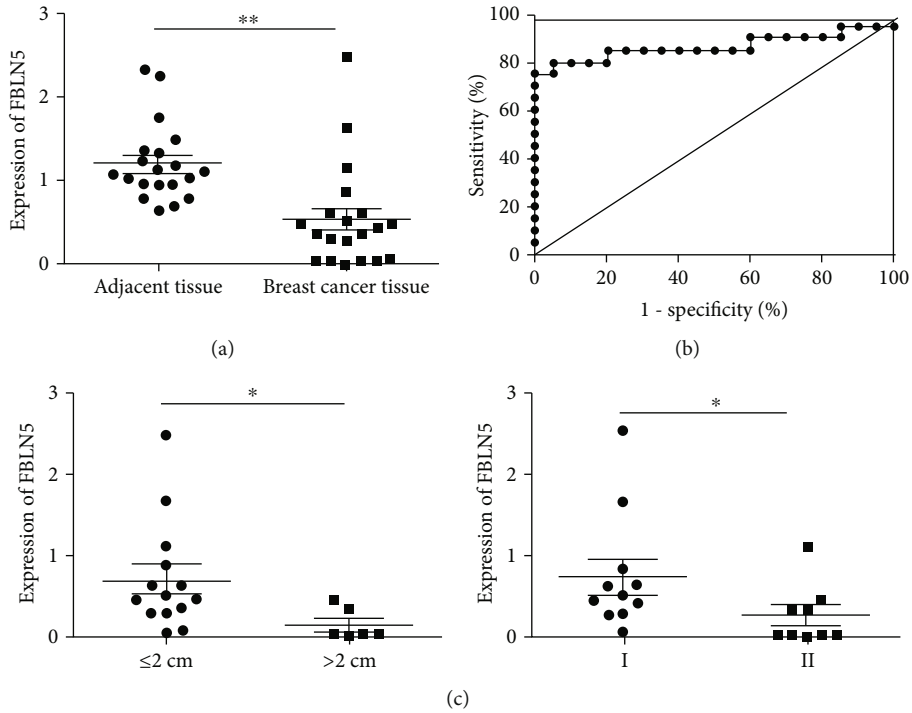

Figure 5: The detection of FBLN5 expression in breast tissues and its clinical correlation analysis. (a) The mRNA levels of FBLN5 in normal tissues adjacent to carcinoma and breast cancer tissues. $^{**}P < 0.01$. (b) The diagnostic values of FBLN5 ROC curves in breast cancer tissues. (c) Correlation between the expression of FBLN5 and clinicopathological parameters (tumor diameter and TNM stage) in breast cancer tissues. $^{*}P < 0.05$.

3.3. miR-370-3p Is Highly Expressed in Breast Tissues, Serum, and Serum Exosomes. To extend current knowledge to clinical patients, we first detected the expression of miR-370-3p in 31 paired breast cancer tissue specimens and adjacent tissue specimens. The results showed that the expression of miR-370-3p in 31 breast cancer tissue specimens exhibited significant increase compared to the paired adjacent tissue specimens (Figure 6(a)). The diagnostic sensitivity, specificity, and area under the ROC curve for miR-370-3p in tissue were 74.19%, 96.77%, and 0.7534, respectively (Figure 6(b)). The expression levels of miR-370-3p were also elevated in clinical serum and exosomes specimens (Figures 6(d) and 6(g)). The expression of miR-370-3p was remarkably upregulated in both samples from breast cancer patients compared with healthy persons. The diagnostic sensitivity, specificity, and area under the ROC curve for miR-370-3p in serum and exosomes were 59.26%, 74.07%, and 0.6735 (Figure 6(e)) and 55.56%, 74.07%, and 0.6797 (Figure 6(h)), respectively. Moreover, our results also showed that diagnostic sensitivity, specificity, and area under the ROC curve of CA153 were 49.18%, 82.54%, and 0.6177 (Figure 6(j)).

Combined with the clinicopathological data, the correlations between the expression of miR-370-3p and clinicopathological parameters in breast cancer tissues, serum, and exosomes were analyzed (Tables 2–4). The expression levels of miR-370-3p in breast cancer patients with lymph node metastasis were higher than those without lymph node metastasis (Figure 6(c)). The expression of miR-370-3p in the lymph node metastasis group and TNM II group was significantly higher than that in breast cancer non-lymph node metastasis and the TNM I group. (Figure 6(f)). The expression of serum exosomal miR-370-3p in tumor diameter > 2 cm, lymph node metastasis, and TNM stage II group were significantly higher than that in breast cancer tumor diameter ≤ 2 cm, non-lymph node metastasis, and TNM stage I group (Figure 6(i)).

4. Discussion

Breast cancer is a complex malignant disease involving tumor formation, proliferation, metastasis, invasion, angiogenesis, recurrence, and other pathological processes [12]. Therefore, it is essential to further investigate the molecular functions and mechanisms in the development of breast cancer.

In this study, we first demonstrated roles of miR-370-3p in breast cancer cells. We found that overexpressed miR-370-3p *in vivo* promoted the proliferation, mobility, and stemness of breast cancer cells, whereas miR-370-3p knockdown significantly offsets these abilities of breast cancer cells, indicating an oncomiRNA in breast cancer. At the same time, we also explored the effects of miR-370-3p on tumor cells of nude mice, which showed that miR-370-3p promoted the proliferation and migration *in vivo*.

We further investigated the molecular mechanism of miR-370-3p in promoting the proliferation, metastasis, and stemness of breast cancer cells. We finally confirmed that FBLN5 is a target gene directly regulated by miR-370-3p. It has been reported that miR-200c is overexpressed in uterine leiomyoma by targeting FBLN5 [13]. Fibulin-5 is expressed

Table 1: Correlation between FBLN5 in breast cancer tissues and clinicopathological parameters.

Clinicopathological information	n	Relative expression of FBLN5	*P*
Tumor diameter			
<=2cm	14	0.481(0.284, 0.915)	0.015
>2cm	6	0.029(0.020, 0.366)	
Age			
<=50	8	0.630(0.273, 1.049)	0.0836
>50	12	0.312(0.029, 0.452)	
Pathological type			
Ductal carcinoma in situ	3	0.338(0.028, 2.489)	0.4472
Invasive ductal carcinoma	14	0.316(0.029, 0.588)	
Others	3	0.624(0.426, 0.636)	
Lymph node metastasis			
0	16	0.439(0.222, 0.627)	0.3694
>=1	4	0.187(0.021, 0.542)	
TNM staging			
I	11	0.503(0.286, 0.844)	0.0227
II	9	0.029(0.028, 0.372)	
ER			
No data	3	0.503(0.053, 1.663)	
-	5	0.028(0.020, 0.643)	0.0651
+	12	0.439 (0.325, 0.627)	
PR			
No data	3	0.503(0.053, 1.663)	
-	5	0.028 (0.020, 0.960)	0.2684
+	12	0.386 (0.284, 0.627)	
HER-2			
No data	4	0.278 (0.047, 0.793)	
+	2	0.435 (0.020, 0.633)	0.6791
++	11	0.451 (0.279, 0.636)	
+++	3	0.338 (0.001, 0.426)	

lowly in colorectal cancer and correlated with clinicopathologic characteristics [14]. Endometrial miR-200c is altered during the transformation into cancerous states and targets FBLN5 [15]. In our study, we found that the expression of FBLN5 in breast cancer cells and breast cancer tissues was lower than that in breast epithelial cells and normal breast tissues, which confirmed that the expression level of FBLN5 was negatively correlated with miR-370-3p. These findings suggested that miR-370-3p promoted the proliferation, migration, and stemness of breast cancer cells, which was related to the underexpressed FBLN5. NF-κB is involved in the inflammatory and immune response and regulates cell apoptosis and stress response. The abnormal expression of NF-κB is closely related to the occurrence and development of cancer [16, 17]. It has been reported that KRAS induced lung adenocarcinoma can activate the NF-κB pathway and promote tumor proliferation [18–20]. NF-κB plays a key role in the proliferation, apoptosis, angiogenesis, and metastasis of colorectal cancer cells [21]. We found that miR-370-3p activated the NF-κB signaling pathway by targeting FBLN5 to promote the proliferation, migration, and stemness of breast cancer cells.

The downregulated expression of FBLN5 in breast cancer suggested that FBLN5 played the role of tumor suppressor gene in breast cancer. FBLN5 is a tumor suppressor that can inhibit the migration and invasion of ovarian cancer cells [22]. Therefore, we studied the expression level of FBLN5 in clinical breast cancer and breast cancer tissues, as well as its correlations with clinicopathological parameters. The results showed that the expression of FBLN5 in breast cancer was lower than that in the adjacent tissues, and the area under the ROC curve was 0.8650, which had better diagnostic efficiency. The sensitivity and specificity of the diagnosis were 80% and 95%, respectively, which indicated that FBLN5 could be a potential candidate of tissue detection for breast cancer diagnosis. In addition, FBLN5 was negatively correlated with tumor diameter and TNM stage of breast cancer, suggesting that low expression of FBLN5 was a predictor of poor prognosis.

The expression level of miRNA is closely related to the morphological characteristics, histopathological parameters, and clinical diagnosis of breast cancer [23]. There are few reports about miR-370-3p in breast cancer. We first measured the expression of miR-370-3p in breast cancer tissues and adjacent tissues. The results showed that the expression of miR-370-3p in breast cancer tissues was significantly higher than that in adjacent tissues, and the ROC curve showed that miR-370-3p had better diagnostic efficiency and higher sensitivity and specificity in breast cancer tissues.

The detection of miR-370-3p in tissues is invasive. Although histopathological detection is the gold standard for breast cancer detection, it is difficult to obtain samples, and the detection cycle is too long to make a timely diagnosis of patients' disease status. miR-370-3p in circulating fluid such as serum and exosomes which come from tissues can well reflect the patient's condition and is easy to obtain and detect.

The results showed that the expression of miR-370-3p in the serum of breast cancer patients was significantly higher than that of healthy people. CA153 is one of the most important specific markers of breast cancer, and we also detected the expression of CA153 in the serum. miR-370-3p in serum had certain diagnostic efficiency that was better than CA153 and higher detection specificity, which can be used as a biological marker for breast cancer diagnosis. Combined with clinicopathological data, we found that the expression level of miR-370-3p was positively correlated with lymph node metastasis and TNM stage.

Exosomes contain many kinds of nucleic acids, especially miRNAs. These miRNAs are selectively sorted into exosomes of different origins [24, 25]. In recent years, many literatures have reported the clinical application of miRNAs in exosomes of breast cancer. Studies have shown that the levels of miR-376a, miR-27a, miR-155, and miR-376c from serum exosomes dynamically reflect the disease status of breast

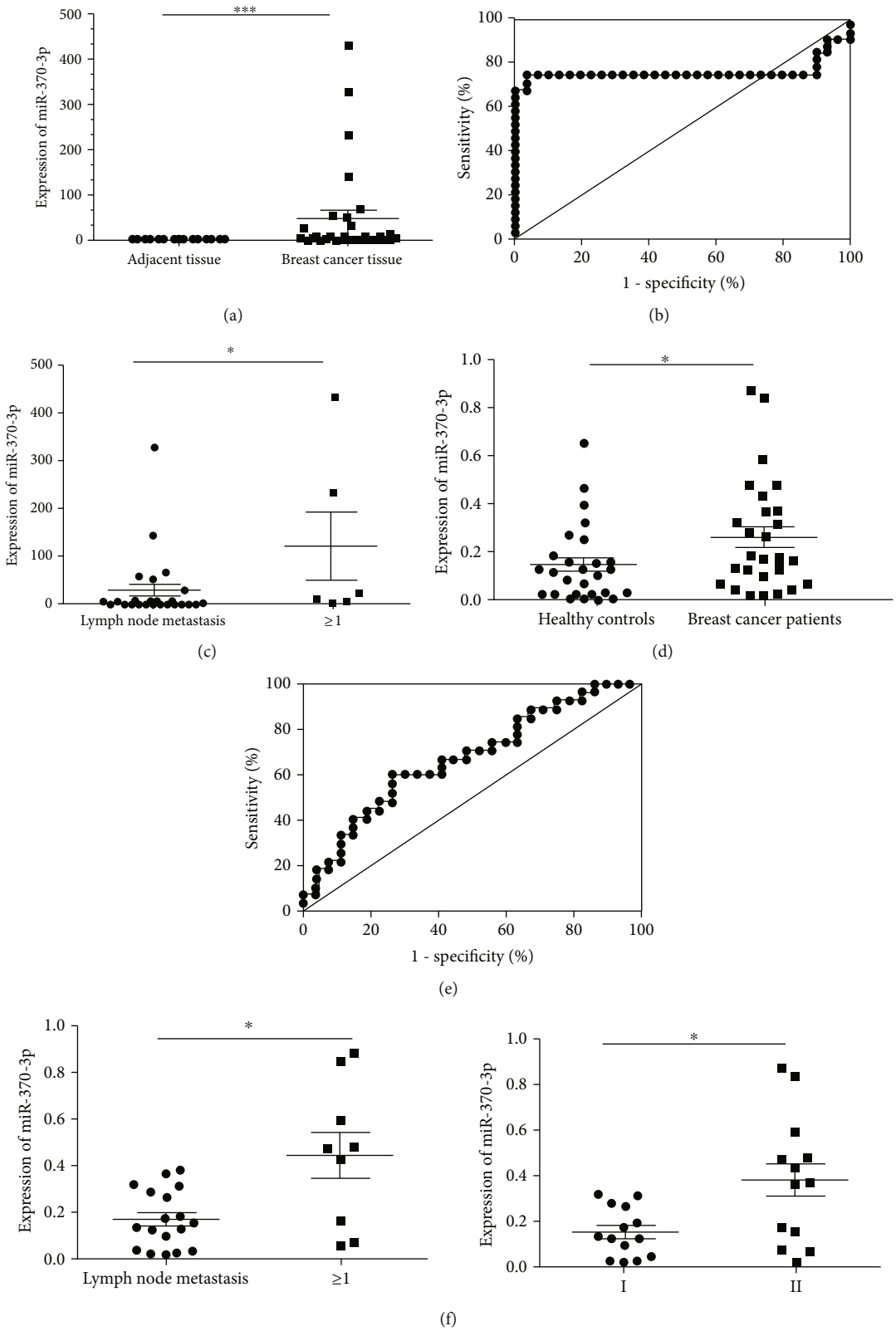

Figure 6: Continued.

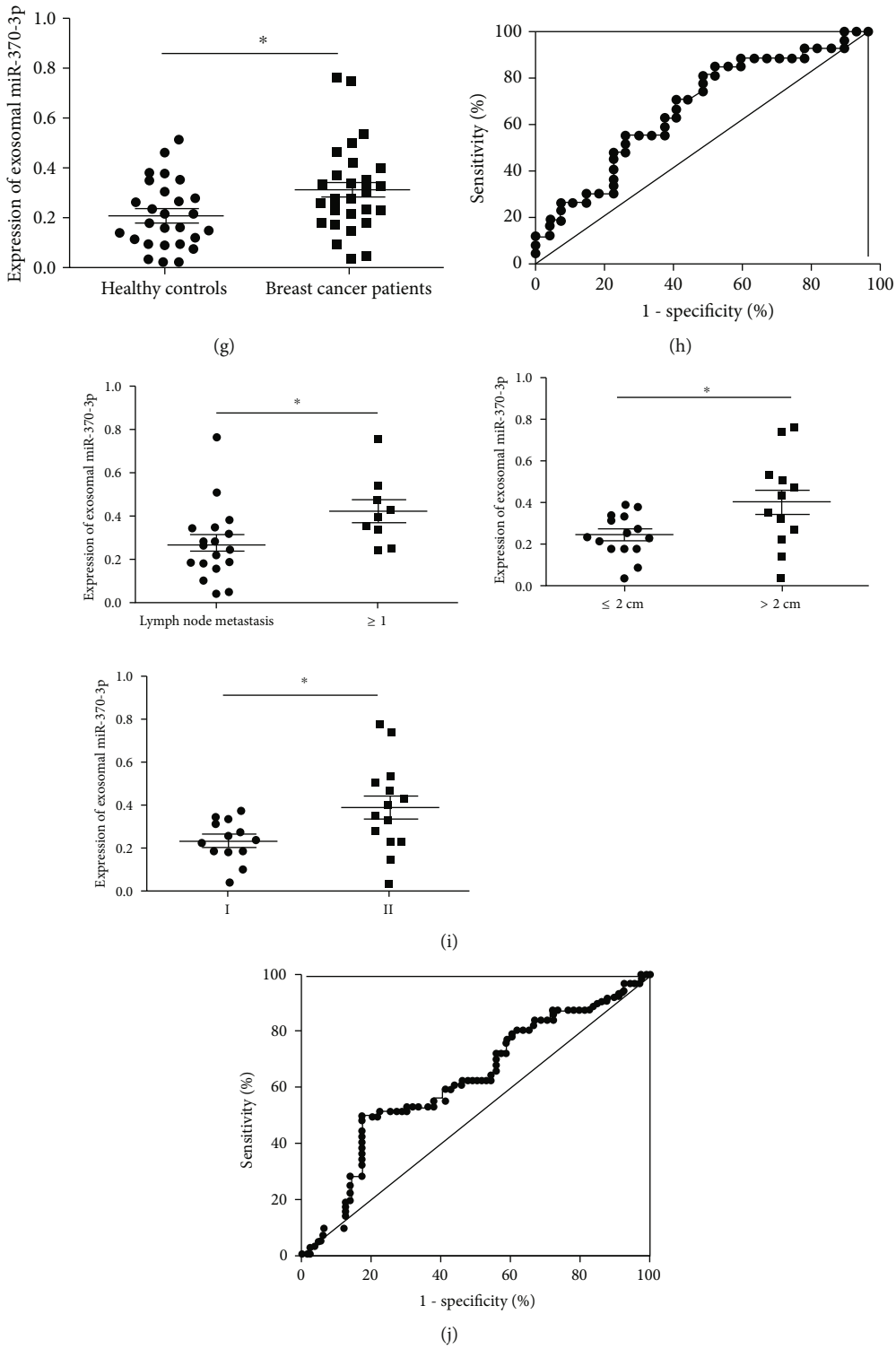

Figure 6: The detection of miR-370-3p expression in breast tissues, serum, and serum exosomes. (a) The levels of miR-370-3p in adjacent normal tissues and breast cancer tissues. $^{***}P < 0.001$. (b) The diagnostic values of miR-370-3p ROC curves in breast cancer tissues. (c) The correlation between the levels of miR-370-3p and clinicopathological parameters (lymph node metastasis) in breast cancer tissues. $^{*}P < 0.05$. (d) The expression of miR-370-3p in the serum of 28 healthy volunteers and that of 28 patients with breast cancer. $^{*}P < 0.05$. (e) The diagnostic values of miR-370-3p ROC curves in breast cancer serum. (f) The correlation between the levels of miR-370-3p and clinicopathological parameters (lymph node metastasis and TNM stage) in breast cancer serum. (g) The levels of miR-370-3p in the exosomes of serum in 28 healthy volunteers and that of 28 patients with breast cancer. $^{*}P < 0.05$. (h) The diagnostic values of miR-370-3p ROC curves in breast cancer serum exosomes. (i) The correlation between the levels of miR-370-3p and clinicopathological parameters (lymph node metastasis, tumor diameter, and TNM stage) in serum exosomes of breast cancer. $^{*}P < 0.05$. (j) The ROC curve of serum CA153.

Table 2: Correlation between miR-370-3p in breast cancer tissues and clinicopathological parameters.

Clinicopathological information	n	Relative expression of miR-370-3p	*P*
Tumor diameter			
<=2cm	18	6.371(1.450, 58.84)	0.1864
>2cm	13	2.389(0.696, 12.60)	
Age			
<=50	8	2.383(1.432, 16.83)	0.4701
>50	23	6.951(1.428, 28.49)	
Pathological type			
Ductal carcinoma in situ	3	1.428(0.068, 28.49)	0.5331
Invasive ductal carcinoma	18	5.507(1.500, 87.45)	
Mucinous adenocarcinoma	2	2.781(1.033, 3.139)	
Others	8	6.549(1.405, 31.13)	
Lymph node metastasis			
0	25	3.506(1.350, 14.18)	0.0483
>=1	6	18.32(6.029, 285.1)	
TNM staging			
I	14	2.781(1.350, 20.94)	0.5515
II	17	6.951(1.249, 33.85)	
ER			
-	10	1.439(0.067, 87.44)	0.1631
+	21	6.951 (2.167, 25.76)	
PR			
-	10	2.478 (0.067, 166.8)	0.6420
+	21	5.792 (1.500, 15.05)	
HER-2			
-	3	0.711 (0.062, 1.500)	0.6175
+	6	0.541 (1.339, 32.54)	
++	15	5.792 (2.389, 11.78)	
+++	6	39.21 (0.968, 87.44)	

cancer patients [26]. In this study, we identified an increased expression of miR-370-3p in serum exosomes of breast cancer than in healthy people, suggesting that exosomal miR-370-3p may be a new marker for diagnosis and prognosis of breast cancer. Serum exosomal miR-370-3p had certain diagnostic efficacy and higher detection specificity, which can be acted as a biological index for the diagnosis of breast cancer. The diagnostic efficiency of serum miR-370-3p and serum exosomal miR-370-3p was better than CA153, which means serum and exosomal miR-370-3p may be a potential biomarker. Combined with clinicopathological data, the results indicated that the expression of miR-370-3p in exosomes was positively correlated with tumor diameter, lymph node metastasis, and TNM stage. Taken together, miR-370-3p was upregulated in different kinds of specimens and promoted breast cancer progression vigorously.

Table 3: Correlation between miR-370-3p in breast cancer serum and clinicopathological parameters.

Clinicopathological information	n	Relative expression of miR-370-3p	*P*
Tumor diameter			
<=2cm	16	0.136(0.058, 0.268)	0.0513
>2cm	11	0.377(0.161, 0.481)	
Age			
<=50	7	0.130(0.054, 0.197)	0.1530
>50	20	0.264(0.093, 0.444)	
Pathological type			
Ductal carcinoma in situ	2	0.079(0.027, 0.102)	0.6287
Invasive ductal carcinoma	9	0.281(0.138, 0.348)	
Invasive cancer	13	0.168(0.091, 0.327)	
Others	3	0.364(0.264, 0.481)	
Lymph node metastasis			
0	18	0.149(0.040, 0.281)	0.0168
>=1	9	0.480(0.143, 0.651)	
TNM staging			
I	14	0.136(0.040, 0.268)	0.0308
II	13	0.377(0.138, 0.508)	

Table 4: The relationship between miR-370-3p in exosomes of breast cancer serum and clinicopathological parameters.

Clinicopathological information	n	Relative expression of miR-370-3p	*P*
Tumor diameter			
<=2cm	15	0.239(0.184, 0.337)	0.0429
>2cm	12	0.389(0.268, 0.514)	
Age			
<=50	7	0.279(0.182, 0.344)	0.3911
>50	20	0.324(0.211, 0.437)	
Pathological type			
Ductal carcinoma in situ	2	0.553(0.258, 0.572)	0.1583
Invasive ductal carcinoma	8	0.379(0.210, 0.478)	
Invasive cancer	14	0.248(0.162, 0.321)	
Others	3	0.351(0.153, 0.374)	
Lymph node metastasis			
0	18	0.247(0.174, 0.339)	0.0193
>=1	9	0.401(0.308, 0.485)	
TNM staging			
I	13	0.237(0.184, 0.322)	0.0273
II	14	0.376(0.238, 0.513)	

5. Conclusions

Collectively, our data firstly demonstrated that miR-370-3p acted as an oncogene in the development of breast cancer by inhibiting FBLN5 expression and activating the NF-κB signaling pathway. This study will offer a new insight into roles of miR-370-3p in breast cancer progression and provide a novel biomarker for the diagnosis and therapy of breast cancer.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

All authors declare no conflict of interest.

Authors' Contributions

JHM and LXW performed and analyzed the experiments and wrote the manuscript. JYW, YCW, HYW, XMZ, and HLF helped to perform the experiments. HY and BW designed and supervised the study. All authors have read and approved the final manuscript. Jiahui Mao and Lingxia Wang contributed equally to this work.

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Supplementary Materials

Table S1: sequences of oligonucleotide fragment and modification. Table S2: primer sequences and amplified fragment products. *(Supplementary Materials)*

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Research Article

Vimentin-Rab7a Pathway Mediates the Migration of MSCs and Lead to Therapeutic Effects on ARDS

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Acute respiratory distress syndrome (ARDS) is difficult to treat and has a high mortality rate. Mesenchymal stem cells (MSCs) have an important therapeutic effect in ARDS. While the mechanism of MSC migration to the lungs remains unclear, the role of MSCs is of great clinical significance. To this end, we constructed vimentin knockout mice, extracted bone MSCs from the mice, and used them for the treatment of LPS-induced ARDS. H&E staining and Masson staining of mouse lung tissue allowed us to assess the degree of damage and fibrosis of mouse lung tissue. By measuring serum TNF-α, TGF-β, and INF-γ, we were able to monitor the release of inflammatory factors. Finally, through immunoprecipitation and gene knockout experiments, we identified upstream molecules that regulate vimentin and elucidated the mechanism that mediates MSC migration. As a result, we found that MSCs from wild-type mice can significantly alleviate ARDS and reduce lung inflammation, while vimentin gene knockout reduced the therapeutic effect of MSCs in ARDS. Cytological experiments showed that vimentin gene knockout can significantly inhibit the migration of MSCs and showed that it changes the proliferation and differentiation status of MSCs. Further experiments found that vimentin's regulation of MSC migration is mainly mediated by Rab7a. Rab7a knockout blocked the migration of MSCs and weakened the therapeutic effect of MSCs in ARDS. In conclusion, we have shown that the Vimentin-Rab7a pathway mediates migration of MSCs and leads to therapeutic effects in ARDS.

1. Introduction

Acute respiratory distress syndrome (ARDS) refers to diffuse lung injury caused by severe infection, trauma, shock, or surgery. The clinical manifestations are acute episodes of dyspnea and refractory hypoxia. The current incidence of hyperemia and acute respiratory failure in adults is about 17.6-64 per 100,000 annually [1]. The disease is characterized by rapid onset and progress and is relatively difficult to treat. Treatment consists mainly of protective lung ventilation strategies and early fluid resuscitation. Despite the continuous improvement in treatment methods, the mortality rate remains as high as 40%-60% [2]. The lack of specific etiological treatment measures is an important factor in the high mortality rate of ARDS. The pathogenesis of ARDS is complicated. Its main pathophysiological changes are damage to alveolar capillary endothelial cells and alveolar epithelial cells, which leads to increased alveolar membrane permeability, causing a large amount of protein-rich fluid to leak out of the lung interstitium and alveoli. Effectively repairing these damaged cells is the key to the early treatment of ARDS.

Mesenchymal stem cells (MSCs) are pluripotent stem cells isolated from various tissues, such as bone marrow, fat, placenta, and umbilical cord blood. They can be induced to differentiate, in vitro, into bone cells, chondrocytes, and chondrocytes. A variety of tissue cells such as muscle cells, fat cells, and fibroblasts. When tissues, such as muscle, fat, and fibroblasts, are damaged, MSCs quickly migrate to the damaged site, induce regeneration into normal cells at the site, and participate in tissue repair. MSCs can also secrete a variety of cytokines to exert anti-inflammatory and repair effects. In recent years, a large number of studies have shown

that MSCs can effectively treat a variety of diseases, including myocardial injury and diabetes [1, 2]. In ARDS, MSCs exert a significant therapeutic effect, both in animals and humans, showing its unique and excellent anti-inflammatory and antidamage properties [3, 4]. However, the mechanism of MSC treatment in ARDS is not clear, particularly the molecular mechanism of MSC migration to the lung injury site.

Vimentin is a cytoskeletal protein that belongs to the type III intermediate filament family. The relative molecular weight of vimentin protein is 57 kD, and the phosphorylation site in the domain is the key to powering vimentin and conducting molecular signals [5]. Vimentin plays an important role in cell migration, contraction, proliferation, protein synthesis, gene expression, cell apoptosis, and mechanical force transmission, among which participation in cell migration is an important prerequisite for tissue damage repair and inflammation control [6, 7]. Some studies have confirmed that vimentin is regulated mainly by Rab7a, a small molecule GTP binding protein. Under the combined action of the Vimentin-Rab7a pathway, cells can migrate, which mediates a variety of biological effects [8, 9]. Therefore, we speculated that Vimentin-Rab7a plays an important role in mediating the migration of MSCs to the damaged lung tissue and the treatment of ARDS, and this aspect of work has not yet been reported.

In this study, we found for the first time that vimentin mediated the migration and colonization of MSCs in damaged lung tissues, thereby playing a role in the treatment of ARDS, and elucidated the mechanism showing that vimentin is regulated by Rab7a to effect the migration of MSCs.

2. Materials and Methods

2.1. Animals, Drugs, Reagents, and Instruments. C57BL/6 (male, 6-8 weeks old) mice weighing 22-25 g were used in this study. Animal breeding conditions meet the requirements of the Experimental Animal Welfare Ethics Committee. The mice had free access to food and water. Mice were housed in an adaptive feeding environment with alternating light and dark for 12 hours, starting after 1 week experiment, at 23.0-25.0°C and 55% ~ 65% relative humidity. Vimentin, Rab7a, and GAPDH (Cat. Nos.: ab92547, ab255423, and ab8245) were all purchased from Abcam (Cambridge, MA, USA).

2.2. Establishment of LPS-Induced ARDS Model. The animal experiment protocol of this study complies with the requirements of the US Guidelines for the Management and Use of Laboratory Animals. The establishment of the mouse LPS-ARDS model refers to the method previously reported in the literature [10]. After the mice were anesthetized by intraperitoneal injection of 50 mg/mL pentobarbital, 50 μL of LPS with a concentration of 2 mg/mL was injected into the airway under the direct vision of a microinjector to establish an LPS-induced ARDS model, and the objects were inhaled with pure oxygen through a mask until fully awakened.

2.3. Experiment Grouping and Processing. After 1 week of adaptive feeding, the C57BL/6 mice were randomly divided into 3 groups, 10 in the normal control group (given the same dose of normal saline intravenously), 10 in the LPS-induced ARDS group, and 10 in the ARDS+MSC treatment group. The tail vein of each experimental animal was injected with 1×10^6 MSCs. At 1, 2, 4, and 16 weeks after LPS-induced ARDS, mouse lung tissues were collected for H&E staining, Masson staining, and α-SMA immunohistochemical staining. Concentrations of TNF-α, TGF-β, and INF-γ were measured in blood collected from the endocanthal vein.

2.4. H&E Staining. Mouse lung tissue was taken from the mice, fixed dehydrated, and embedded in paraffin. The samples were sliced into 5 μm sections. 4 slices from each group were dewaxed with xylene and rehydrated using an ethanol gradient. The nuclei were stained with hematoxylin, and eosin was used to stain the cytoplasm. Following routine dehydration and washing, the samples were sealed with a neutral gum sheet. The specimens were numbered, and the pathological changes of lung tissue structure were observed through an optical microscope. Three specimens were selected from each group, and eight fields were randomly selected under an optical microscope at 400x magnification. The degree of lung tissue damage was quantitatively analysed according to the Ashcroft score [11].

2.5. Masson Staining. Masson trichrome staining was performed using the Trichrome Stain (Masson) Kit (Sigma-Aldrich, St. Louis, MO, USA). Ashcroft scores were blindly assigned based on a modified system of grades using slides stained with Masson trichrome [12].

2.6. MSC Isolation and Culture. After C57BL/6 mice were sacrificed and disinfected, they were placed on a clean bench to separate mouse femurs. Bone marrow was washed thoroughly using the medium and placed in a petri dish. According to the experimental design, the washed cells were cultured in an incubator at 37°C incubator with 5% CO_2. Cell growth was observed, and cells were photographed daily. The cells were passaged when they reached 70-80% confluency.

2.7. Detection of MSC Surface Markers by Flow Cytometry. MSCs were dissociated with trypsin and centrifuged to concentrate. The cells were resuspended in PBS to a concentration of approximately 1×10^6 cells/mL. 100 μL of cells were transferred to another EP tube, and the appropriate amount of antibody was added (2 μL each for CD105, CD90, CD31, and CD45). The cells were incubated with antibody for 30 min in the dark at RT. 500 μL of Stain Buffer was added to each tube; the sample cell count was acquired by flow cytometry.

2.8. GFP Labeled MSCs. 2×10^5/mL MSCs were plated in a petri dish to prepare for transfection. When the cells reached 70-80% confluency, they were transfected using polybrene and 6 μg/mL GFP viral vector. After 18-20 hours, the culture medium was changed. After 24 hours, the cells were photographed under a fluorescence microscope (excitation at 488 nm), and the number of fluorescent cells and fluorescence intensity was measured.

2.9. Enzyme-Linked Immunosorbent Assay (ELISA Experiment). The double antibody sandwich ABC-ELISA method was used to detect the corresponding cytokines in mouse serum. Coated on the ELISA plate, the standard and corresponding target molecules in the samples which were to be tested were combined with the corresponding monoclonal antibody in the ELISA plate, which was coated with anti-mouse TNF-α, TGF-β, INF-γ, and other cytokine monoclonal antibodies. The sample was subsequently incubated with the corresponding secondary antibody. The primary antibody and the secondary antibody combined to form an immune complex and adhere to the plate. HRP-labeled streptavidin bounds to biotin. The substrate working solution was added to produce a blue color, and the reaction was stopped with the addition of sulfuric acid. The absorbance (450 nm) was then measured in each well. The OD values were proportional to the concentration of the molecules of interest, and the corresponding cytokine concentration in the sample was calculated against a standard curve.

2.10. Western Blotting. Cells or tissues were lysed with RIPA (Beyotime Biotechnology, China) and subjected to protein concentration determination using BCA assay. The obtained protein samples were loaded at 40 mg/well for SDS-PAGE. Proteins were electrotransferred onto a PVDF membrane at a constant 70 V. The PVDF membrane was placed into blocking solution containing 5% skim milk for 2 hours at room temperature and incubated with primary antibody solution overnight at 4°C. The membrane was washed and incubated with secondary antibody for 2 hours. Protein bands were visualized via ECL.

2.11. Statistics. All experiments were repeated three times to ensure the reliability of the results. All data were presented as mean ± SD. SPSS version 19.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. The independent sample t-test was used for comparison between the two groups; $P < 0.05$ indicated significant differences.

3. Results

3.1. Construction of LPS-Induced ARDS Model in Mice. We constructed an LPS-induced acute lung injury model in mice according to the method of Rittirsch et al. [10]. After induction of LPS (2 mg/mL) tracheal instillation, H&E and Masson staining were performed at 1 W, 2 W, 4 W, and 16 W to assess pneumonia and pulmonary fibrosis in mice. The results showed that LPS can induce lung tissue damage and small amounts of inflammation. The manifestations included lung tissue congestion, edema, alveolar exudation, increased red blood cells, and increased inflammatory cell infiltration. By the 4th and 16th weeks, the alveolar separation was obvious thickening, which indicated an increase in the degree of non-fibrosis (Figures 1(a) and 1(c)). Additionally, Masson staining (blue indicated increased collagen) showed that the lung septum of mice gradually thickened after LPS induction, and the blue area also gradually increased. The blue area increased significantly, especially at the 16th week, which indicated that the degree of fibrosis was increased (Figures 1(b) and 1(d)). The above results indicated that the LPS-induced acute lung injury model in mice was successfully constructed.

3.2. MSCs Effectively Treated ARDS in Mice. Based on the establishment of the LPS-induced acute lung injury model in mice, we further studied the treatment of ARDS with MSCs. Mice were infused with MSCs for 1 hour, and lung tissues were taken at 1, 4, and 16 weeks to observe the damage degree. The results show that MSCs can effectively reduce LPS-induced lung injury, lung congestion, and edema and relieve the exudation of inflammatory cells (Figures 2(a) and 2(b)). Masson staining and α-SMA staining also showed that the treatment of MSCs can reduce tissue damage. The positive area of Masson (Figures 2(c) and 2(d)) and α-SMA (Figures 2(e) and 2(f)) indicated that MSCs effectively alleviated pulmonary fibrosis.

3.3. Vimentin Knockout Inhibited the Proliferation of MSCs and Changed the Differentiation State of MSCs. In order to confirm the key role of vimentin in mediating MSC treatment of ARDS, we constructed a vimentin knockout mouse model. We tested the difference in proliferation and differentiation between MSCs and wild-type mouse MSCs of vimentin knockout mice. The results showed that under the conditions of in vitro culture, the growth of vimentin knockout mouse MSCs was significantly weaker than normal MSCs from day 5, indicating that vimentin knockout had a certain effect on the proliferation of MSCs (Figures 3(a) and 3(b)). MSCs were labelled with CD105, CD90, CD31, and CD45, and it was found that MSCs of wild-type and vimentin gene knockout mice were positive for CD105 and CD90, while CD31 and CD45 were negative, indicating that the purity of the obtained MSCs was relatively high. However, the positive rates of CD105 and CD90 in MSCs of vimentin gene knockout mice were significantly lower than those of wild-type MSCs, suggesting that vimentin gene knockout had a certain effect on the differentiation of mouse MSCs.

3.4. Vimentin Knockout Attenuated the Therapeutic Effect of MSCs on ARDS in Mice. We compared the therapeutic effects of wild-type mouse MSCs and vimentin knock-out mouse MSCs on LPS-induced ARDS. The results showed that, compared with wild-type MSCs, the MSCs of vimentin knockout mice had a significantly weaker therapeutic effect, showing increased edema and inflammatory cell exudation (Figures 2(a) and 2(b)) and significantly increased the degree of fibrosis (Figures 2(c)–2(f)). Mouse alveolar lavage fluid was collected on day 2 after LPS induction, and flow cytometry was used to detect the content of F4/80 positive macrophages. On day 2 after LPS induction, alveolar CD11b and F4/80 were found to be increased, while the MSC treatment group decreased, indicating that MSCs can reduce lung inflammatory cell infiltration caused by LPS, but the knockout of vimentin offsets the protective effect of MSCs (Figure 2(g)). TNF-α and TGF-β are important indicators of LPS-induced pneumonia, and their increase can reflect the state of pneumonia, especially as TGF-β is considered to be important for fibrosis. Experimental results showed that

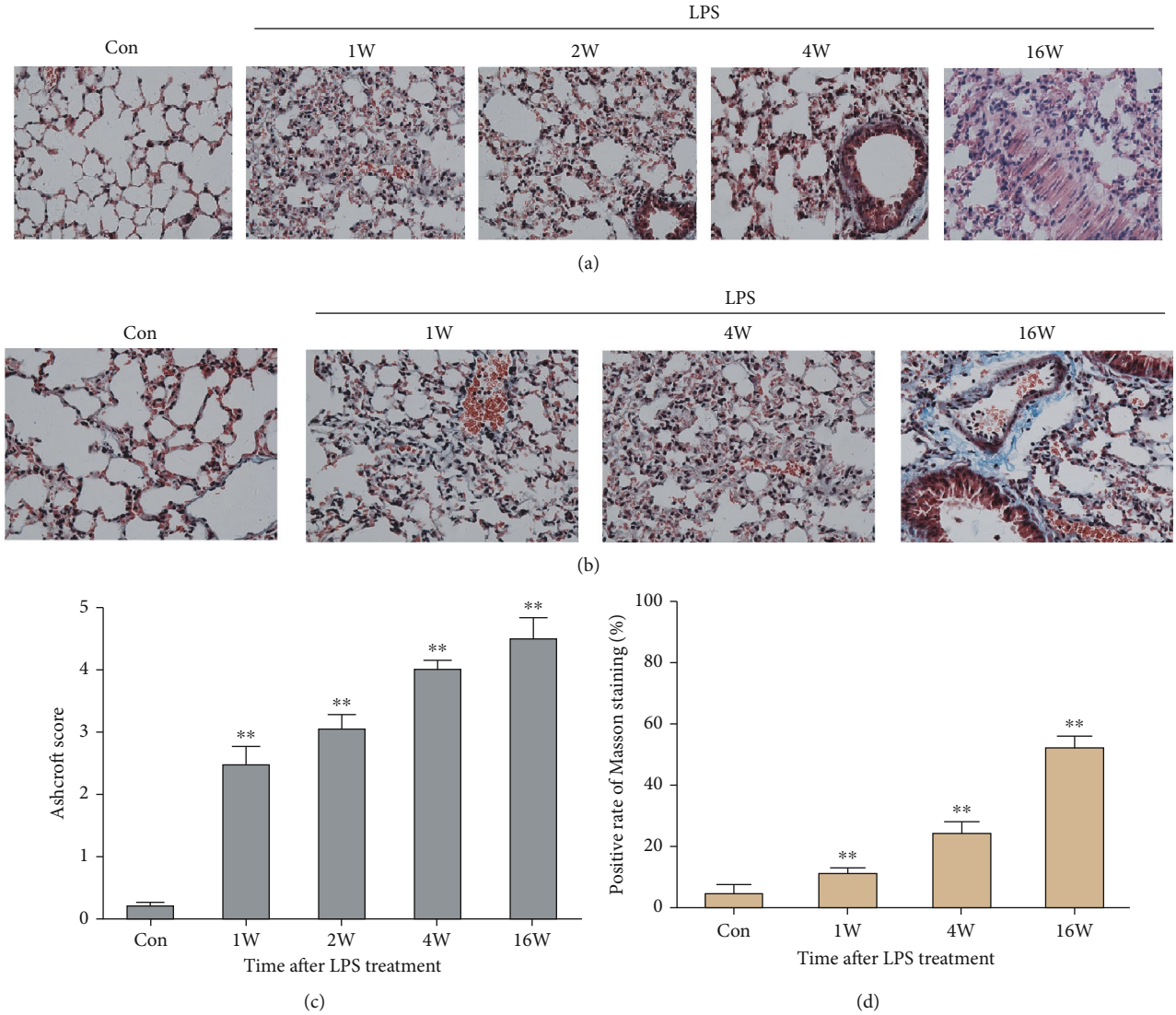
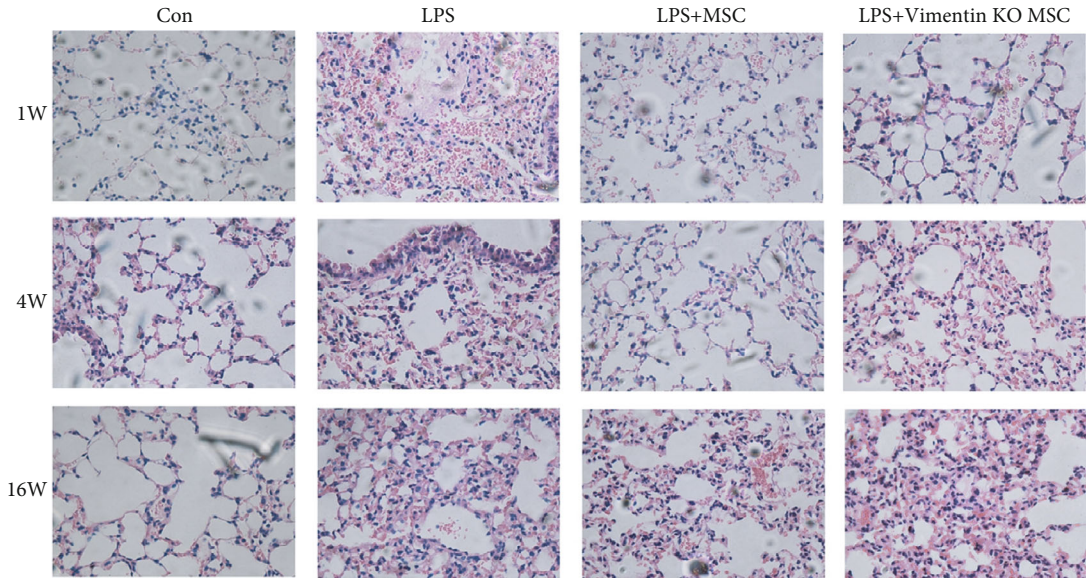


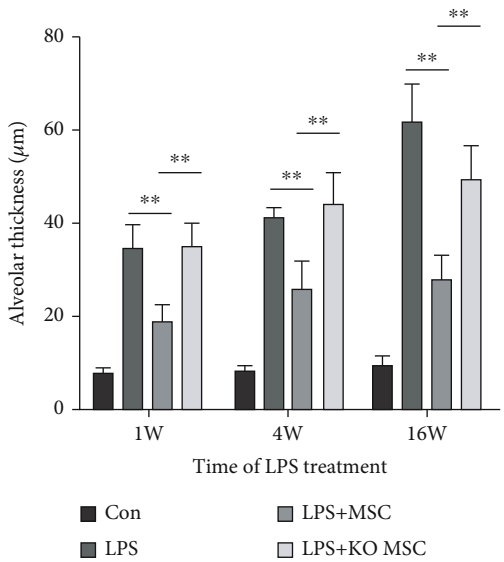
Figure 1: Construction of LPS-induced ARDS model in mice. 50 μL of LPS at a concentration of 2 mg/mL was injected into the airway of mice. Left lung tissues of mice were taken at 1 W, 2 W, 4 W, and 16 W for H&E staining (a) and quantified using the Ashcroft score (c). Masson staining was used to assess the degree of fibrosis in the left lung tissue at 1 W, 4 W, and 16 W after LPS-induced ARDS (b) and quantified by calculating the blue stained area (d). Statistical results are represented by mean ± SD. $^{**}P < 0.01$, $n = 10$.

TNF-α increased with time after LPS induction, reached a peak at 1 W, and then dropped. Meanwhile, TGF-β began to increase after 1 W, and the increase was more obvious as time progressed. As a protective factor for pneumonia, especially fibrosis, INF-γ also increased in the late stage of LPS induction. The above results are consistent with the typical manifestations of LPS-induced pneumonia. On this basis, the use of different MSC treatments found that wild-type mouse MSCs can block the increase of TNF-α and TGF-β and increase the content of INF-γ in the later stage, but the effect of vimentin knockout mouse MSCs is reduced (Figures 2(h)–2(j)). After two mouse MSCs were labelled with GFP, they were injected into the tail vein of LPS-induced ARDS mice, and lung tissues were taken at different times to observe the fluorescent (green) signal. Wild-type mouse MSCs were detected in the lungs at 24 h, peaked at 48 h, and decreased after 96 h. Meanwhile, the MSCs of vimentin knockout mice were less colonized, indicating that vimentin plays an important role in MSC homing (Figure 2(k)).

3.5. Rab7a Affects the Migration of MSCs by Acting on Vimentin. As the upstream regulator of vimentin [8, 9], Rab7a has been proven to play an important role in cell migration. We explored whether the Vimentin-Rab7a pathway affects the migration of MSCs. First, we verified the combination of Rab7a and vimentin using immunoprecipitation and found that Rab7a was detected in the precipitated vimentin, and its expression was upregulated under the induction of LPS. It shows that vimentin bound to Rab7a, and LPS upregulated the expression of Rab7a (Figure 4(a)). Furthermore, we constructed Rab7a knockout MSCs (Figure 4(b)) and used the scratch test to determine the effect of Rab7a on the migration ability of MSCs. The results showed that compared with the NC group, the Rab7a knockout group



(a)



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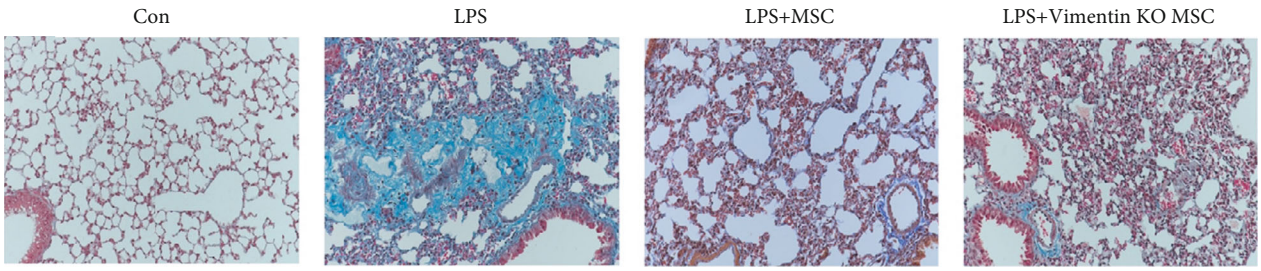

(c)

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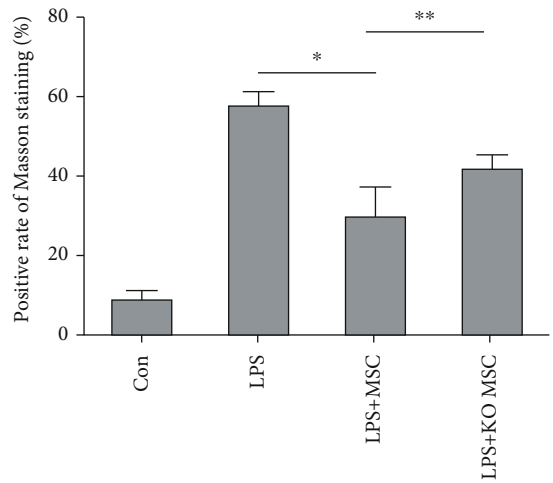
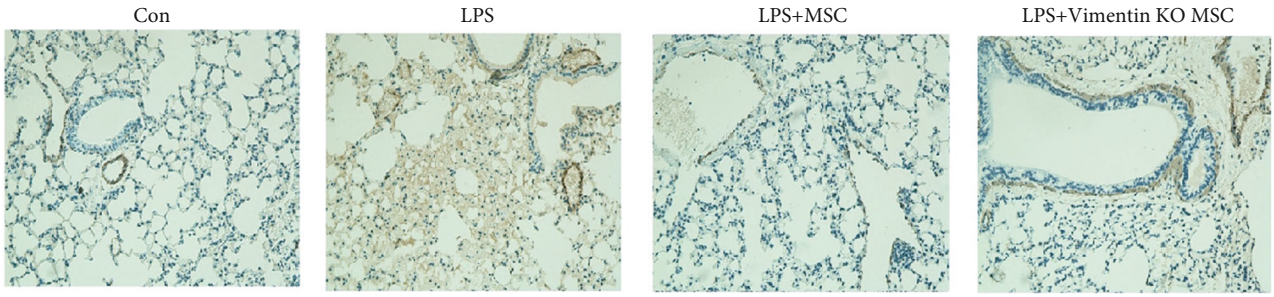


(d)



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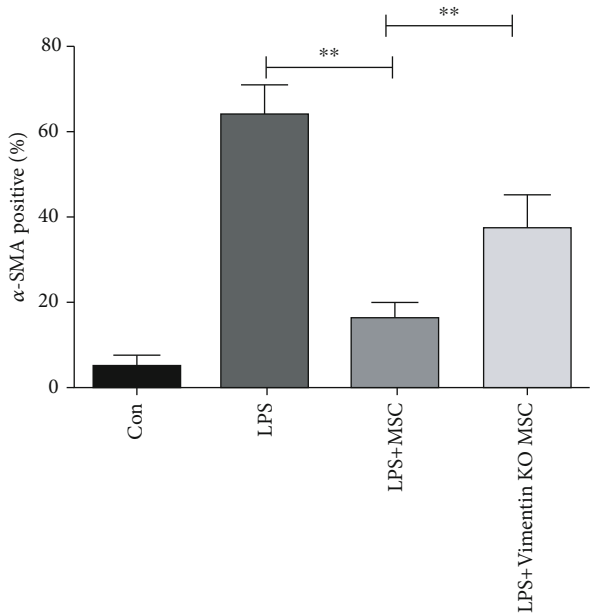

(f)

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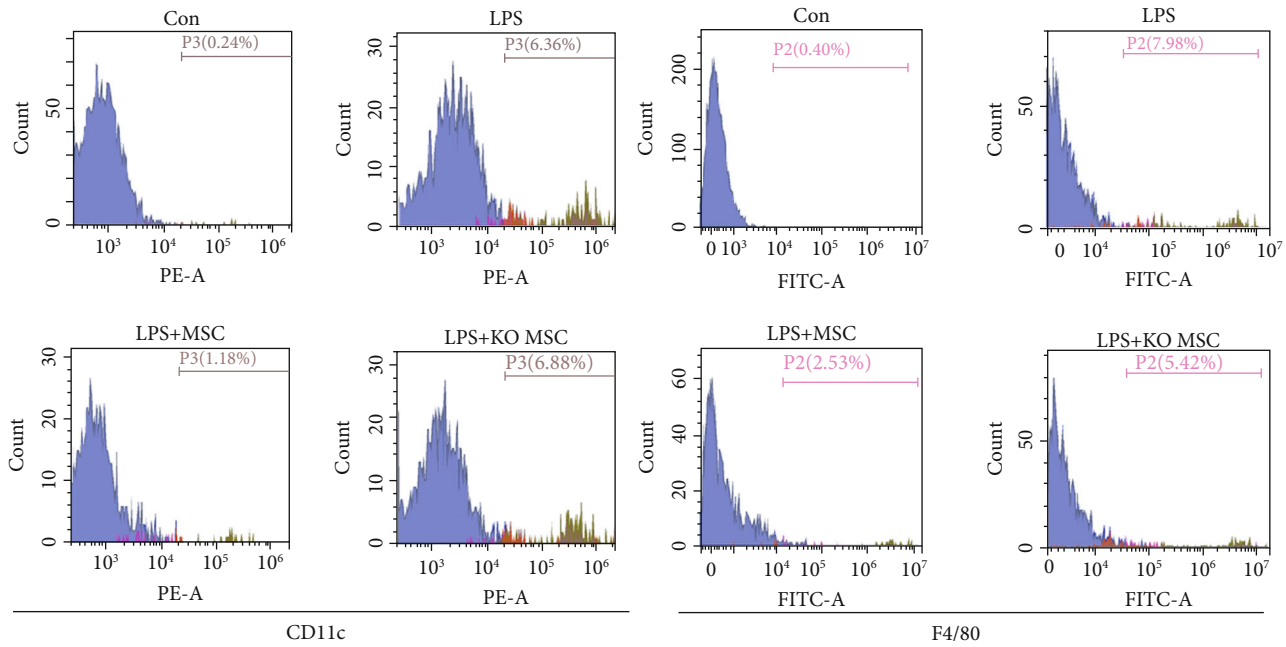
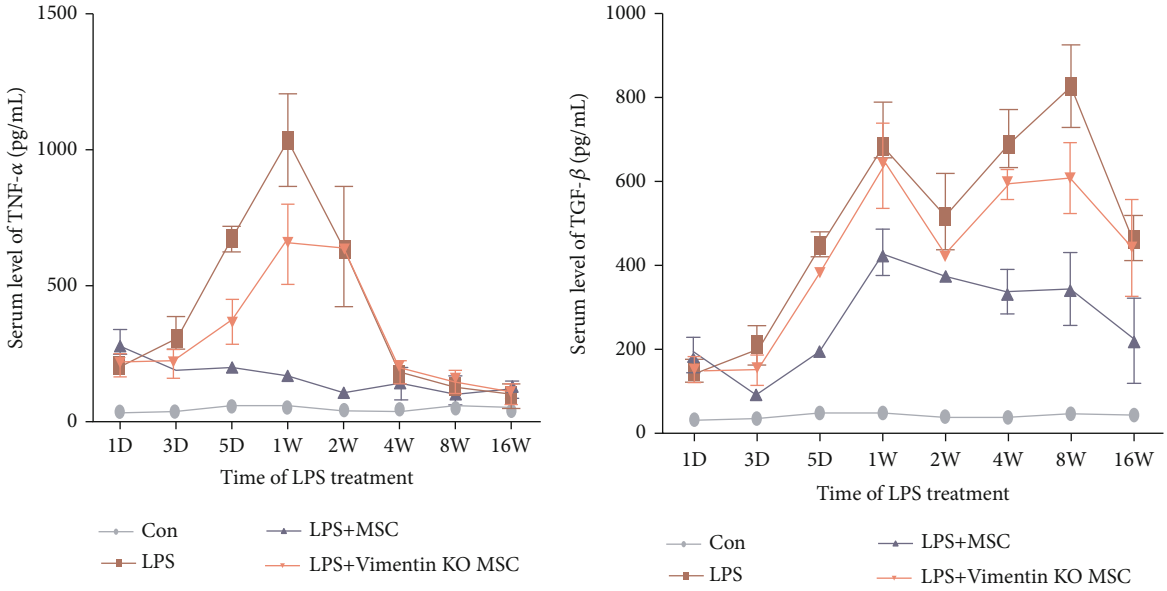


(g)



(h) (i)

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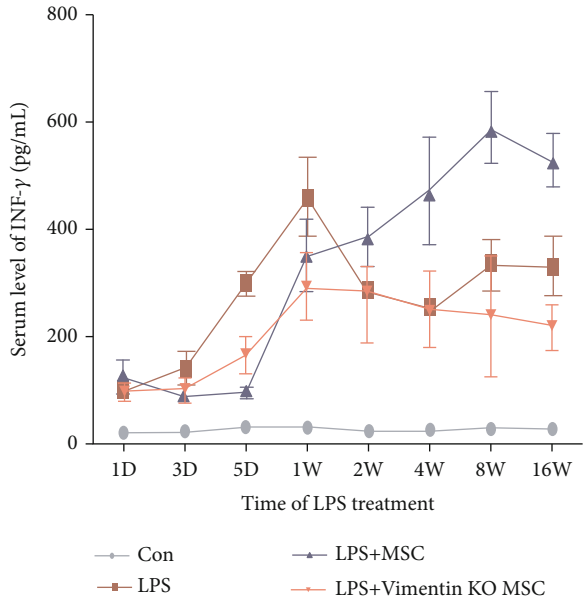
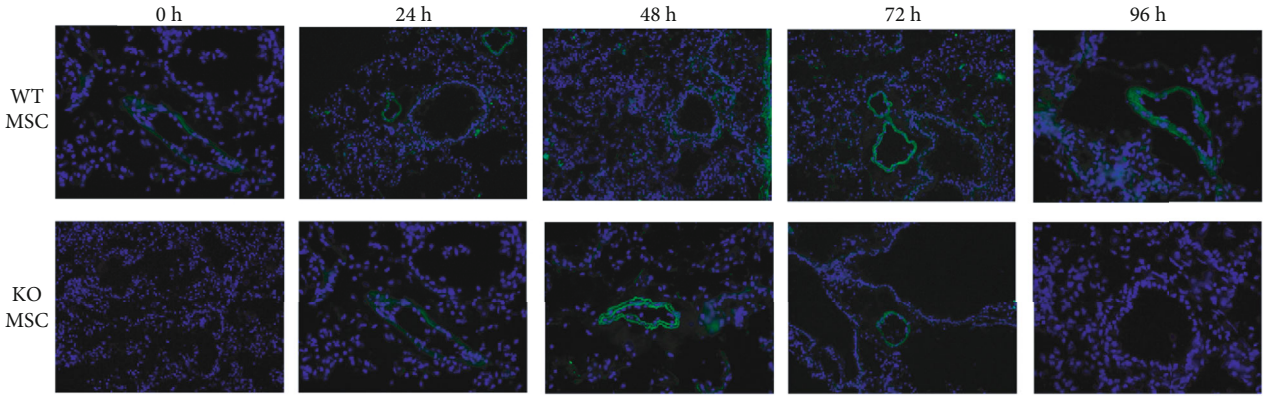


(j)



(k)

Figure 2: Vimentin knockout alleviated the therapeutic effect of MSCs on ARDS. Two hours after inducing ARDS in mice, MSCs from wild-type mice and vimentin knockout mice were injected, and the mice were housed for 1 W, 4 W, and 16 W. Lung tissue was stained by H&E (a) and quantified by calculating alveolar thickness (b). Masson staining (c, d) and α-SMA immunohistochemical staining (e, f) were performed on the 16 W lung tissue to assess the degree of pulmonary fibrosis. On the second day after LPS induction of ARDS, the alveolar lavage fluid of each group of mice was collected, labelled with CD11c and F4/80, and then counted via flow cytometry (g) to clarify the differentiation of MSCs. In order to observe the effect of MSCs on the release of ARDS inflammatory factors, the peripheral serum of mice was collected at 1 D, 3 D, 5 D, 1 W, 2 W, 4 W, 8 W, and 16 W after LPS-induced ARDS, and TNF-α (h), TGF-β (i), and INF-γ (j) contents were tested. Two hours after the ARDS model was constructed, MSCs from GFP-labeled wild-type mice and vimentin knockout mice were injected through the tail vein of the mouse. At 24 h, 48 h, 72 h, and 96 h, the left lung tissue of the mouse was obtained and observed under a British crown microscope. GFP-labelled cells (k). Statistical results are represented by mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$.

significantly inhibited scratch healing (Figure 4(c)), indicating that Rab7a played an important role in mediating MSC migration.

3.6. Rab7a Knockout Weakens the Therapeutic Effect of MSCs on ARDS. We tested the effect of Rab7a knockout MSCs on ARDS. The Rab7a knockout MSCs were injected into mice with LPS-induced pneumonia, and the lung tissues of the mice were taken at 1 W, 4 W, and 8 W. H&E pathological examination showed that the knockout of Rab7a significantly weakened the therapeutic effect of MSCs in mice with LPS-induced pneumonia (Figures 5(a) and 5(b)). Masson staining (Figures 5(c) and 5(d)) and α-SMA staining (Figures 5(e) and 5(f)) results were consistent with H&E results, indicating that Rab7a knockout attenuates the therapeutic effect of MSCs on LPS-induced pulmonary fibrosis in mice.

4. Discussion

Alveolar epithelial injury is the main pathophysiological basis of ARDS. The alveolar type II epithelial cells remaining after injury can proliferate and differentiate into alveolar type I epithelial cells to repair lung injury, but, in moderate to severe ARDS, alveolar type II cells are severely damaged,

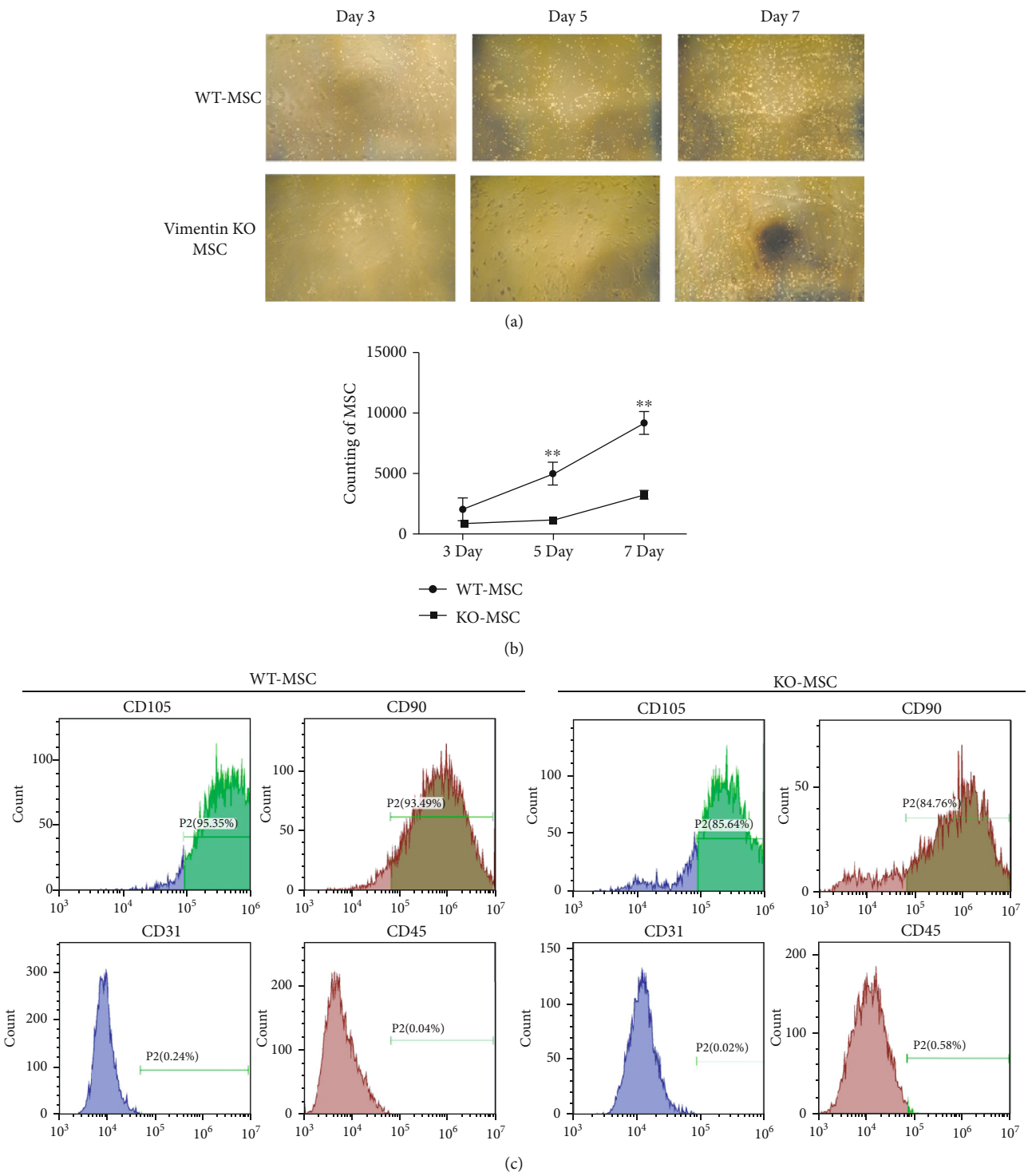


Figure 3: The effect of vimentin knockout on the characteristics of mouse MSCs. MSCs were isolated and cultured in vitro in wild-type mice and vimentin gene knockout mice. On the 3rd, 5th, and 7th day after culture, the morphological changes were examined via microscopy (a) and counted (b) at the same time. The MSCs cultured to the 7th day were labelled with CD105, CD90, CD31, and CD45, and the relevant expression levels were detected by flow cytometry. Statistical results are represented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 3$.

and their number is insufficient for repair. For damaged alveolar epithelium, supplementation of exogenous repair seed cells is a viable option [13]. MSCs are stem cells with multidirectional differentiation potential derived from the mesoderm. Previous studies have shown that exogenous MSCs given in ARDS animal models can indeed repair damaged alveolar epithelium and even reduce the mortality rate, but the number of MSCs homing to the lungs is relatively small. Short lung retention time and low differentiation ratio remain difficult challenges faced to utilize MSCs in the

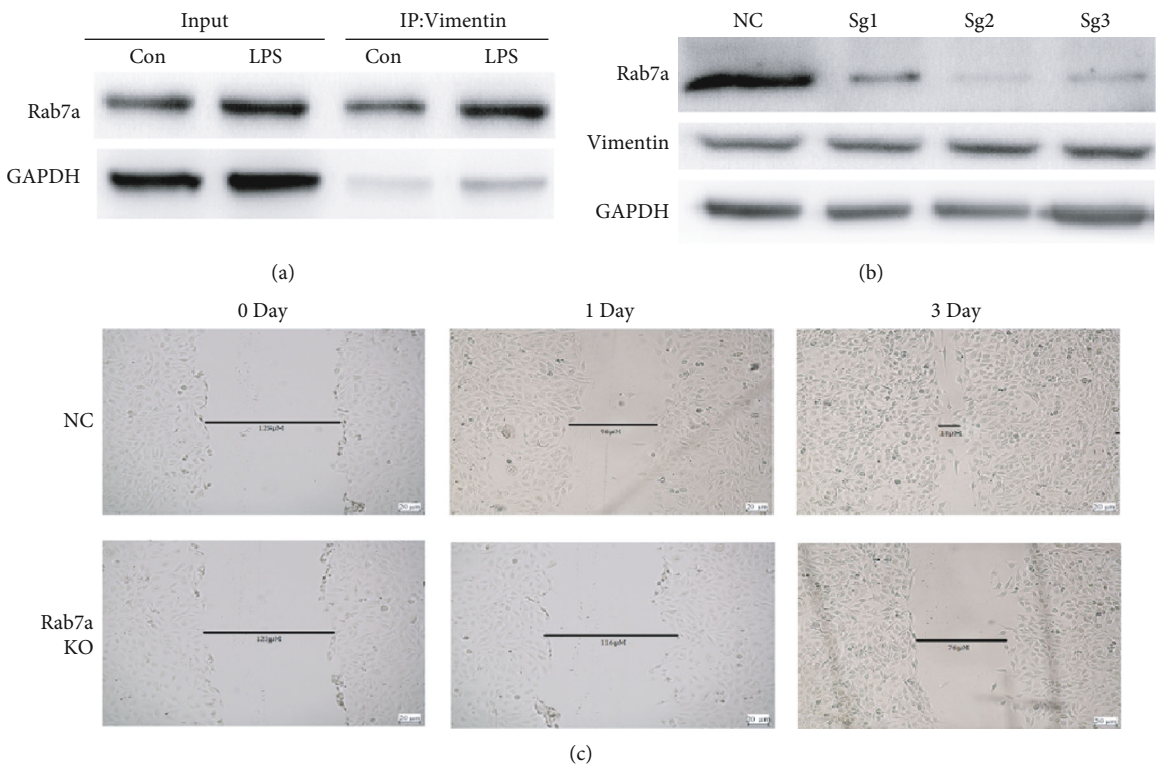


Figure 4: Rab7a knockout inhibited the migration of MSCs. In order to detect the binding of Rab7a and vimentin, the state of Rab7a contained in vimentin was detected by immunoprecipitation method (a). Lentiviral transfection was used to knock out Rab7a and confirm knockout (b). The scratch test was used to determine the impact of Rab7a knockout on scratch healing, a measure of cell migration capability (c).

treatment of ARDS [14]. Previous studies have shown that vimentin acts on cell migration, contraction, proliferation, protein synthesis, and protein synthesis.

Molecules that play an important role in gene expression, apoptosis, and mechanical force transmission [15] also play an important role in the migration of MSCs [16]. Therefore, the regulation of MSCs by targeting vimentin is expected to further improve the lung protection effect of MSCs. This study was verified by in vivo and in vitro experiments.

The current study found that mice with vimentin gene knockout had slower MSC growth than wild-type mice, and their differentiation status also differed. This suggested that there may be a considerable difference between the therapeutic effects of vimentin knockout mouse MSCs and wild-type mouse MSCs. The results confirmed that vimentin gene knockout weakened the therapeutic effect of MSCs in ARDS. Furthermore, we used GFP-labelled MSCs to observe their colonization in damaged lung tissues. The results showed that the homing effect of MSCs in vimentin knockout mice was severely weakened. We have been able to be relatively clear that the effect of vimentin on the therapeutic effect of MSCs is mainly reflected in the change of the migration effect of MSCs into damaged lung tissue. Presently, there have been many reports on vimentin-mediated MSC migration [17, 18], but the role of vimentin-mediated MSC regulation has not been clear. Although some studies, such as Dave et al. [19], reported that PIAS1 may play an important role in vimentin-mediated cell migration, MSCs are different from other cells. They are a kind of immature cell that is induced under different conditions. Under varying circumstances, their state will change, so the genetic law presented is often in an unstable state. Furthermore, MSCs secrete many cytokines, and many of these cytokines, such as IL-6, IL-8, and MCP-1, promote migration [20]. Some studies have reported that the effect of vimentin on cells not only promotes migration and proliferation but also has an important effect on the secretion of cytokines [21]. Therefore, vimentin-mediated MSC migration may be more complicated than the experimental results show and will require further investigation.

In addition to exploring the downstream mechanism of vimentin-mediated MSC migration, we investigated changes in the upstream signalling of vimentin. Many studies have shown that vimentin, as a skeletal protein, has many complex molecules that regulate its expression, which depends on different signal stimuli and different cell functions. In the regulation of cell migration, it is currently believed that Rab7a (a small molecule GTP binding protein) may be an important vimentin regulatory molecule. Some studies have shown that the role of Rab7a in regulating vimentin is not to affect its expression but to change the polarity of its molecules, thereby inducing cells to move in different directions. Studies have also shown that Rab7a can phosphorylate vimentin, thereby affecting the function of vimentin [9]. Obviously, the Vimentin-Rab7a pathway plays an important role in cell migration, but its mechanism is also very complicated. At present, there are no reports on the role of the Vimentin-

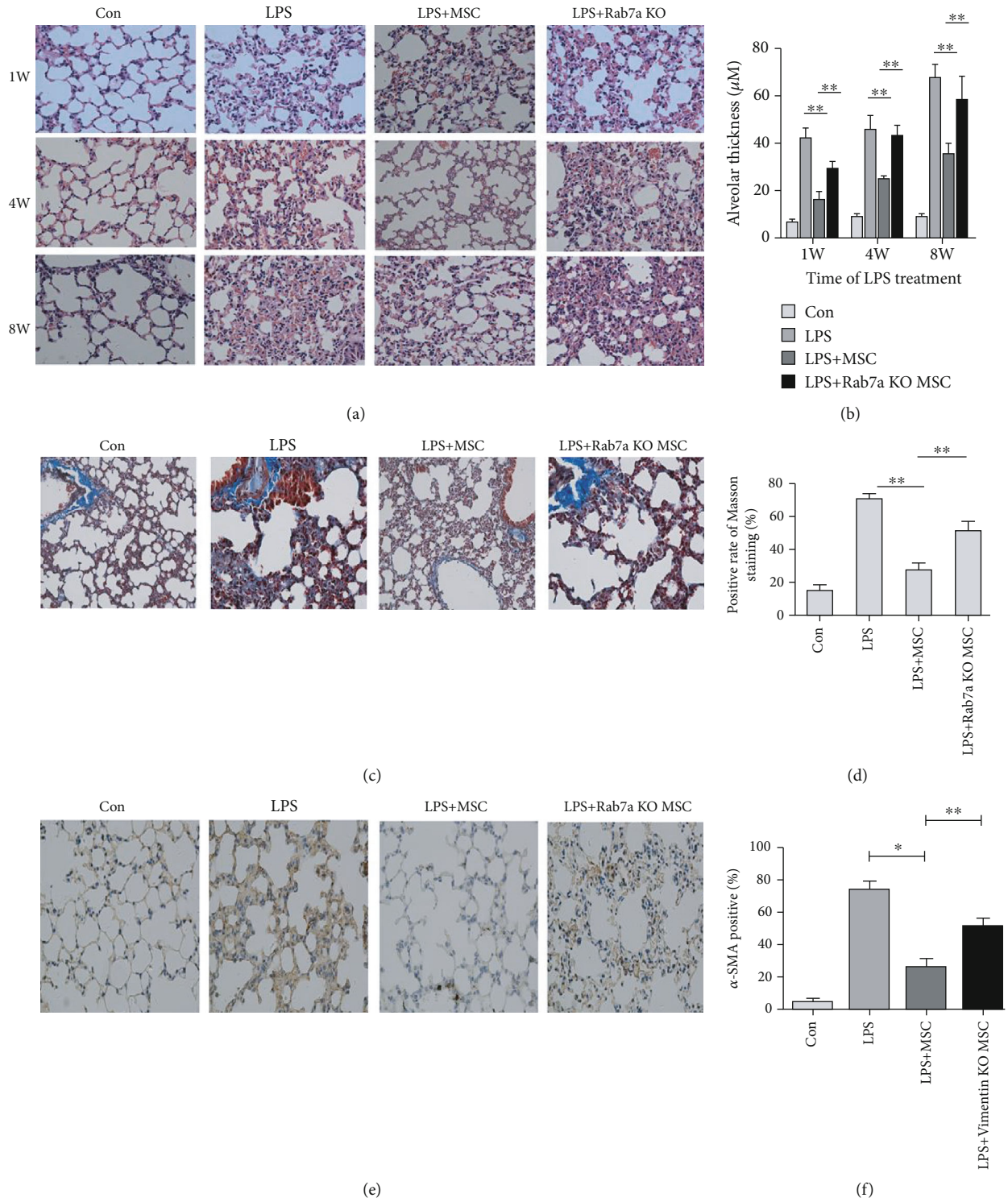


Figure 5: Rab7a knockout weakened the therapeutic effect of MSCs on ARDS. Two hours after the ARDS model was constructed, normal MSCs and Rab7a knockout MSCs were injected into the tail vein of mice. Left lung tissues of the mice were collected at 1 W, 4 W, and 8 W for H&E staining (a, b), Masson staining (c) at 8 W (d), and α-SMA immunohistochemical staining (e, f). Statistical results are represented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $n = 5$.

Rab7a pathway on MSC migration. In our research, we observed the binding of Rab7a and vimentin in MSCs, for the first time, and used genetic blocking to prove that vimentin is indeed a downstream molecule of Rab7a. Interfering with the function of Rab7a also affected the migration of MSCs, affecting the therapeutic effect of MSCs in ARDS. This phenomenon also illustrates, however, that Rab7a may have a more important function than vimentin.

5. Conclusions

In summary, this study proved for the first time that Vimentin-Rab7a can enhance the migration ability of MSCs and affect its homing effect in damaged lung tissue, thereby mediating the treatment of ARDS. The findings of this study provide new insight into intervention targets for enhancing the therapeutic effect of MSCs in ARDS.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that no conflicts of interest exist in the submission of this manuscript.

Authors' Contributions

All authors have approved the manuscript for publication.

Acknowledgments

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Review Article

The Effects of Mesenchymal Stem Cell on Colorectal Cancer

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Colorectal cancer (CRC) is a common malignant tumor of the gastrointestinal tract with nonobvious early symptoms and late symptoms of anemia, weight loss, and other systemic symptoms. Its morbidity and fatality rate are next only to gastric cancer, esophageal cancer, and primary liver cancer among digestive malignancies. In addition to the conventional surgical intervention, other therapies such as radiotherapy and chemotherapy and new treatment methods such as biologics and microbiological products have been introduced. As a promising cell therapy, mesenchymal stem cell (MSC) has attracted extensive research attention. MSCs are early undifferentiated pluripotent stem cells, which have the common features of stem cells, including self-replication, self-division, self-renewal, and multidirectional differentiation. MSCs come from a wide range of sources and can be extracted from a variety of tissues such as the bone marrow, umbilical cord, and fat. Current studies have shown that MSCs have a variety of biological functions such as immune regulation, tissue damage repair, and therapeutic effects on tumors such as CRC. This review outlines the overview of MSCs and CRC and summarizes the role of MSC application in CRC.

1. Introduction

Since the discovery of MSCs, researchers have deeply discussed their physiological characteristics. According to the definition of the International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell (ISCT MSC) committee, MSCs are different from mesenchymal stromal cells [1]. MSCs are heterogeneous cell groups with the ability of self-renewal and differentiation. Besides bone marrow-derived MSCs (BM-MSCs) and umbilical cord-derived MSCs (UC-MSCs), which are widely studied, there are several other sources of MSCs including peripheral blood, adipose tissue, and minced pulp tissues [2–5]. According to their donors and passages, MSCs derived from diverse sources have different surface markers. The ISCT committee suggests that the minimum criteria for identification is plastic adhesion, the ability to express CD73, CD90, and CD105, but not expressing CD11b, CD14, CD19, CD34, CD45, CD79a, and HLA-DR [1, 6].

Compelling evidence indicates that MSCs have great potential in regenerative medicine. MSCs can participate in tissue repair and regeneration [7], immune homeostasis regulation [8], inflammation resistance, and tumor inhibition [9, 10]. Exosomes derived from MSCs, as one of the paracrine products, have attracted researchers' attention recently because of their similar functions to MSCs. Exosomes, the 30-150 nm vesicles, are formed by endocytosis of cells, which encapsulate nucleic acids, proteins, lipids, and metabolites from the original cells when discharged from them [11, 12]. It has been found that exosomes can participate in information transmission between cells through packaged biologically active substances such as nucleic acid and protein [13, 14]. Through receptors and ligands, exosomes target specific cells and achieve information exchange by their integration into cell membranes [15]. Exosomes derived from MSCs possess the functional ability to be applied for tissue damage repair and cancer inhibition and avoid the adverse reactions

associated with cellular therapy [16–18]. As a cell-free therapy, exosomes have great prospects in cancer treatment.

CRC is among the top five cancers in the world in morbidity and mortality. Although the specific pathogenic factors that cause CRC remain unclear, its incidence is related to heredity, smoking, and eating pickled products, among other environmental factors [19, 20]. CRC begins insidiously and is usually screened for with stool tests, radiology, blood tests, and colonoscopy [21, 22]. Traditional chemotherapy has a poor curative effect on CRC with associated high toxicity and drug resistance [23]. Researchers have found that natural compounds, such as resveratrol and curcumin can treat CRC without huge adverse reactions through a series of animal models [24]. However, the lack of clinical trials hinders the application of these compounds. MSCs and their derived exosomes possess the ability to regulate the tumor microenvironment through paracrine and direct contact to inhibit CRC cells [25, 26]. MSCs can also be used as adjuvant therapy for tumor treatment, where they enhance the tumor cell-cytotoxic effect of antitumor drugs via cytokine secretion [27]. In this review, we aim to discuss the current knowledge of the effect of MSCs and their exosomes on CRC, including the mechanism of MSCs' inhibitory function in the CRC microenvironment, and the tumor-promoting effect of MSCs that cannot be ignored. This will bring up-to-date data on the status of the effects of MSCs and MSC-derived exosomes on CRC.

2. Overview of MSCs

2.1. Sources of MSCs. MSC is favored by the field of regenerative medicine because of its ability of self-renewal and directional differentiation under specific regulations. The characteristics of MSCs differ according to their different sources. Studying the characteristics of MSCs may help better selection of the suitable source, regulate their differentiation, and perhaps reduce the side effects of clinical application.

The sources of MSCs are varied, among which BM-MSCs, UC-MSCs, and MSCs from adipose tissue (AD-MSCs) are the most widely explored and applied [2]. The bone marrow provides an abundant source of MSCs, although this sampling method is invasive and causes great pain to patients. Alternatively, femoral shaft MSCs obtained during total hip arthroplasty have similar characteristics to iliac crest aspirate MSCs and can secrete cytokines such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and insulin-like growth factor (IGF) [28]. The method of extracting AD-MSCs is also invasive, while the success rate and colony frequency of isolating AD-MSCs are relatively high [2]. MSCs can also be separated from neonatal-related tissues, such as the umbilical cord, umbilical cord blood, placenta, and amniotic membrane [29]. MSCs derived from umbilical cord blood (UCB-MSCs) and UC-MSCs are separated from the umbilical cord-related tissue and are widely used because their collection causes no pain to patients. At the same time, UCB-MSCs have a relatively longer survival time and higher differentiation potential [2, 29]. MSCs from neonatal tissues have better application potential than those from adult tissues [30].

Other noninvasive sources of MSCs are MSCs derived from endothelium (eMSCs), Wharton's Jelly (WJ-MSCs), and menstrual blood (MenSCs), which are considered to regulate the innate and adaptive immune system both in vivo and in vitro [31, 32]. MSCs derived from pluripotent stem cells (PSCs) have been shown to overlap with BM-MSCs in gene expression and have similar immunomodulatory and inflammatory suppressive functions. Most importantly, they have the highest proliferation efficiency and longer passage time, which can be widely used as the source of MSCs [33]. MSCs derived from dental tissue, including dental pulp (dPSCs) [34], periodontal ligament (PDLSCs), gingiva (GSCs), apical papilla (APSCs) [35], and dental follicles (DFSCs)[10], have not been widely studied, while they have great potential in dental tissue regeneration. Sufficient research on the source of MSCs can help in the selection of suitable MSCs for specific disease treatment, establish a matching culture and separation system, and accelerate the development of MSC therapy. The sources and characteristics of MSCs are summarized in Table 1.

2.2. Differentiation of MSCs. One of the reasons MSCs are popular in the field of regenerative medicine is their multiple differentiation ability. MSCs have the ability to differentiate into osteoblasts, chondroblasts, and adipocytes and even develop a relatively mature induction system for different target cells [54, 55]. The regulation of MSCs differentiation is a complex system involving series of factors including cytokines, transcription factors, and nucleic acids. In theory, MSCs can be differentiated into cells through precise regulation [56]. For example, the osteogenic differentiation of MSCs could be active through calcium signaling [57]; miR-27a is involved in the differentiation of MSCs from osteogenesis to adipogenesis in postmenopausal osteoporosis [58]; transcription factors, including Runt-related transcription factor 2 (Runx2), SRY-related high-mobility group-box gene 9 (Sox9), the adipogenic-specific peroxisome proliferation-activated receptor γ (PPARγ), the member of the helix-loop-helix family transcription factors, MyoD, and the GATA zinc finger transcription factor family, GATA4 and GATA6, play a significant role in the differentiation of MSCs as corroborated by Almalki and Agrawal [59]; intercellular adhesion molecule-1 (ICAM-1) has been shown to inhibit MSC differentiation into adipocytes by activating the extracellular signal-regulated kinase (ERK) pathway and maintain MSCs undergoing adipogenesis through the p38 pathway [60].

MSCs are recruited in injury repair, and their ability of multidirectional differentiation is used in the reconstruction of injured tissues, with most of such reports described in MSCs from autologous sources [61]. Allogeneic MSCs disappear soon after injection in vivo, which may limit their curative effect [62]. MSCs play a therapeutic role by secreting active factors, and their differentiation ability in vivo is rarely reported, although in vivo studies of MSCs in the tumor microenvironment have been explored [63]. In the tumor microenvironment, the differentiation of MSCs could result in tumor promotion. For example, the infiltration of multipotential MSCs was found during the transformation of

Table 1: The sources and characteristics of MSCs.

Name	Source	Surface marker	Separation	Reference
BM-MSCs	Bone marrow	Stro1(+), Stro4(+), CD271(+), CD146(+), CD106(+), CD73(+), CD105(+), SSEA3(+), FZD9(+), SUSD2(+), LEPR(+), GD2(+), 3G5(+), CD90(+), CD140b(+), CD340(+), CD349(+), CD44(-), CD31(-), CD34(-), CD45(-), Lin(-), CD140a(low/-)	Prospective isolation based on monoclonal antibodies Density-gradient centrifugation	[36–40]
AD-MSCs	Adipose tissue	CD271(+), CD146(+), TM4SF1(+), CD44(+), CD73(+), CD90(+), CD105(+), CD36(+), CD163(+), CD200(+), CD273(+), CD274(+), TM4SF1(+), CD24(+), CD140B(+), CD34(-), Stro-1(-), SSEA-4(-)	Enzymatic digestion Mechanical centrifugation	[39–43]
UCB-MSCs	Umbilical cord blood	CD105(+), CD73(+), CD90(+), GD2(+), SSEA-4(+/-), CD146(+), CD49f(+), PODXL(+), TM4SF1(+), Stro1(-), CD45(-), CD34(-), CD14(-), HLA-DR(-), CD79a(-), CD11b(-), CD271(-), CD19a(-)	Density-gradient purification	[39, 40, 44]
UC-MSCs	Umbilical cord	CD146(+), CD29(+), CD44(+), CD73(+), CD90(+), CD105(+), Stro-1(-), CD271(-), SSEA-4(-)	Enzyme digestion	[39, 45, 46]
eMSCs	Endometrium	(PDGFRβ)/CD140b(+), CD146(+), SUSD2(+), CD29(+), CD44(+), CD73(+), CD90(+), NTPDase2(+), CD105(+), CD31(-), CD34(-), CD45(-), Stro-1(-)	Enzyme digestion	[39, 47–50]
MenSCs	Menstrual blood	CD56(+), CD73(+), CD90(+), CD105(+), CD146(+), SSEA-4(+)	Isolated cells were seeded into culture flasks	[51]
dPSCs	Dental pulp	CD9(+), CD10(+), CD13(+), CD29(+), CD44(+), CD59(+), CD73(+), CD9(+), CD105(+), 3G5(+), CD106(+), CD146(+), Stro-1(+), SSEA-4(+), CD166(+), CD271(+), CD14(-), CD19(-), CD24(-), CD31(-), CD34(-), CD45(-), CD117(-), CD133(-)	Enzymatic digestion of the pulp tissue Explant method	[39, 52, 53]

human prostate tumor, which suggests a possible role of the multipotential differentiation in cancer promotion [64]. Researchers found that limiting MSCs' differentiation potential may become a new target for cancer treatment [65]. It is revealed that CXCR4/TGF-β1 can mediate the self-differentiation of human BM-MSCs into cancer-related fibroblasts (CAFs) in CRC, thus playing a role in promoting cancer [66]. Research focuses on the differentiation function of MSCs in CRC which is conducive to finding more cancer targets; hence, more related research is needed to explore the field in search good CRC treatment.

2.3. General Effect of MSCs. From inflammation, infection, abnormal metabolism, immune disorder, and tissue damage to the tumor, MSCs have obvious curative effects in the treatment of these diseases. It is generally believed that MSCs exert therapeutic effects through direct contact and paracrine action, where MSC-derived extracellular vehicles (EVs) are reported as the mechanism behind these effects [67, 68]. In the immunoregulatory process, monocytes can rapidly phagocytize MSCs injected in vitro, changing their phenotype, expressing interleukin- (IL-) 10 and programmed death ligand-1 (PDL-1), and reducing the expression of tumor necrosis factor- (TNF-) α. These monocytes can migrate through the whole body via the circulatory system and play a follow-up immunoregulation effect [69]. Macrophage phenotype could also respond to the effect of MSCs by switching from proinflammatory "M1" to anti-inflammatory "M2" [70]. TGF-β and polyethylene glycol- (PEG-) 2 secreted by MSCs complete this process through the Akt/FoxO1 pathway [71, 72]. At the same time, the direct contact between proinflammatory macrophages and MSCs can enhance the secretion of tumor necrosis factor-stimulated gene-6 (TSG-6), and the production of anti-inflammatory T cells and macrophages [73]. MSCs play a role in relieving pain, inhibiting inflammation, sustaining proliferation, and regenerating matrix and cartilage in the process of bone regeneration with the regulation of V-Akt murine thymoma viral oncogene homolog (AKT), ERK, and the serine/threonine kinase AMP-activated protein kinase (AMPK) signaling [74].

For the treatment of tumors, the role of MSCs is complex. Compelling evidence indicated that MSCs can play a role in the process of tumor suppression, tumor growth, and drug resistance. The microRNA- (miR-) 100 secreted by MSCs is related to the downregulation of vascular endothelial growth factor (VEGF) in tumor angiogenesis [75], and miR-23b promotes the dormancy of metastatic breast cancer cells [76]. However, microRNA does not always play a positive role in inhibiting tumors. This is confirmed by several studies including that of Wei et al., who reported that microRNA-375 increases the invasion and metastasis of CRC via regulating the target gene RECK [77]. The fusion of MSCs with benign tissue cells may be beneficial to the repair of tissue damage, but after MSCs are recruited into tumor tissues, the fusion with tumor cells may be related to tumor metastasis [78]. This means that the direct contact and paracrine pathway of MSCs may not be able to alleviate the tumor. Further studies are needed to explore the mechanism of MSCs' functional effects to enhance the antitumor application of MSCs and avoid the tumor-promoting effect.

2.4. General Clinical Application of MSCs. With the deepening of the understanding of the mechanism of MSCs' functions, their clinical application is gradually standardized, and their safety has been greatly improved. Therefore, clinical trials of MSCs are constantly carried out. Intravenous

injection and intralesional injection account for the majority of MSCs administration. A three-year follow-up after intravenous infusion of UC-MSCs showed there were no abnormalities in blood routine, liver and kidney function, and immunoglobulin in the treatment of rheumatic immune diseases. Moreover, the health index and joint function index significantly improved [79]. For patients with multiple sclerosis, MSCs can improve the quality of life of patients without serious adverse reactions, and intrathecal administration is more effective than intravenous injection [80, 81]. More MSC-related clinical trials presented so far in the field of the cardiovascular system, digestive system diseases, nervous system, and endocrine system prove its effectiveness (Table 2).

The reduction of side effects in the clinical use of MSCs does not mean that it is completely safe. More attention should be paid to the safety research of MSCs application. Matthay et al., demonstrated that one dose of intravenous allogeneic BM-MSCs is safe in patients with moderate to severe acute respiratory distress syndrome (ARDS) [82]. The safety and efficacy of the intravenous infusion of UC-MSCs in patients with heart failure are also demonstrated by Bartolucci et al. [83]. Researchers using modified MSCs to treat gastrointestinal cancer found that MSCs were safe and well tolerated in patients with gastrointestinal cancer. However, due to the small number of patients and the heterogeneity of tumors, the treatment received by patients may affect the interaction between tumor microenvironment and MSCs; no improvement in tumor activity was observed [84]. Another group of researchers found no dose-limiting toxicity in MSC treatment of prostate cancer, while the effectiveness is still worth further exploring [85]. There are few clinical applications of MSCs in tumor treatment, which may be due to the lack of standard MSCs separation and infusion standards, the inability to inhibit the tumor-promoting effect of MSCs, the lack of cognition of MSCs homing mechanism, and the heterogeneity of the tumor. In the application of tumor therapy, there is a need to explore MSCs and tumors for a long time.

3. Overview of CRC

3.1. The Pathogenesis of CRC. CRC is a heterogeneous malignant tumor of the colon and rectum, the fourth most common cancer around the world contributing to 9.7% of the global cancer burden [108] and the third most frequent malignant tumor in China [109]. Bad living habits and environmental pollution contribute to the prevalence of CRC. It is reported that up to 90% of the morbidity risk of CRC is related to environmental factors such as diet [110]. CRC occurrence is linked with inappropriate dietetic habits such as high consumption of heme-iron foods and alcohol and low consumption of fruits, vegetables, fiber, fish, dairy products, and vitamin C. Furthermore, unhealthy lifestyles such as obesity and lack of exercise increase CRC risk [111].

Besides diet and lifestyle, genetics and certain diseases can also contribute to CRC. In all CRCs, the genetic predisposition genes with high cancer risk are 2–8%. When the pathogenic mutations in high- and moderate-penetrance genes are added, the ratio will rise to 6–10% [112]. The risk of developing CRC increases in patients with inflammatory bowel disease (IBD), where the inflammation involved with IBD serves as a hazard for CRC onset [110, 113, 114]. Diabetes is positively relevant to the risk of CRC in men (HR = 1.17 ; 95% CI: 1.08-1.26; $I^2 = 0\%$) and women (HR = 1.13; 95% CI: 0.82-1.56; $I^2 = 46\%$) [115]. Microsatellite instability and microRNAs play important roles in the occurrence of CRC [114, 116]. Furthermore, intestinal microbiota highly impacts the state of CRC, where a proper balance in the diversity and composition positively correlates with a good CRC prognosis [117, 118]. The influencing factors of CRC occurrence are shown in Figure 1.

Individual habits such as eating red meat and consuming alcohol increase CRC risk, while eating dairy products, fish, and vitamin C-rich foods reduces the risk. Intestinal flora also plays a dual role in the development of CRC. Gene mutation and some noncoding RNAs can promote the occurrence of CRC.

3.2. Current Therapeutic Interventions in CRC. In addition to surgery, there are several current and emerging treatment options for CRC including chemotherapy, radiotherapy, targeted therapy, and immunotherapy, among others [119]. These CRC treatment interventions are summarized in Table 3.

4. MSC Studies on Tumors

The biological functions of MSCs have been studied and applied in clinical trials, including the study of their effects on tumors. A search of the terms "mesenchymal stem cell and tumor" in the PubMed database revealed a total of 594 documents published in nearly 15 years, from January 1st, 2006, to December 31st, 2020 (Figure 2). Further statistical analysis of the MSC studies on different tumors in the last 15 years showed approximately 8% of literature reports on the role of MSCs on CRC (Figure 3).

The increasing attention given to MSC studies correlates with the rising application in cancer studies. Over the past few years, the number of published researches on the effect of MSCs on tumors has steadily been increasing.

This figure represents studies on MSCs and different tumors in the past 15 years, where CRC represents 8% of total studies. Those with a proportion less than 4% are classified as others.

5. The Effect of MSCs on CRC

According to the literature, MSCs possess a double-edged sword property on CRC. In effect, MSCs can significantly inhibit the proliferation, migration, and infiltration of tumor cells to prevent the occurrence and progression of CRC, while in other conditions, MSCs serve as promoting agents for CRC progression (Table 4).

5.1. Inhibitory Effects on CRC. Studies show that under certain treatment conditions, MSCs can inhibit the proliferation of CRC cells and promote apoptosis, thus inhibiting the

Table 2: The general clinical application of MSCs.

Systems	Disease	Effect	Mechanism	Reference
Respiratory system	Bronchopulmonary dysplasia; non-small cell lung cancer; ARDS; asthma inflammation; diabetic lung fibrosis	Improve lung function; reduce pulmonary fibrosis; relieve pulmonary hypertension	Increase the "M2" macrophages; mitochondrial transfer; adjusting Sirt3-mediated responses; exosomal transfer of miR-144	[70, 86–89]
Digestive system	IBD; intestinal ischemia-reperfusion injury (IRI)	Suppression of inflammatory responses; improve I/R-induced intestinal damage; improve gut barrier function	IL-10; macrophage polarization; TSG-6 through hyaluronan-CD44 interactions in an Akt-dependent manner; promote Claudin-3, Claudin-2, and ZO-1 expression; NLRP3-related signaling pathways	[90–94]
Endocrine system	Type 2 diabetes mellitus (T2DM)	Reduce blood glucose levels; reverse insulin resistance	Inhibition of STZ-induced β-cell apoptosis; activation of autophagy via the AMPK pathway; blockade of the NLRP3 inflammasome activation	[95–97]
Immune system	Rheumatoid arthritis; systemic lupus erythematosus (SLE); allergic asthma	Reduce joint destruction; improve the immune system	Restore the balance between memory T cells populations; miRNA-150-5p; release TGF-β1 to generate CD4 + CD25 + Foxp3 + T-reg cells; expand IL-10 producing lung interstitial macrophages	[98–102]
Nervous system	Stroke; neuroinflammation	Improve neurological impairment and long-term neuroprotection; attenuate neuroinflammation	Inhibiting STAT3-dependent autophagy; microRNA cluster miR-17-92	[103–107]

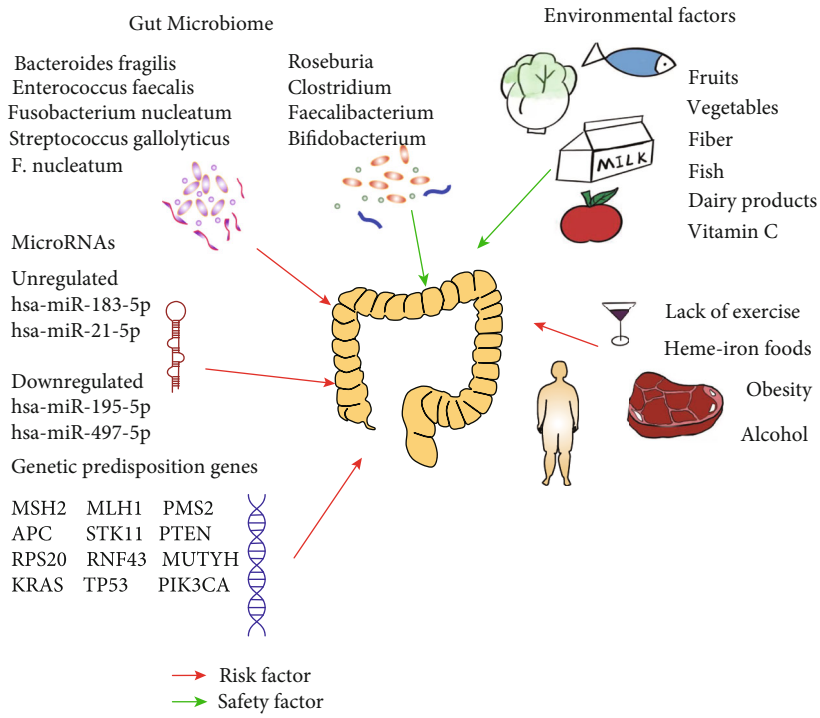

Figure 1: The influencing factors of CRC occurrence.

progression of CRC. A study by Chen and colleagues indicates the hypothesis that MSCs improve tumorigenesis in IBD by inhibiting the expression of proinflammatory cytokines and activation of STAT3 [133]. In a study of azomethane- (AOM-) induced carcinogenesis, exogenous MSCs were demonstrated to possess inherent antitumor properties. Specifically, the MSCs could induce apoptosis by blocking the cell cycle in the G1 phase, and the intervention

Table 3: The different treatment options in CRC.

Therapeutic method	Effects	Reference
Surgery	The cornerstone of CRC treatment	[120]
Chemotherapy	Prolongs survival and improves symptoms and quality of life	[121]
Radiotherapy	Achieves local control and improves long-term prognosis	[122]
Targeted therapy	Reduces potential liver metastasis associated with CRC (antiviral therapy)	[123]
Immunotherapy	Achieves long-term durable remission in patients	[124, 125]
Probiotics	Enhance the immune barrier, regulate the intestinal immune state, inhibit pathogenic enzyme activity, regulate CRC cell proliferation and apoptosis, regulate redox homeostasis, and reprogram intestinal microbial composition	[126]
Prebiotics	Stimulate the growth and/or activity of specific bacteria in the gut, improve host health, possess prebiotic potential, modulate gut microbiota composition, a product of fermentation metabolites, antiadhesive properties against pathogens, and alter the gene expression profile	[127]
Postbiotics	Modulate the composition of the gut microbiota and the functionality of the immune system, promote the CRC treatment effectiveness, and reduces its side effects in CRC patients	[128]
Antibiotics	Improve the treatment efficacy of oxaliplatin-based therapy and reduce cancer severity through controlling *F. nucleatum*	[129, 130]
Nonsteroidal anti-inflammatory drugs (NSAIDs)	Prolong survival time	[131]
Fecal microbiota transplantation (FMT)	Restores the sensitivity of patients to anticancer drugs and enhances the immune response	[132]

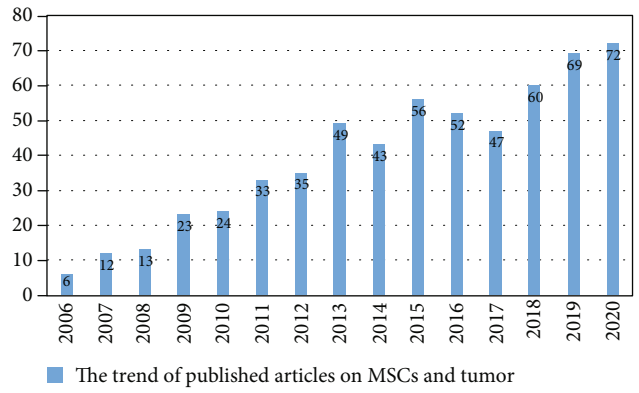

Figure 2: The trend of research on the application of MSCs in tumors.

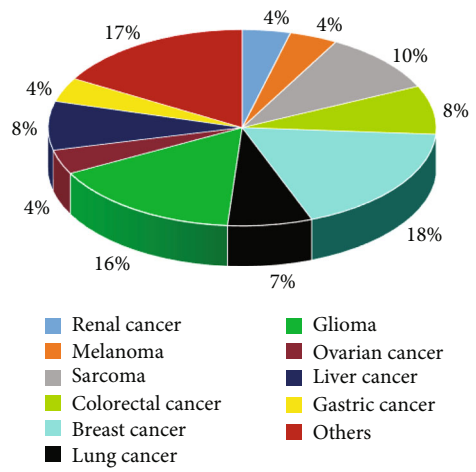

Figure 3: MSCs studies on the different kinds of tumors.

of MSCs could lead to the dysregulation of the Wnt and TGF-β-Smad signaling pathways in the body, thus interfering with tumor initiation [134]. In the study of Feng and colleagues, low doses of ultraviolet radiation and X-ray irradiation caused BM-MSCs to secrete specific cytokines (TNF-α, IFN-γ) to inhibit CRC cell proliferation and induce apoptosis, showing antitumor effects [26].

Moreover, MSCs can play a tumor-suppressive role through microRNAs contained in exosomes, a paracrine mode. Yan et al. presented that miR-16-5p overexpression in BM-MSC-derived exosomes inhibits the proliferation, migration, and invasion of CRC cells and promotes the apoptosis of CRC cells by downregulating ITGA2 expression [135]. Chen and colleagues also demonstrated in in vitro cell experiments that BMSC-derived exosomal miR-4461 inhibits CRC cell proliferation, migration, and invasion by reducing COPB2 expression, suggesting that miR-4461 may be a potential target for diagnosis and treatment of CRC [136]. In addition, MSC-exosome-derived miR-3940-5p inhibits CRC cell invasion, EMT, and tumor growth and metastasis by targeting ITGA6 and subsequent TGF-β1 inactivation [137]. ADMSC-derived EVs carrying miR-15a inhibit the immune escape of CRC cells by the downregulation of the KDM4B/HOXC4/PD-L1 axis [138].

In addition to the anticancer effect of MSC exosomal miRNAs, Luetzkendorf and colleagues showed that human MSCs could be induced by the third-generation lentiviral vector system to produce TRAIL- (tumor necrosis factor-related apoptosis-inducing ligand-) MSCs. It has been demonstrated that TRAIL-MSCs can reduce tumor growth of

Table 4: The effects of MSCs within the CRC microenvironment.

MSC-CRC interaction	Mode of function	Mechanisms	References
Inhibition	Cell-to-cell contact	Inhibiting the expression of proinflammatory cytokines and STAT3 activation	[133]
	Cell-to-cell contact	Induces apoptosis and interferes with tumor initiation through the dysregulation of Wnt and TGF-β-Smad signaling pathways	[134]
	Paracrine	miR-165-p overexpression in BM-MSC-exosomes inhibited the proliferation, migration, and invasion and promoted apoptosis of CRC cells by downregulating ITGA2 expression.	[135]
	Paracrine	miR-4461 in BM-MSC-exosomes inhibits the proliferation, migration, and invasion of CRC cells by reducing the expression of COPB2.	[136]
	Paracrine	MSC-exosome-derived miR-3940-5p inhibited CRC cell invasion, EMT, and metastasis by targeting ITGA6 and subsequent TGF-β1 inactivation.	[137]
	Paracrine	miR-15a carried by AD-MSC-EVs restricted CRC immune escape by downregulating the KDM4b/HOXC4/PD-L1 axis.	[138]
Promotion	Cell-to-cell contact	Regulating cell cycle and inhibiting apoptosis through the activation of NF-κB mediated by AMPK/mTOR	[143]
	Cell-to-cell contact	Promoting the progression of CRC cells through IL-6/JAK 2/STAT3 signal	[144]
	Cell-to-cell contact	The activation of JAK/STAT3 stimulated by the TGF-β1 or CXCR4/TGF-β1 axis can induce MSCs to differentiate into CAFs, which can promote the progression of CRC.	[66, 145, 152]
	Cell-to-cell contact	Interacting with CRC cells through CCL3/4/5 -CCR5 to promote the growth of CRC tumors in vivo	[148]
	Cell-to-cell contact	Promoting the EMT process of CRC through the CCL5/β-catenin/Slug pathway and SPARC	[147, 151]
	Cell-to-cell contact	Activating the HER2/HER3-dependent PI3K/Akt signaling cascade in CRC cells by releasing soluble NRG1.	[149]
	Cell-to-cell contact	Affecting the early xenograft growth of CRC cells with specific α-catenin deficiency by secreting extracellular matrix	[150]
	Cell-to-cell contact	Regulating the P53/P21 pathway through posttranscriptional regulation helps CRC resist senescence.	[153]
	Cell-to-cell contact	Promoting CRC angiogenesis through paracrine's high levels of the proangiogenic factor IL-8	[154]
	Paracrine	miR-222 targets ATF3 and inhibits the transcriptional activity of AKT1, thereby promoting malignant invasion and immune escape of CRC cells.	[157]
Affection	Paracrine	Inducing morphological and functional changes in colon mesenchymal stem cells by secreting exosomes	[155]

CRC cells in vivo by inducing apoptosis [139]. Another study that supported the hypothesis showed that TRAIL-MSCs can induce the apoptosis of TRAIL-CRC-resistant cells and overcome the tumor resistance to TRAIL in clinical treatment, suggesting that MSCs can be used as a carrier for clinical cancer treatment [140]. Zheng et al. revealed that CXCR4 overexpression by BM-MSCs increases the ability of stem cells to nest in the intestinal tract and improves colitis-related tumorigenesis in mice [141]. Due to the limited data available, the clinical application of MSCs in the treatment of CRC remains controversial. The issue of the clinical therapeutic value of MSCs is an intriguing one that could be usefully explored in further research to promote the development of stem cell therapies.

5.2. Promoting Effects on CRC. Tumor-stromal interaction plays a key role in the biology of CRC. With the deepening of research on MSCs, recent studies show that MSCs are recruited from the bone marrow into tumor stroma and form an important component of the tumor microenvironment, being the main source of CAFs. It is involved in the regulation of intestinal inflammation, epithelial proliferation, stem cell maintenance, angiogenesis, and extracellular matrix remodeling and metastasis [142]. Wu et al. illustrated that the protumor effect of MSCs is attributable to the altered expression of cyclin and the inhibition of apoptosis, possibly through the AMPK/mTOR-mediated activation of NF-κB to promote the progression of CRC [143]. Similarly, Zhang and colleagues found that human CRC-derived MSCs promote the progression of CRC cells through IL-6/JAK2/STAT3 signaling and activate PI3K/AKT signaling [144]. A recent study showed that TGF-β1 can induce the differentiation of MSCs to CAFs through the activation of the JAK/STAT3 signaling pathway and promote migration and invasion of CRC cell lines HCT116 and HT29 cells [145]. Tan et al. revealed that the CXCR4/TGF-β1 axis plays an important role in the

transformation of the tumor microenvironment by mediating the differentiation of MSCs to CAFs, promoting the growth and metastasis of CRCs [66].

In addition, studies by Nishikawa and colleagues have shown that MSCs interact with CRC cells through CCL3/4/5-CCR5, thereby promoting the growth of CRC tumors in vivo [146]. Chen et al. found that inflammation-activated human MSCs promote the epithelial-mesenchymal transformation (EMT) process and progression of CRC through the CCL5/β-catenin/Slug pathway [147]. Similarly, it is reported that BM-MSCs are implanted in nude mice after subcutaneous injection of HCT116-cancer stem cells (CSCs) to construct xenograft tumors. BM-MSCs can promote the migration and invasion of CSCs in CRC, suggesting that it can be a potential therapeutic target for CRC [148]. Further studies by De Boeck and colleagues found that BM-MSCs stimulate the invasion, survival, and tumorigenesis of CRC cells by releasing soluble NRG1 and activating HER2/HER3-dependent PI3K/Akt signaling cascade in CRC cells, and the high expression of NRG1 is associated with poor prognosis [149].

In a study by Widder and colleagues, MSC-CRC interaction promoted the formation of three-dimensional globules in CRC cells with a dysfunctional E-cadherin system. Further analysis showed that MSCs may affect the early xenotransplantation growth of specific α-catenin-deficient CRC cells by secreting extracellular matrix and ultimately play a cancer-promoting role [150]. EMT is an important mechanism for the progression of CRC, and SPARC is an important EMT-related factor in CRC, which is involved in the interaction between tumor cells and stromal cells. Naito and colleagues confirmed that MSCs induce the tumor-stromal formation and EMT process by expressing secreted protein acidic and rich in cysteine (SPARC) [151] and demonstrating a stronger ability to attack peripheral tissues through the mediation of newly expressed surface TGF-β on MSCs after coculture with tumor cells [152], thereby promoting the occurrence and development of CRC.

In addition to MSCs playing a role in the proliferation, invasion, and migration of CRC cells, Li and colleagues also found that MSCs can regulate the P53/P21 pathway through posttranscriptional regulation to help CRC resist senescence [153]. MSCs promote CRC angiogenesis and tumor growth through high levels of paracrine proangiogenic factor IL-8 [154]. Interestingly, besides the regulatory effects of MSCs on CRC cells, CRC cells can also induce morphological and functional changes of colon MSCs through secretion of exosomes, which is conducive to the growth and malignant progression of tumors [155], confirming the mutual promotion between the two. In addition to MSCs promoting the EMT process of CRC through direct cell-to-cell contact [156], recent studies by Li and colleagues found that miR-222 carried by extracellular vesicles derived from MSCs targets ATF3 binding and suppresses the transcriptional activity of AKT1, thereby promoting malignant invasion and immune escape of CRC cells [157].

6. Conclusion

Concerning CRC, various factors such as diet, environment, and genetic susceptibility greatly influence the constantly increasing incidence of CRC. The current cure rate of CRC is low, and the available treatment interventions are associated with a lot of side effects. MSC therapy has been highly regarded as a promising method for the treatment of clinical diseases including tumors. They have excellent conditions for the treatment of CRC due to their low immunogenicity, strong immune regulatory function, self-renewal ability, and easy accessibility. MSC-derived exosomes also express certain RNAs that participate in the inhibition of CRC growth and progression. These vesicles could also be engineered to serve as effective carriers of drugs and other therapeutic molecules to the CRC cells. However, like other malignancy studies, research on the application of MSCs in CRC is confronted with many challenges including the complex pathogenesis of CRC, the dual regulatory effects of MSCs on CRC, and the uncertainties of therapeutic dosage, administration mode, and adverse reactions shown in certain studies. Therefore, it is imperative to explore solutions to these hindrances, in addition to further investigating the regulatory mechanism and cargo sorting, functional modification, and carrier potentials of MSCs in CRC therapy for novel treatment interventions.

Conflicts of Interest

The authors declare that they have no competing interests

Authors' Contributions

Jintao Yuan, Zhiping Wei, and Xinwei Xu contributed equally to this work.

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Review Article

Regulatory Effect of Mesenchymal Stem Cells on T Cell Phenotypes in Autoimmune Diseases

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Research on mesenchymal stem cells (MSCs) starts from the earliest assumption that cells derived from the bone marrow have the ability to repair tissues. Several scientists have since documented the crucial role of bone marrow-derived MSCs (BM-MSCs) in processes such as embryonic bone and cartilage formation, adult fracture and tissue repair, and immunomodulatory activities in therapeutic applications. In addition to BM-MSCs, several sources of MSCs have been reported to possess tissue repair and immunoregulatory abilities, making them potential treatment options for many diseases. Therefore, the therapeutic potential of MSCs in various diseases including autoimmune conditions has been explored. In addition to an imbalance of T cell subsets in most patients with autoimmune diseases, they also exhibit complex disease manifestations, overlapping symptoms among diseases, and difficult treatment. MSCs can regulate T cell subsets to restore their immune homeostasis toward disease resolution in autoimmune conditions. This review summarizes the role of MSCs in relieving autoimmune diseases via the regulation of T cell phenotypes.

1. Introduction

There are abundant sources of MSCs including the umbilical cord, placenta, bone marrow, adipose tissue, gums, endometrium, menstrual blood, synovium, periosteum, skeletal muscle, and ligamentum cruciatum, among other tissues [1–5]. Besides, human induced pluripotent stem cells (iPS) also serve as a source of MSCs. Although there are variations in the criteria of surface markers of MSC from different sources, the literature shows that classic MSCs express CD105, CD73, CD90, CD34, and CD44, but not CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-DR [6, 7]. MSCs have the potential of self-renewal and multidirectional differentiation, and their differentiation potential depends on the tissues from which they originate (Table 1). Because of the low immunogenicity and homing capabilities of MSCs, they are used to treat multiple disease conditions. For instance, human umbilical cord-derived mesenchymal stem cells (UC-MSCs) have the potential to transform into cardiomyocyte-like cells. According to Peter et al., the cardiomyocyte-like contractile cells are produced in vitro by MSC differentiation and aggregation on the cardiomyocyte feeder layer and that only the young MSCs could maintain their low immunogenicity after differentiation into cardiomyocyte-like cells [8]. Similarly, Yu and colleagues found UC-MSCs have higher liver differentiation potential than bone marrow MSCs (BM-MSCs), hence their superiority to BM-MSCs in the treatment of end-stage liver disease [9]. This indicates the differential ability of MSCs from varying sources towards specific tissue repair and regeneration.

Also, the proliferation and differentiation potential of MSCs can be enhanced by the culture environment via modification techniques; therefore, the establishment of various modified culture systems makes the application of MSCs in regenerative medicine even more promising. These modification approaches can be roughly divided into genetic modification and preconditioning modification (using drugs,

Table 1: The surface markers and differentiation potential of different kinds of MSCs.

Sources	Surface marker	Differentiation potential	Reference
BM-MSCs	CD271(+), CD59(+), CD81(+), CD47(+), CD151(+), CD147(+), CD98(+), CD143(-), Lin (-) CD45(-), CD140a (PDGFRα) (low/-)	Strong adipogenic and osteogenic potential, poor chondrogenic potential, strong differentiation potential of corneal epithelial cells, and cardiac progenitors	[14–18]
UC-MSCs	CD73(+), CD90(+), CD105(+), CD44(+), CDH-1(+), CD29(+), CD34(-), CD45(-)	Muscle, neurogenic cells, hepatocyte-like cells, endothelial lineage	[19–23]
AD-MSCs	CD45(-), HLA-DR(-), CD44(+), CD106(+), CD34(+), CD90(+), CD105(+)	Strong adipogenic and osteogenic potential, poor chondrogenic potential, poor differentiation potential of corneal and muscle	[14, 16, 17, 24–26]
DP-MSCs	TRO-1(+), CD146(+), CD29(+), CD90(+), CD105(+), CD44(+), CD59(+), CD73(+), CD146(-), CD34(-), CD45(-), CD11b(-), CD45(-)	Osteogenic, adipogenic, chondrogenic, fibroblast lineage, neural stem cells	[27, 28]
SD-MSCs	CD9(+), CD10(+), CD13(+), CD44(+), CD54(+), CD55(+), CD90(+), CD105(+), CD166(+), D7-FIB(+), CD14(-), CD20(-), CD45(-), CD133(-)	Strong chondrocyte, osteocyte, and adipocyte differentiation ability, as well as muscle differentiation	[16, 29]

growth factors, and other molecules), which can improve the inherent biological activities concerning migration, homing to target site, adhesion, and survival and reduce premature senescence [10]. Existing research has shown that MSCs communicate with other cells through direct contact and paracrine signaling. In effect, MSCs repair tissue by directly contacting, adhering, and subsequently differentiating into the injured cells. It also exerts its anti-inflammatory, repairing, and immunomodulatory effects by secreting extracellular vesicles (EVs) or paracrine factors and mitochondrial transfer [11].

Autoimmune diseases are caused by imbalanced homeostasis of the autologous environment including T cells. While peripheral regulatory T cell (pTreg) and T helper type 17 (Th17) cell share a common precursor cell (the naïve CD4 T cell) and require a common signal for initial differentiation (tumor growth factor- (TGF-) β), they turn to elicit opposite functions via terminal differentiation: Treg is anti-inflammatory, inhibits autoimmunity, and maintains immune homeostasis, whereas Th17 cell causes autoimmunity and inflammation [12]. Moreover, the instability within T cell phenotypes such as Treg alongside their cellular plasticity and tissue-specificity also affects the development of autoimmune diseases [13]. Detailed exploration of T cell interaction with both immune and nonimmune cells presents not only deeper insight into disease pathogenesis but new therapeutic strategies as well.

MSC-based therapy is widely used in refractory immune diseases and has achieved encouraging results. They effectively restore T cell balance within the autoimmune environment, enhancing inflammation resolution through the complemented effect of both cells. This paper examines the application of MSCs in autoimmune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE), among others, and particularly highlights their modulatory effects on T cell phenotypes and the resultant contribution towards therapeutic strategies.

BM-MSCs: bone marrow-derived MSCs; UC-MSCs: umbilical cord-derived MSCs; AD-MSCs: adipose-derived MSCs; DP-MSCs: human dental pulp-derived MSCs; SD-MSCs: synovium-derived MSCs.

2. Application of MSCs in Autoimmune Diseases

Autoimmune disease is a diverse kind of complex and heterogeneous abnormal condition caused by immune system disorder. Common examples include IBD, RA, SLE, type 1 diabetes (T1D), and multiple sclerosis (MS), among others. In addition to the difficulties in early detection and poor curative effect, patients with autoimmune diseases are also faced with complicated pathogenesis [30]. At present, due to the low immunogenicity and multidirectional differentiation of MSCs, it is promising to study its therapeutic effect on patients with autoimmune diseases.

2.1. Application in IBD. IBD refers to a group of chronic and heterogeneous intestinal inflammatory disorders, including ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of IBD is highly complex and not completely clear, but current literature shows that it is related to several factors such as genetic susceptibility, environmental triggers, intestinal flora, diet, psychology, and immunity [31, 32]. The incidence and prevalence of IBD vary from region to region. European countries such as Norway and Germany have the highest global prevalence of 505/100,000 in UC and 322/100,000 in CD, respectively, followed by the North American countries, the United States of America with a UC prevalence of 286/100,000, and Canada with CD prevalence of 319/100,000. Due to the recent modernization of Asian and Latin American countries, the prevalence of IBD in such countries is also constantly rising [33–36].

At present, there are several clinical interventions for IBD patients, including drug therapy such as immunomodulators, steroids, and antibiotics, surgical treatment, and fecal microbiota transplantation (FMT) as a novel therapy. However, these treatment approaches are insufficient in curing IBD. Drug therapies are less effective and often associated with adverse reactions. Surgical procedures have certain

requirements for patients' state, and FMT is relatively safe but linked with many problems such as patient acceptance [37–40]. In the phase of these challenges, autologous or allogeneic MSCs emerged as a potentially effective therapy for IBD because of their anti-inflammatory and tissue repair effects, excellent immunomodulatory properties, and low immunogenicity [41]. Experimental studies illustrate that MSCs can repair dextran sulfate sodium- (DSS-) induced acute and chronic colitis in mouse models and prevent the recurrence of experimental IBD. In this process, MSCs regulate immune response, reduce inflammatory cell infiltration, regenerate intestinal epithelial cells, blood vessels, and lymphatic vessels, and change gut microbiota [42–44]. A myriad of clinical trials also shows that MSC therapy is well tolerated with promising efficacy and safety profile. Most recorded adverse effects described for MSCs are mild and transient [45].

A randomized, double-blind, parallel-group, placebo-controlled trial divided 212 patients having a CD with refractory perianal fistula into two groups, which were treated with either autologous AD-MSCs or placebo. The comprehensive remission rates of patients treated with AD-MSCs were higher than those of the placebo group, 50% and 34%, respectively. The incidence of treatment-related adverse events was 17% in AD-MSCs and 29% in placebo groups [46]. Clinical trials by Park et al. also demonstrated the effectiveness of MSCs for perianal fistula repair. A large number of systematic reviews and meta-analyses have also reported the effectiveness and safety of MSCs as IBD-related treatment, especially with long-term effects [47–49]. A study designed to investigate the occurrence of adverse events related to acute infusion toxicity, long-term adverse events, and efficacy of human amnion-derived MSCs (AMSCs) was carried out among CD patients who only achieve partial symptomatic relief with traditional therapy. The results of this phase I/II trial study will be beneficial to further promote the clinical application of AMSCs in IBD [50]. Different doses of BM-MSCs were injected locally in CD patients with refractory perianal fistula to determine the effective dosage that promotes healing of perianal fistulas. The authors concluded that no severe adverse events were associated with the allogeneic MSCs administered and the injection of $3 \times 10(7)$ MSCs appeared to promote healing of perianal fistulas [51].

However, it is important to note that regardless of the success witnessed in MSC therapy in IBD, it is still confronted with several challenges such as severe adverse events, encouraging tumor growth and metastasis, among other reactions as detailed in recent reviews by Ocansey et al. [52, 53]. There is the need to identify supportive or combined therapies of MSC transplantation and also choose the most appropriate stem cell and treatment approach to enhance effectiveness while avoiding the occurrence of serious adverse events [54].

2.2. Application in RA. RA is a systemic autoimmune disease, which mainly affects and damages joints and bones. The main clinical manifestations are pain, swelling, deformation of joints, and dyskinesia. In RA, there are unresolved immune cells infiltrating joints and unregulated autoantibody levels. Additionally, RA also affects other organs, including the blood vessels, kidneys, heart, and lungs, resulting in severe pain in patients. The complexity of the definition of RA makes it difficult to study its incidence. According to the criteria defined by the American Rheumatology Society 1987, the global prevalence of RA is about 0.24% (95% CI 0.23% to 0.25%), and the number of patients with RA will reach 4.8 million by 2010 (95% CI 3.7 million to 6.1 million). However, after the revision of the diagnostic criteria of RA in 2010, the number of patients with RA increased further [55, 56]. The main risk factors of RA are gender (women usually have a higher risk than men), heredity, environment, and psychological factors [57]. Recent studies have also shown that RA has a strong correlation with periodontal diseases [58, 59].

At present, there is no effective therapy for RA. The use of nonsteroidal anti-inflammatory drugs and cortisol can alleviate the symptoms of pain and stiffness, but cannot delay the progress of the disease. The use of disease-modifying antirheumatic drugs such as methotrexate and sulfasalazine can delay the disease progression. For example, the depletion of B cells and the development of B cell inhibitory antibodies, IL-6 inhibitors, and T cell-targeted drugs can bring a glimmer of hope for the treatment of RA, but the adverse reactions and toxic side effects of these drugs still hinder their application [60]. Presently, the application of MSCs as a treatment option for RA has demonstrated unique advantages in a host of clinical trials.

According to Wang et al., patients with insufficient response to traditional RA drugs were divided into two groups: patients injected with traditional antirheumatic drugs plus culture medium not containing UC-MSCs and patients injected with traditional antirheumatic drugs and culture medium containing UC-MSCs. They found that the group that received UC-MSCs was significantly relieved, and only one infusion capably achieved the relief effect for 3-6 months, with no major adverse reactions [61]. Another group of researchers selected refractory RA patients in phase Ib/IIa clinical trials and reported similar results without serious side effects in the short term after intravenous injection of AD-MSCs [62]. These demonstrated safety and efficacy studies indicate the encouraging development of MSC therapy in RA, regardless of challenges that demand further exploration in areas such as maintaining the therapeutic effect for a long period.

2.3. Application in SLE. SLE, a common autoimmune disease, involves an inflammatory disorder of multiple organs and systems of the body such as the kidney, lung, and skin. It mostly affects females, and some patients develop symmetrical butterfly erythema or other rashes on their faces. The main cause of mortality and morbidity of SLE patients at the end stage is lupus nephropathy [63]. SLE also has the characteristics of complex pathogenesis which largely remains unclear. Notwithstanding, it is agreed that the pathogenesis of SLE is related to the imbalance of factors such as heredity, environment, and endocrine and autoimmune system [64]. Although many genes related to SLE have been found, including complement component-related genes

(C1q, C1r, C1s, and C4) and HLA-DR, the specific effects of each gene are still unknown [65, 66]. Besides, estrogen and prolactin have also been identified as risk factors of SLE, which is consistent with the higher prevalence in women than men. Ultraviolet radiation can also increase the risk of SLE. Most SLE patients produce autoantibodies that are related to the clearance defect of apoptotic cells [67]. The global incidence and prevalence of SLE vary with gender, age, race, and time, which are partly explained by the differences in genetic and environmental risk factors [68].

Current treatment of SLE includes immunomodulators and immunosuppressants such as hydroxychloroquine and other drugs that prevent complications. While symptomatic treatment is given to subside various systemic manifestations [69], monoclonal antibody therapy is administered to target and deplete B cells as a proposed treatment of lupus nephritis [70]. Both the traditional and newly developed therapies have limitations related to drug administration, as well as gradual drug reduction until withdrawal, both of which may affect the balance between disease activity control and organ damage caused by long-term and/or unbalanced immunosuppression [71, 72].

MSCs have been used in the treatment of lupus nephritis and refractory SLE patients for more than ten years. Most documented clinical trials are self-controlled studies with only a few being randomized controlled trials. In a meta-analysis study that evaluated the efficacy and safety of MSC treatment in SLE patients, the researchers report that the MSC group showed significantly decreased SLE disease activity index, as well as decreased urine protein, and increased complement C3 [73]. A meta-analysis of the animal model of lupus nephritis also confirmed that MSC treatment resulted in lower levels of disease-associated elements such as double-stranded DNA (ds-DNA), antinuclear antibody (ANA), serum creatinine (Scr), blood urea nitrogen (BUN), proteinuria, and renal sclerosis score, as well as higher albumin levels [53]. Several other studies have reported the promising effect of MSCs in SLE experimental studies and clinical trials [74]. These current pieces of evidence show that MSCs capably improve the disease activity, hypocomplementemia, and proteinuria in SLE patients. However, large-scale and high-quality randomized controlled trials are required to validate the efficacy and safety of MSC treatment in SLE patients. It is also worth noting that allogeneic and autologous MSC treatment of SLE may have opposite effects; hence, allogeneic rather than autologous MSC transplantation could be potentially advantageous for SLE patients [63, 75, 76].

2.4. Application in Other Autoimmune Diseases. Recent studies demonstrate the remarkable therapeutic effectiveness of MSCs towards several other autoimmune diseases such as type 1 diabetes [77], multiple sclerosis [78], Hashimoto's autoimmune thyroiditis [79], autoimmune hepatitis, primary biliary cirrhosis [80], and vitiligo [81], among others. For example, MSCs have been shown to prevent inflammation and neurodegeneration in animal models of multiple sclerosis (MS). These experimental studies have set the ground for clinical trials such as a recent randomized, double-blind, placebo-compared phase I/II clinical trial with autologous BM-MSCs in MS which is currently ongoing (ClinicalTrials.gov NCT01854957) [82]. Autoimmune destruction of insulin-producing B cells in the pancreas results in type 1 diabetes, a disease condition that demands more than a mere administration of exogenous insulin to gently and sensitively regulate blood glucose concentration. MSCs can transdifferentiate into insulin-producing cells, support the regeneration of residual B cells via production of growth and trophic factors, or participate in the suppression of the autoimmune reaction against B cells [83, 84]. Hashimoto's thyroiditis (HT) is a disease wherein lymphocytes mediate the autoimmune damage and destruction of the thyroid gland. MSCs have been demonstrated to improve HT via reducing the level of thyroid autoantibody partly by regulating Th17/Treg interactions [79].

A detailed exposition of research progress on MSC therapy in autoimmune diseases indicating remarkable therapeutic effectiveness has recently been reviewed by Chen and colleagues [85]. MSCs also capably home to the disease site, regulating the balance of T cells through direct contact and secretion of active factors. Table 2 presents some of the documented studies of MSC's role in immune regulation of selected autoimmune diseases.

T1D: type 1 diabetes mellitus; TGF-β/MSCs: TGF-β engineered MSCs; MS: multiple sclerosis; SS: Sjögren's syndrome; PBC: primary biliary cirrhosis; HT: Hashimoto's thyroiditis.

3. Regulatory Effect of MSCs on T Cells in IBD

In the experimental IBD model, MSCs regulate the generation of T cell subsets to alleviate intestinal inflammation [102, 103]. For instance, the coculture of peripheral blood mononuclear cells (PBMCs) and MSCs strongly inhibits the proliferation of CD4+ and CD8+ T cells, as well as natural killer (NK) cells. The researchers found that the mechanism involved is not dependent on cell contact, but rather activated by interferon-gamma (IFN-γ) produced by lymphocytes. IFN-γ stimulates MSCs to produce indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), and IL-10, wherein the IDO principally inhibits the proliferation of T lymphocytes [104, 105]. MSCs homing in colon tissue can promote the proliferation of intestinal epithelial cells and the regeneration of intestinal stem cells. This effect has been shown to be related to the downregulation of Th1/Th17. Other molecules reduced in the process owing to the anti-inflammatory effect of IFN-γ include interleukin- (IL-) 2, tumor necrosis factor- (TNF-) α, IFN-γ, T-bet, IL-6, IL-17, and retinoic-acid-receptor-related orphan nuclear receptor gamma (RORγt) [106]. However, in most cases, IFN-γ is still considered a proinflammatory factor. It has been reported that MSCs significantly inhibit the secretion of IFN-γ and promote the production of IL-10 by T cells. IL-10 acts with dendritic cells (DCs) to promote anti-inflammatory effect [107, 108]. These findings show the complex role of IFN-γ in MSC-mediated immune regulation including its role in inducing T cell inhibition via MSC regulation.

Regulatory T cells (Tregs), the special T cell subset for immunosuppression, specifically express transcription factor

Table 2: The application of MSCs in other autoimmune diseases.

Disease	Source of MSCs	Effects	Reference
T1D	UC-MSCs TGF-β/MSCs	MSCs were safe and tolerable Hyperglycemia was significantly controlled	[77, 86–89]
MS	BM-MSCs	Clinically feasible and relatively safe and could immediately produce immune regulation	[90–94]
SS	UC-MSCs	Effective in treatment	[95, 96]
PBC	BM-MSCs UC-MSCs	MSCs were well tolerated and no obvious side effects were found Symptoms were significantly alleviated	[97–99]
HT	AD-MSCs	MSCs inhibited inflammation and helped recover from injury	[79, 100, 101]

forkhead box P3 (FoxP3) in the nucleus and CD25 and CTLA-4 (cytotoxic T lymphocyte-associated protein 4) on the cell surface [109]. Tregs play a crucial role in the inhibition of inflammation associated with several diseases such as IBD [110]. The combination of MSCs and Tregs in experimental treatment results in longer survival time for exogenous Tregs, upregulation of endogenous Tregs, and downregulation of proinflammatory Th17 cells [111, 112]. It is worth noting that endogenous CD4+CD25+Foxp3+ T cells are differentiated from CD4+ T cells, rather than natural Treg amplification, which involves TGF-β and/or programmed cell death- (PD-) 1/PD-L1 mechanism [45, 105].

Studies by Sarah and others indicate that cytokines secreted by MSCs intensely participate in the immunomodulatory role on T cells. TGF-β1, a soluble cytokine produced by MSCs, can induce Tregs under TCR (T cell receptor) costimulation, promote the activation of monocytes, and enhance monocyte differentiation into type II macrophages. Macrophages produce a large amount of IL-10 and CCL-18 (C-C motif chemokine ligand-18), which has been shown to play an important role in Treg induction; IL-10 further inhibits the pathogenicity of Th17 [113]. Macrophages can significantly inhibit the proliferation of CD4+ T cells and reduce the content of inflammatory factors TNF-α and IFN-γ [114]. TGF-β can also induce Foxp3 expression, inhibit Th17 differentiation, and stimulate Treg development [103, 115], via its biological activities through transcription regulation of several genes. Activated TGF-β binds to TGF-β1 and TGF-β2 receptors, followed by induction of the formation of phosphor-mothers against decapentaplegic homolog 2 (pSmad2), pSmad3, and Smad4 complexes, thus activating the intracellular signal activation of TGF-β signaling [105, 116]. Studies have shown that the lack of TGF-β1 leads to severe colonic inflammation, while the restoration of TGF-β1 activity improves the resolution of colitis [117, 118]. MSCs can block the induction of inflammatory-associated TNF and interleukins while promoting T cells to secrete anti-inflammatory cytokine like IL-10. The secretion of polyethylene glycol (PEG) by MSCs in the process of inflammation resolution has also increased significantly, as PEG is an important regulator to maintain immune homeostasis [119].

The immunomodulatory effect of MSCs is not only due to the role of soluble factors but also the effect of intercellular contact. MSCs constitutively express FasL and PD-L1. FasL induces apoptosis of activated T cells, while PD-L1 on the surfaces of MSCs combine with PD-1 on the surfaces of T cells, exerting immunosuppression through major histocompatibility complex II (MHC II). MSCs can also secrete IFN-β to increase the expression of PD-1 on the surface of T cells and strengthen the inhibition of T cells [105, 107, 120]. Mice with PD-1 gene knocked out produce autoimmune diseases, which also prove that MSCs can inhibit the activation, expansion, and cytokine production of T cells through the PD-1/PD-L1 pathway [121]. Experiments prove that tonsil-derived MSCs (T-MSCs) weaken the differentiation of Th17 and directly regulate the phosphorylation of signal transducer and activator of transcription 3 (STAT3) through the PD-L1 expression [122]. The imbalance of the IL6/IL6R-STAT3-SOCS3 (suppressor of cytokine signaling 3) pathway is closely related to IBD-related diseases [123, 124]. MSCs express NOD2 (nucleotide-binding oligomerization domain-containing protein 2), and its binding with ligand MDP (muramyl dipeptide) enhances the production of PEG2 and increases the production of IL-10 and Tregs through NOD2-RIP2 (receptor-interacting protein 2) pathway. In several experimental colitis models in mice, MSCs have been demonstrated to highly express Jagged-1, induce Notch signaling of T lymphocytes, reduce the activity of NF-κB, reduce the production of IL-2 and IFN-γ, and hinder the proliferation of T lymphocytes [125–128].

Intercellular adhesion molecule-1 (ICAM-1), also known as CD54, is involved in signal transmission between cells, regulates immune response, and mediates cell differentiation, development related to lymphocyte homing and circulation. Generally, ICAM-1 is not expressed on the surface of MSCs, but ICAM-1 is upregulated in the inflammatory microenvironment. MSCs overexpressing ICAM-1 significantly reduced the percentage of Th1 and Th17 cells in the spleen, increasing the number of Tregs of IBD mice. Further analysis revealed remarkably reduced mRNA levels of INF-γ and IL-17A and promoted expression of Foxp3, thus alleviating the experimental colitis [129].

Nitric oxide (NO) produced by MSCs can also inhibit the expression of CD25 in T cells by regulating LKB1- (liver kinase B1-) AMPK- (adenosine 5′ monophosphate-activated protein kinase-) mTOR pathway. It is reported that the deletion of LKB1 decreases AMPK phosphorylation level and activates mTORC1, which leads to T cell activation and inflammation, while MSCs can increase LKB1 and AMPK phosphorylation level, thus exerting inhibitory effects on inflammatory T cell proliferation and increasing anti-

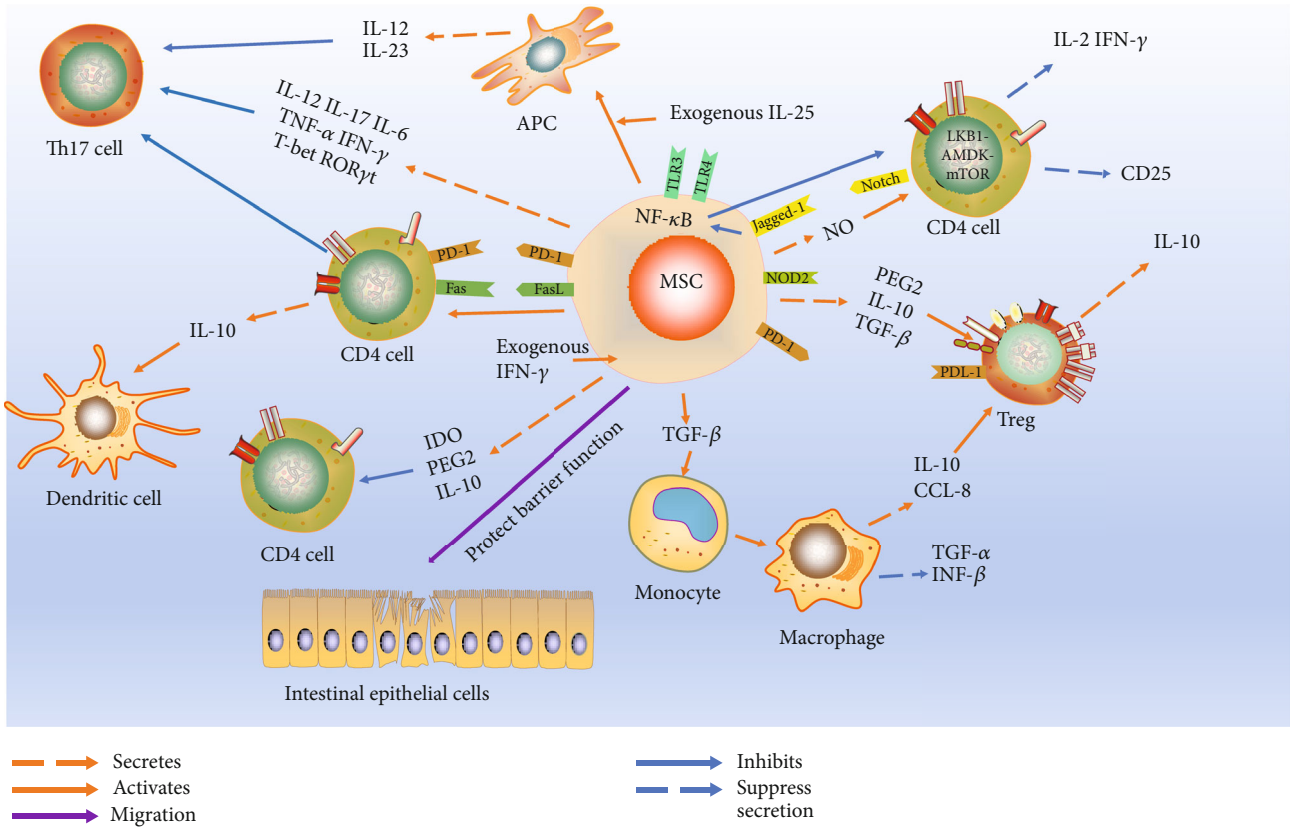

Figure 1: MSCs alleviate IBD by regulating T cells. MSCs induce CD4 T cells to differentiate into Treg and maintain Th17/Th1 balance through a series of cytokines and cell-to-cell contact. This results in decreased inflammatory activities to repair intestinal inflammation.

inflammation [130]. IL-25 can inhibit TNF-γ and IL-17A produced by CD4+ T cells of IBD patients, promote the secretion of anti-inflammatory IL-10, and inhibit the differentiation of CD4+ T cells of IBD into proinflammatory Th1 and Th17 cells [131, 132].

It is worth mentioning that peripheral circulating T lymphocytes play an important role in the MSC treatment mechanism of IBD. There are groups of intestinal intraepithelial lymphocytes (IELs) that are similar to peripheral lymphocytes in the intestinal tract and have complex cell subsets, including TCR-positive and TCR-negative cells. Every ten intestinal epithelial cells (IECs) in the small intestine contain about one IEL, which is lower in the colon. IELs reside in intestinal epithelial cells and do not participate in the circulation. They are associated with the maintenance of the intestinal mucosal immune barrier [133, 134]. The increase of IELs can be observed in the intestinal tract of children with IBD [135]. The imbalance of IEL subsets is related to the pathogenesis of IBD, but it is still unknown whether MSCs can regulate IELs.

IDO plays an important role in the regulation of MSCs on experimental enteritis in mice. MSCs can secrete IDO, which is a rate-limiting enzyme that catalyzes tryptophan metabolism. IDO and its downstream metabolites kynurenine (KYN) and kynurenic acid (KYNA) play a powerful role in inhibiting T cell proliferation and Treg differentiation [136, 137]. IDO can alleviate DSS-induced enteritis by regulating tryptophan metabolites KYN and KYNA in MSCs, activating transcription factor aryl hydrocarbon receptor (AhR), and upregulating the expression of TNF-stimulated gene 6 (TSG-6) [138]. Under the action of the inflammatory microenvironment, MSCs enhance the glycolytic pathway and upregulate the IDO level through the Janus kinase (JAK)/STAT1 pathway, which plays an immunosuppressive role [139, 140]. The activities of MSCs in IBD as discussed above are summarized in Figure 1.

4. Regulatory Effect of MSCs on T Cells in RA

The mechanism by which MSCs regulate T cells to relieve RA overlaps with the mechanism of regulating IBD. Just as reported in other autoimmune diseases, there is an imbalance of T cell subsets in RA patients too, including Th17/Treg cells which are capably regulated by MSCs. The expression of TNF-α inducible protein 3 (TNFAIP3), also known as A20, from BM-MSCs of RA patients has been found to be reduced. TNFAIP3 is a protective protein of chronic arthritis, which can negatively regulate the NF-κB pathway and reduce the expression of IL-6. MSCs overexpressing A20 can inhibit the expression of IL-6, thus restoring Th17/Treg balance. A20 deficiency also increases Th17 and decreases Tregs, while Th1 and Th2 are not affected. Specifically, inflammatory cytokines induce A20 expression in MSCs. Mechanism studies show that knocking out A20 in MSCs can inhibit the activation of the p38 mitogen-activated protein kinase (MAPK) pathway, effectively promote the production of

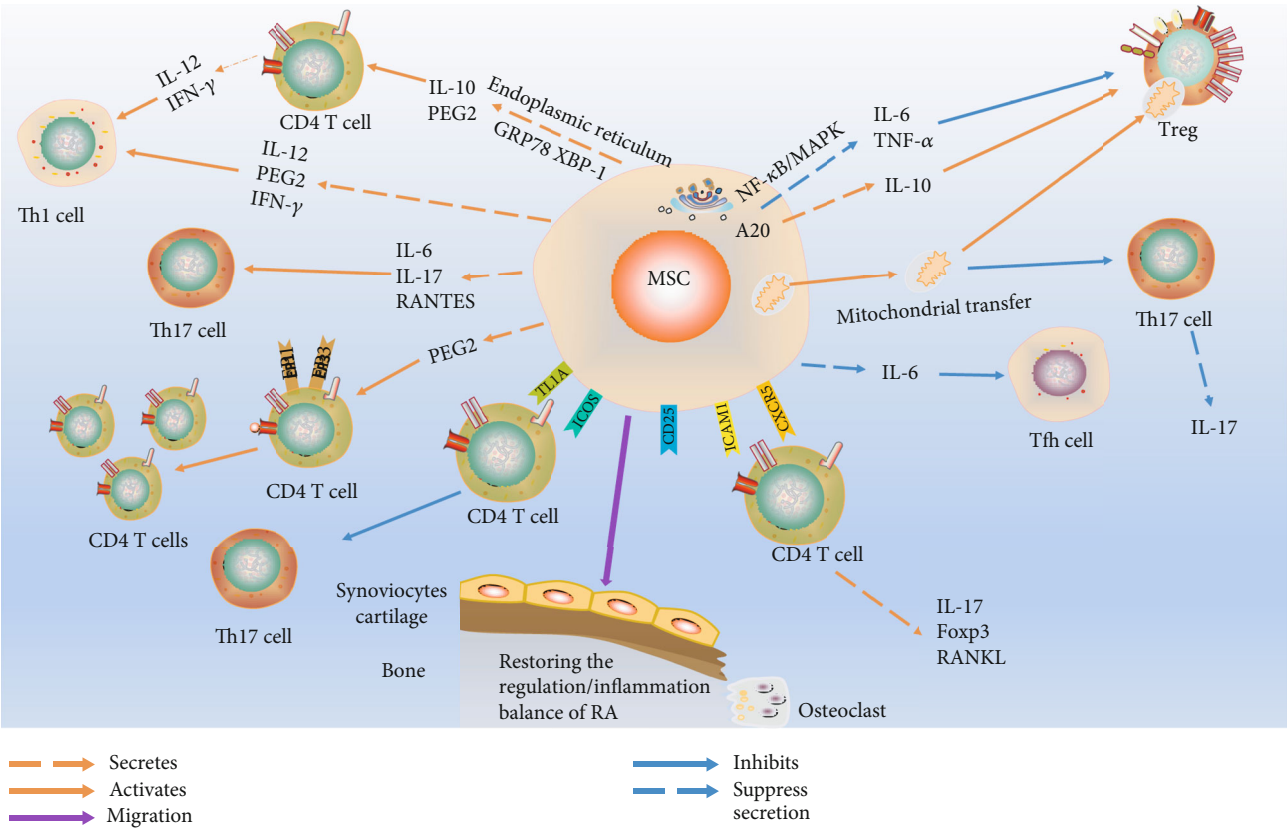

Figure 2: MSCs relieve RA by regulating T cells. MSCs can regulate the balance of T cells by homing to the articular cavity and secreting a series of cytokines that increase the anti-inflammatory activity of the environment. T cells are also regulated via the transfer of mitochondria from MSCs to T cells. Additionally, MSCs under endoplasmic reticulum stress can also play a regulatory role in inducing T cells.

TNF-α, and inhibit the production of IL-10. It is worth noting that the therapeutic effect of MSCs on RA may be different due to the different expression of A20 [76, 141]. Mitochondrial transfer from MSCs to Th17 cells, as a mechanism of MSC regulating immunity, can occur through intercellular contact, resulting in increased oxygen consumption of Th17 and reduced production of IL-17. At the same time, the mitochondria of Treg markers on the surface of Th17 cells increase, indicating that mitochondria from MSCs can increase the production of anti-inflammatory phenotype [142]. Inflammatory microenvironment can lead to the activation of the PI3K/Akt/mTORC1 pathway, which is closely related to cell metabolism and can enhance glycolysis and activate lymphocytes. Mitochondrial metastasis can transform energy metabolism into oxidative phosphorylation, and Treg-related markers are upregulated while proinflammatory markers are downregulated [143, 144].

In addition to Th17 cells, T follicular helper (Tfh)/T follicular regulatory (Tfr) cells are also closely related to RA. It is reported that the number of Tfh and Tfr in RA patients is increased, but the ratio of Tfr/Tfh is decreased, with a significantly increased number of circulating B cells related to Tfh [145, 146]. The production of autoantibodies in RA patients, such as antirheumatoid factor (RF) and anticyclic citrullinated peptide (CCP), leads to the deposition of immune complexes, while Tfh cells can migrate to the germinal center (GC) to maintain the differentiation of B cells. Tfh is closely related to the production of autoantibodies in B cells. Tfh cells express high levels of CXCR5, PD-1, IL-21, and other characteristic markers, and their cellular differentiation is regulated by a complex network of transcription factors, including positive factors (Bcl6, ATF-3, Batf, IRF4, c-Maf, etc.) and negative factors (Blimp-1, STAT5, IRF8, Bach2, etc.) [147, 148]. On the other hand, Tfr is a type of cell in the Treg subgroup, which can inhibit the reaction in GC and the production of high-affinity antibody. As an inflammatory factor, IL-6 plays a role in the pathogenesis of RA, by phosphorylating STAT3 and participating in Tfh differentiation [149]. MSCs can significantly reduce the production of IL-6 in vivo, which may have an alleviating effect by regulating Tfh/Tfr. Whether MSCs can regulate these transcription factors and participate in the regulation of Tfh cells in RA patients is rarely reported at present, which is also the direction of future research.

MSCs can exert their immune function and relieve autoimmune diseases through PEG2, TGF-β, HGF, IL-10, and IDO, which are found in RA, IBD, and other autoimmune diseases. Studies have shown that endoplasmic reticulum- (ER-) stressed MSCs can produce higher levels of IL-10 and PEG2 than ordinary MSCs and downregulate CD4+CXCR5+ICOS+ T cells (Tfh) in RA patients. It may be that glucose-regulated protein 78 (GRP78) and X-box binding protein 1 (XBP-1) are strongly induced in ER-stressed MSCs, resulting in a large amount of PEG2 production. PEG2 receptors, membrane-bound G

protein-coupled receptors termed EP1, EP2, EP3, and EP4, are expressed on CD4+ T cell surface. PEG2 can also increase the levels of IL-12 and IFN-γ, which triggers Th1 cells to differentiate. The anti-T cell proliferation effect is realized by the EP/COX2/PEG2 axis, wherein COX2 is upregulated under the condition of inflammatory stress [150–152].

In RA patients, MSCs can phagocytize apoptotic cells (ACs) in an actin-dependent way and secrete IL-6, IL-17, and RANTES (regulated upon activation, normal T cell expressed and presumably secreted). In this process, MSCs can express CXCR4, CXCR5, and ICAM-1 and migrate to inflammatory joints through the SDF/CXCR4 pathway, which makes synovial CD4+CD25+CD69+ T cells increase and makes them express IL-17, FoxP3, and RANKL, known to promote the increase of osteoclasts. Th17 cell differentiation depends on IL-6 and IL-1β, and intercellular contact mediated by costimulatory molecules CD25, ICOS (inducible costimulatory), and TL1A (TNF-like ligand 1A) can also participate in Th17 cell differentiation. The MSCs induced by RA can express IL-6 and MHCII and then increase the level of IL-17. Cytokines and intercellular contact promote Th17 cells and osteoclast formation. In other studies, RA-induced MSCs did not change the number of CD4+FOXP3+Treg; therefore, MSCs may enhance the pathogenic effect of RA in patients; hence, MSC therapy for RA should be carefully considered [153, 154].

CD4+ T cells expressed by the granulocyte-macrophage colony-stimulating factor (GM-CSF) play certain roles in RA induction. AD-MSCs can reduce the number of GM-CSF+CD4+ T cells. MSCs participate in regulating immune response by promoting early adaptive regulatory T cell signals, which is characterized by a decrease in the level of T cells secreting pathogenic GM-CSF, an increase in the number of Tregs, and the development of effector Th17 cells towards IL-10-driven anti-inflammatory response, thus restoring the regulation/inflammation balance of RA [155]. The effects of MSCs within the RA environment are illustrated in Figure 2.

5. Regulatory Effect of MSCs on T Cells in SLE

Literature indicates that the disorder of AC clearance mechanism may be one of the pathogenesis of SLE [67]. It has been demonstrated that MSCs phagocytize ACs and regulate immune homeostasis in vivo [154], in a time- and dose-dependent manner. MSCs exposed to ACs activate the NF-κB pathway by recognizing phosphatidylserine, which leads to highly expressed COX2, associated with the production of a large amount of PEG2. MSCs activated by ACs can also inhibit the proliferation of CD4+ T cells more strongly than controls, which are related to soluble cytokines IFN-γ and IL-17. However, how ACs activate the NF-κB pathway in MSCs is still unknown.

The decreased expression of CD4, CD25, and Foxp3 indicates a reduced number of Tregs in SLE patients. While MSCs recover Tregs through secretion of TGF-β, Tregs inhibit the response and the production of autoantibodies by B cells through the induction of apoptosis related to the expression of granzyme A, granzyme B, and perforin [156, 157].

Abnormal methylation of T cells in SLE patients leads to overexpression of methylation-sensitive autoimmune genes CD70, ITGAL (integrin subunit alpha L) (CD11a), selectin-1, IL-4, and IL-13 in lupus. CD70, a costimulatory factor, activates B cell response. ITGAL is related to the self-activation of T cells. Studies have shown that MEK/ERK pathway defects and T cell methylation changes in SLE patients lead to increased immune disorders. The MEK/ERK pathway of PBMC from SLE patients can be active after coculture with BM-MSCs. After coculture, DNA methyltransferase 1 DNMT1 was upregulated, and CD7, CD70, integrin, ITGAL, selectin-1, and IL-13 were downregulated in PBMC of patients. BM-MSCs downregulate the expression of methylation-related genes and reduce the self-activation of PBMC through the MEK/ERK pathway [158–160].

Several other studies have investigated the Th17 and Treg imbalance found in SLE patients. Stress response and immune regulation molecule heme oxygenase-1 (HO-1) are involved in the induction of Tregs. MSCs can express HO-1 and participate in the induction of Treg cell subsets, but there are big individual differences. Perhaps HO-1 expressed by MSCs can play certain roles in SLE patients, and the specific mechanism needs to be studied [161, 162].

6. Conclusion

As a cell-based therapy, MSCs possess the potential of ameliorating injury or possibly offering a cure for patients with immune-mediated conditions. Available document on the contribution of MSCs in restoring T cell balance within the autoimmune environment is promising, as the MSCs exert their immunoregulatory effects via direct contact and secretion of active factors. The mechanism of action of MSCs overlaps and has quite a few differences in various autoimmune diseases, which may be related to the origin of MSCs and the heterogeneity of autoimmune diseases. Additionally, MSCs treated with certain factors to overexpress desired cytokines result in stronger regulation of T cell immunity. Further explorations of key targets of MSCs during T cell regulation and their associated mechanisms in autoimmune diseases are needed to enhance understanding towards improving the therapeutics of MSCs.

Conflicts of Interest

All authors declare no conflict of interest.

Authors' Contributions

Zhiping Wei and Jintao Yuan contributed equally to this work. All authors approved the final version of the article.

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