Transplantation of ACE2$^-$ mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia

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Abstract

A coronavirus (HCoV-19) has caused the novel coronavirus disease (COVID-19) outbreak in Wuhan, China, Preventing and reversing the cytokine storm may be the key to save the patients with severe COVID-19 pneumonia. Mesenchymal stem cells (MSCs) have been shown to possess a comprehensive powerful immunomodulatory function. This study aims to investigate whether MSC transplantation improve the outcome of 7 enrolled patients with COVID-19 pneumonia in Beijing YouAn Hospital, China from Jan 23, 2020. to Feb 16, 2020. The clinical outcomes, as well as changes of inflammatory and immune function levels and adverse effects of 7 enrolled patients were assessed for 14 days after MSC injection. MSCs could cure or significantly improve the functional outcomes of seven patients with COVID-19 pneumonia in 14 days without observed adverse effect. The pulmonary function and symptoms of all patients with COVID-19 pneumonia were significantly improved in 2 days after MSC transplantation. Among them, two common and one severe patient were recovered and discharged in 10 days after treatment. After treatment, the peripheral lymphocytes were increased and the overactivated cytokine-secreting immune cells CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells were disappeared in 3-6 days. And a group of CD14+CD11c+CD11b^{mid} regulatory DC cell population dramatically increased. Meanwhile, the level TNF-α is significantly decreased while IL-10 increased in MSC treatment group compared to the placebo control group. Furthermore, the gene expression profile showed MSCs were ACE2^{-} and TMPRSS2^{-} which indicated MSCs are free from COVID-19 infection. Thus, the intravenous transplantation of MSCs was safe and effective for treatment in patients with COVID-19 pneumonia, especially for the patients in critically severe condition.

Key words

COVID-19, ACE2 negative, mesenchymal stem cells, cell transplantation, immunomodulatory, function recovery

Introduction

The novel coronavirus disease 2019 (COVID-19) has grown to be a global public health emergency since patients were first detected in Wuhan, China, in December 2019. Since then, the number of COVID-19 confirmed patients have sharply increased not only in China, but also worldwide, including Germany, South Korea, Vietnam, Singapore, and USA[1]. Currently, no specific drugs or vaccines are available to cure the patients with COVID-19 infection. Hence, there is a large unmet need for a safe and effective treatment for COVID-19 infected patients, especially the severe cases.

Several reports demonstrated that the first step of the HCoV-19 pathogenesis is that the virus specifically recognizes the angiotensin I converting enzyme 2 receptor (ACE2) by its spike protein[2-4]. ACE2-positive cells are infected by the HCoV-19, like SARS-2003[5,6]. In addition, a research team from Germany revealed that the cellular serine protease TMPRSS2 for HCoV-19 Spike protein priming is also essential for the host cell entry and spread[7], like

the other coronavirus (i.e. SARS-2003)[8,9]. Unfortunately, the ACE2 receptor is widely distributed on the human cells surface, especially the alveolar type II cells (AT2) and capillary endothelium[10], and the AT2 cells highly express TMPRSS2[9]. However, in the bone marrow, lymph nodes, thymus, and the spleen, immune cells, such as T and B lymphocytes, and macrophages are consistently negative for ACE2[10]. The findings suggest that immunological therapy may be used to treat the infected patients. However, the immunomodulatory capacity may be not strong enough, if only one or two immune factors were used, as the virus can stimulate a terrible cytokine storm in the lung, such as IL-2, IL-6, IL-7, GSCF, IP10, MCP1, MIP1A, and TNFα, followed by the edema, dysfunction of the air exchange, acute respiratory distress syndrome, acute cardiac injury and the secondary infection[11], which may led to death. Therefore, avoiding the cytokine storm may be the key for the treatment of HCoV-19 infected patients. MSCs, owing to their powerful immunomodulatory ability, may have beneficial effects on preventing or attenuating the cytokine storm.

MSCs have been widely used in cell-based therapy, from basic research to clinical trials[12,13]. Safety and effectiveness have been clearly documented in many clinical trials, especially in the immune-mediated inflammatory diseases, such as graft versus-host disease (GVHD)[14] and systemic lupus erythematosus (SLE)[15]. MSCs play a positive role mainly in two ways, namely immunomodulatory effects and differentiation abilities[16]. MSCs can secrete many types of cytokines by paracrine secretion or make direct interactions with immune cells, leading to immunomodulation[17]. The immunomodulatory effects of MSCs are triggered further by the activation of TLR receptor in MSCs, which is stimulated by pathogen-associated molecules such as LPS or double-stranded RNA from virus[18,19], like the HCoV-19.

Here we conducted an MSC transplantation pilot study to explore their therapeutic potential for HCoV-19 infected patients. In addition, we also explored the underlying mechanisms using a 10× Genomics high throughput RNA sequencing clustering analysis on MSCs and mass cytometry.

Materials and Methods

Study design

A pilot trial of intravenous MSC transplantation was performed on seven patients with COVID-19 infected pneumonia. The study was conducted in Beijing YouAn Hospital, Capital Medical University, China, and approved by the ethics committee of the hospital (LL-2020-013-K). The safety and scientific validity of this study “Clinical trials of mesenchymal stem cells for the treatment of pneumonitis caused by novel coronavirus” from Shanghai University/ PUMC have been reviewed by the scientific committee at International Society on Aging and Disease (ISOAD) and issued in Chinese Clinical Trial Registry (ChiCTR2000029990).

The Patients

The patients were enrolled from Jan 23, 2020 to Jan 31, 2020. All enrolled patients were

confirmed by the real-time reverse transcription polymerase chain reaction (RT-PCR) assay of HCoV-19 RNA in Chinese Center for Disease Control and Prevention using the protocol as described previously[11,20]. The sequences were as follows: forward primer 5′-TCAGAATGCCAATCTCCCCAAC-3′; reverse primer 5′-AAAGGTCCACCCGATACATTGA-3′; and the probe 5′CY5-CTAGTTACACTAGCCATCCTTACTGC-3′BHQ1.

We initially enrolled patients with COVID-19 (age 18–95 years) according to the guidance of National Health and Health Commission of China (Table 1).

If no improvement signs were observed under the standard treatments, the patient would be suggested to receive the MSC transplantation. Patients were ineligible if they had been diagnosed with any kind of cancers or the doctor declared the situation to belong to the critically severe condition. We excluded patients who were participating in other clinical trials or who have participated in other clinical trials within 3 months.

Cell preparation and transplantation

The clinical grade MSCs were supplied, for free, by Shanghai University, Qingdao Co-orient Watson Biotechnology group co. LTD and the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. The cell product has been certified by the National Institutes for Food and Drug Control of China (authorization number: 2004L04792，2006L01037，CXSB1900004). Before the intravenous drip, MSCs were suspended in 100 ml of normal saline, and the total number of transplanted cells was calculated by 1×10^6 cells per kilogram of weight. The window period for cell transplantation was defined as the time when symptoms or/and signs still were getting worse even as the expectant treatments were being conducted. The injection was performed about forty minutes with a speed of ~40 drops per minute.

The patients were assessed by the investigators through the 14-day observation after receiving the investigational product. The clinical, laboratory, and radiological outcomes were recorded and certified by a trained group of doctors. The detailed record included primary safety data (infusional and allergic reactions, secondary infection and life-threatening adverse events) and the primary efficacy data (the level of the cytokines variation, the level of C-reactive protein in plasma and the oxygen saturation). The secondary efficacy outcomes mainly included the total lymphocyte count and subpopulations, the chest CT, the respiratory rate, and the patient symptoms (especially the fever and shortness of breath). In addition, the therapeutic measures (i.e. antiviral medicine and respiratory support) and outcomes were also examined.

Statistical analysis

MIMICS 21.0 (Interactive medical image control system of Materialise, Belgium) was used to evaluate the chest CT data. The analysis of Mass cytometry of the peripheral blood mononuclear cells is described in Supplementary Material 1. The analysis of the 10 x RNA-seq survey is described in Supplementary Material 2. Data were analyzed by SPSS software (SPSS 22.0). Differences between two groups were assessed using unpaired two-tailed t tests. Data

involving more than two groups were assessed by analysis of variance (ANOVA). P values <0.05 indicated statistical significance.

Results

MSC treatment procedure and general patient information

This study was conducted from Jan 23, 2020, to Feb 16, 2020. Seven confirmed COVID-19 patients, including 1 critically severe type (patient 1), 4 severe types (patient 2, 3, 6, 7) and 2 common types (patient 4, 6) were enrolled. The timepoint of MSC transplantation for each patient is as shown in Figure 1. The general information of the 7 patients is listed in Table 1. Hitherto, the critically severe patient had completed the MSC treatment. This patient had a 10-year medical history of hypertension with the highest-level of 180/90 mmHg recorded. All the treatment information of the patients was collected.

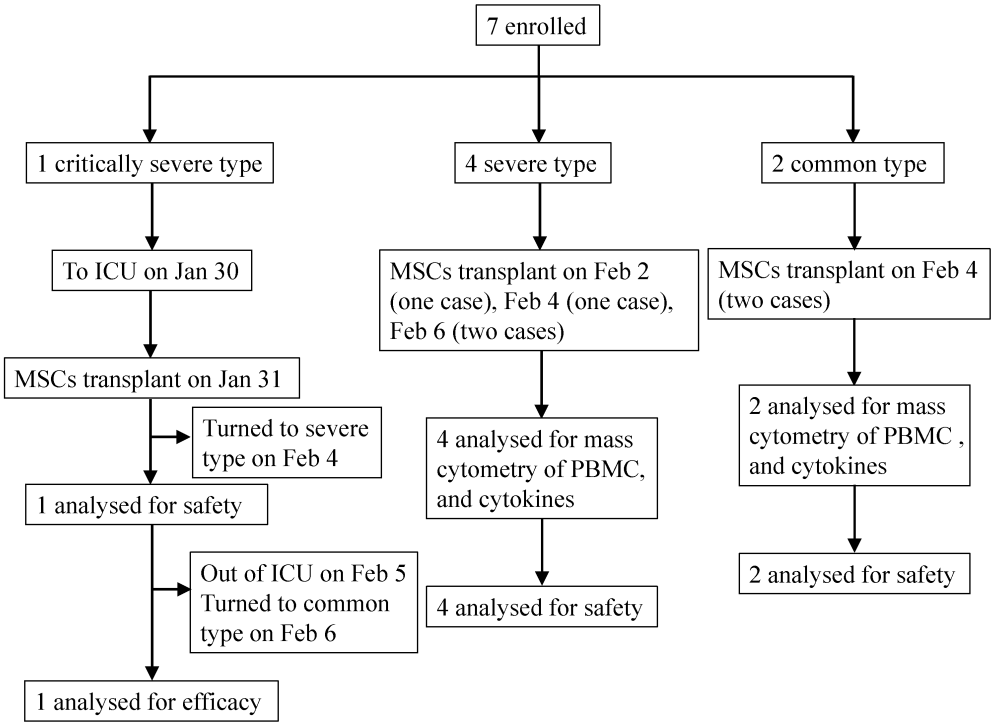

Figure 1. The flow chart of the cell transplantation treatment

The primary safety outcome

Before the MSC transplantation, the patients had symptoms of high fever (38.5℃ ± 0.5℃), weakness, shortness of breath, and low oxygen saturation. However, 2~4 days after transplantation, all the symptoms were disappeared in all the patients, the oxygen saturations rose to ≥ 95% at rest, without or with oxygen uptake (5 liters per minute). In addition, no acute infusion-related or allergic reactions were observed within two hours after transplantation. Similarly, no delayed hypersensitivity or secondary infections were detected after treatment.

The detailed diagnosis and treatment procedures of the critically severe patient are shown in Supplementary Material 3. The main symptoms and signs are shown in Table 3.

The efficacy outcome

The immunomodulating function of MSCs contributed to the main efficacy outcome and the transplantation of MSCs showed impressive positive results (Table 3). For the primary outcome in the critically severe patient 1, the plasma C-reaction protein level decreased from 105.5 g/L (Jan 30) to 10.1 g/L (Feb 13), which reached the highest level of 191.0 g/L on Feb 1, indicating that the inflammation status was alleviating quickly. The oxygen saturation, without Supplementary oxygen, rose from 89% (Jan 31) to 98% (Feb 13), which indicated the pulmonary alveoli regained the air-change function.

The secondary outcomes were also improved (Table 4). Considering, for example, the critically severe patient 1, the lymphopenia was significantly improved after the cell transplantation. The patient was isolated in the hospital isolation ward with a history of hypertension and blood pressure reaching grade 3 hypertension. On Feb 1, biochemical indicators in the blood test showed that aspartic aminotransferase, creatine kinase activity and myoglobin increased sharply to 57 U/L, 513 U/L, and 138 ng/ml, respectively, indicating severe damage to the liver and myocardium. However, the levels of these functional biochemical indicators were decreased to normal reference values in 2~4 days after treatment (Table 4). On February 13, all the indexes reached to normal levels, namely 19 U/L, 40 U/L, and 43 ng/ml, respectively. The respiratory rate was decreased to the normal range on the 4th day after MSC transplantation. Both fever and shortness of breath disappeared on the 4th day after MSCs transplantation. Chest CT imaging showed that the ground-glass opacity and pneumonia infiltration had largely reduced on the 9th day after MSC transplantation (Figure 2).

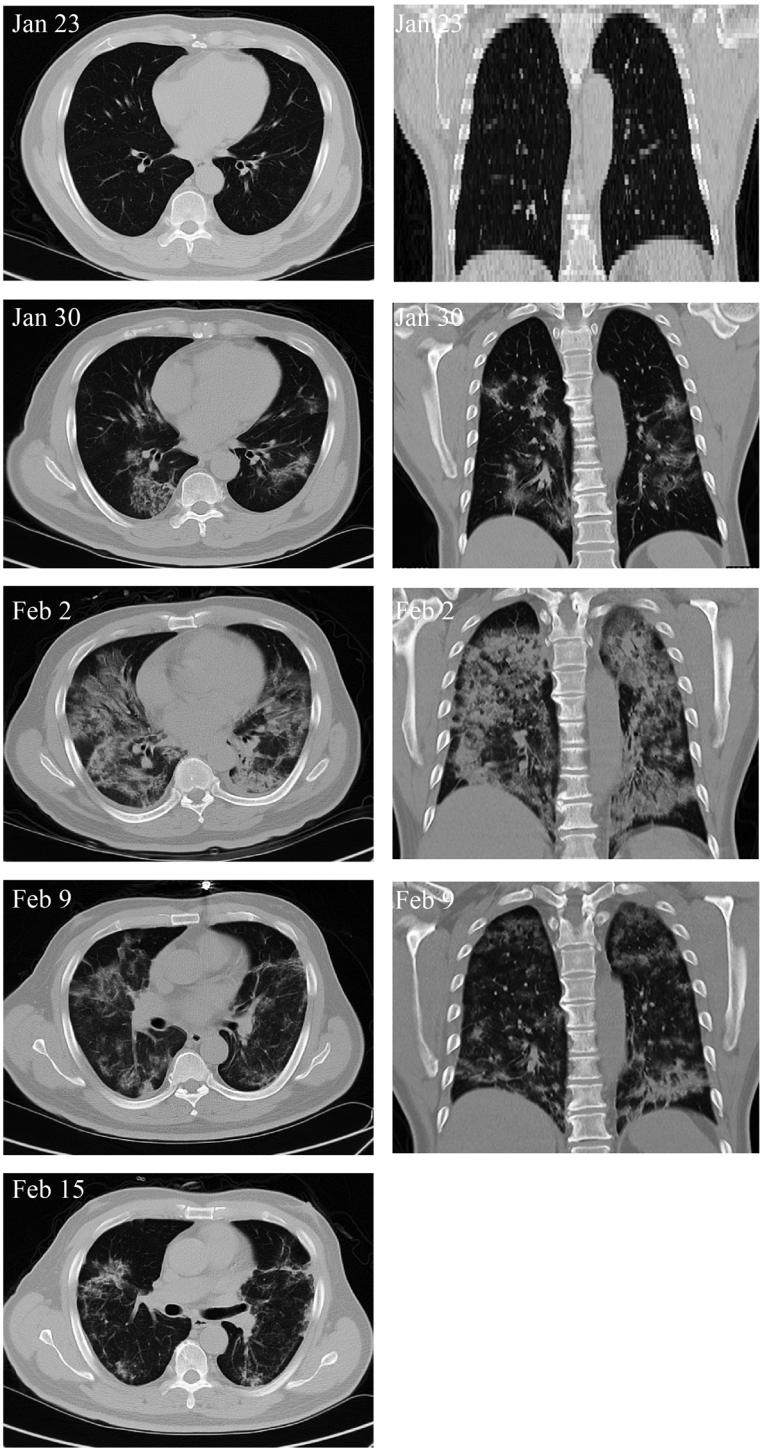

Figure 2. Chest computerized tomography (CT) images of the critically severe COVID-19 patient. On Jan 23, no pneumonia performance was observed. On Jan 30, ground-glass opacity and pneumonia infiltration occurred in multi-lobes of the double sides. Cell transplantation was performed on Jan 31. On Feb 2, the pneumonia invaded all through the whole lung. On Feb 9, the pneumonia infiltration faded away very much. On Feb 15, only little ground-glass opacity was residual in local.

HCoV-19 nucleic acid detection

RT-PCR analysis of HCoV-19 nucleic acid was performed before and after MSC transplantation. For the critically severe patient, before transplantation (Jan 23) and 6 days after transplantation (Feb 6), HCoV-19 nucleic acid was positive. 13 days after transplantation (Feb 13), HCoV-19 nucleic acid turned to be negative. The patient 3, 4,5 also turned to be negative result of HCoV-19 nucleic acid until this report date.

Mass cytometry (CyTOF) analysis of the patients' peripheral blood

To investigate the profile of the immune system constitution during MSC transplantation, we performed the CyTOF to analyze immune cells in the patients' peripheral blood before and after transplantation. CyTOF revealed that there was nearly no increase of regulatory T cells ($CXCR3^-$) or dendritic cells (DC, $CXCR3^-$) for the two patients of common type (Patient 4 and 5). But in the severe patients, both the regulatory T cells and DC increased after the cell therapy, especially for the critically severe patient. Notably, no significant $CXCR3^-$ DC enhanced after placebo treatment in three severe control patients. Moreover, for the critically severe patient, before the MSC transplantation the percentage of CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells in the patient's PBMC were remarkably increased compared to the healthy control, which caused the inflammatory cytokine storm. However, 6 days after MSC transplantation, the overactivated T cells and NK cells nearly disappeared and the numbers of the other cell subpopulations were almost restored to the normal levels, especially the $CD14+CD11c+CD11b^{mid}$ regulatory dendritic cell population (Figure 3).

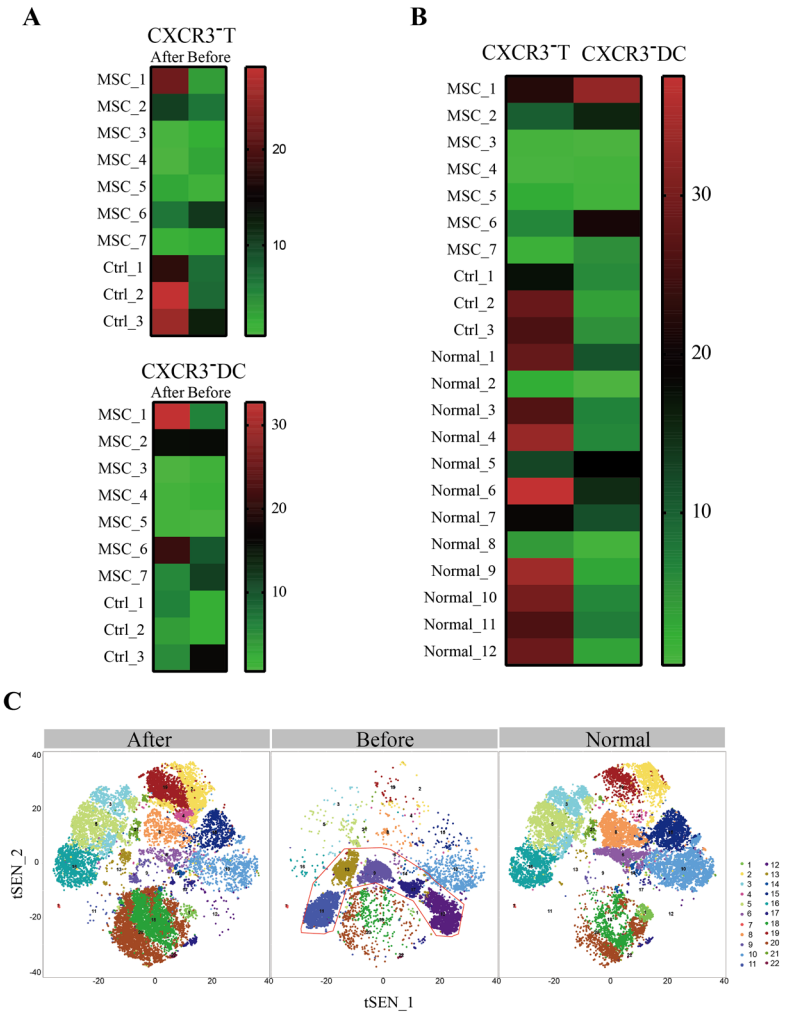

Figure 3. The mass cytometry results of peripheral blood mononuclear cells of the enrolled patients (A, B) and the critically severe patient (C). No increase of regulatory T cells (CXCR3-) or dendritic cells (DC, CXCR3-) for the two patients of common type (Patient 4 and 5, Figrue 3A). But in the severe patients, both the regulatory T cells and DC increased after the cell therapy, especially for the critical severe patient 1 (Figure 3B). Moreover, for the critically severe patient 1, before the MSC transplantation the percentage of overactivated CXCR3+CD4+ T cells (#9), CXCR3+CD8+ T cells (#17), and CXCR3+ NK cells (#12) in the patient's PBMC were remarkably increased compared to the healthy control (Figure 3C). However, 6 days after MSC transplantation, the overactivated T cells and NK cells nearly disappeared and the numbers of the other cell subsets were almost reversed to the normal levels, especially the CD14+CD11c+CD11b^{mid} DC (#20) population. Normal: health individuals, MSCs: mesenchymal stem cells transplant group, Ctrl: placebo control group.

Serum Cytokine/Chemokine/Growth Factor Analysis

After intravenous injection of MSCs, the decrease ratio of pro-inflammatory cytokine in serum TNF-α before and after MSC treatment was significant ($p<0.05$). Meanwhile, the increase ratio of anti-inflammatory IL-10 ($p<0.05$) also showed remarkably in the MSC treatment group. The

serum levels of chemokines like IP-10 and growth factor VEGF were both increased, though not significantly (Figure 4).

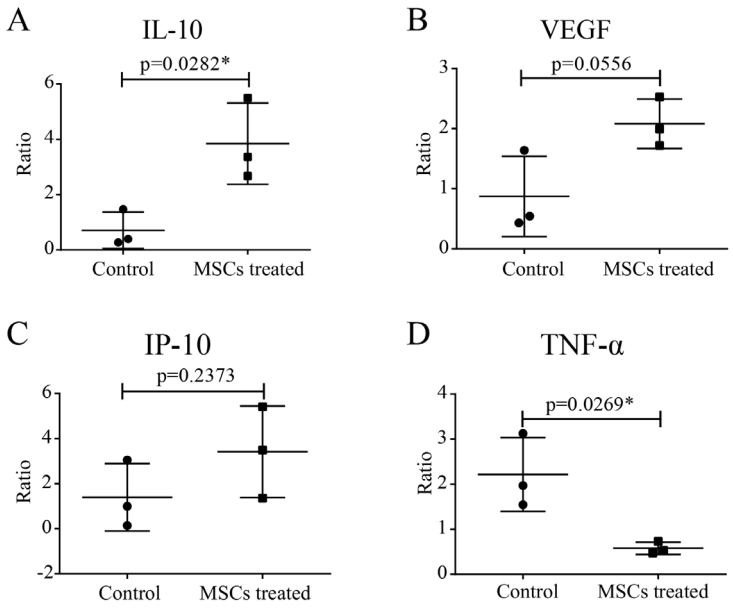

Figure 4. The ratio of serum cytokines IL-10 (A), growth factor VEGF (B), the chemokine IP-10 (C) and TNF-α (D) before and after MSCs treatment were detected in severe patients compared with the control group without MSCs by panel assay analysis, respectively. Ctrl: placebo control group. P-values were determined using the student's t-test. *P < 0.05.

10 x RNA-seq analysis for transplanted MSCs

To further elucidate the mechanisms underlying MSC-mediated protection for COVID-19 infected patients, we performed the 10 x RNA-seq survey for transplanted MSCs. The 10 x RNA-seq survey captured 12,500 MSCs which were then sequenced with 881,215,280 raw reads totally (Supplementary Material 4). The results revealed that MSCs are ACE2 or TMPRSS2 negative, indicating that MSCs are free from COVID-19 infection. Moreover, anti-inflammatory and trophic factors like TGF-β, HGF, LIF, GAL, NOA1, FGF, VEGF, EGF, BDNF, and NGF were highly expressed in MSCs, further demonstrating the immunomodulatory function of MSCs. Moreover, SPA and SPC were highly expressed in MSCs, indicating that MSCs might differentiate to AT2 cells (Figure 5). KEGG pathway analysis showed that MSCs were closely involved in the antiviral pathways (Supplementary Material 4).

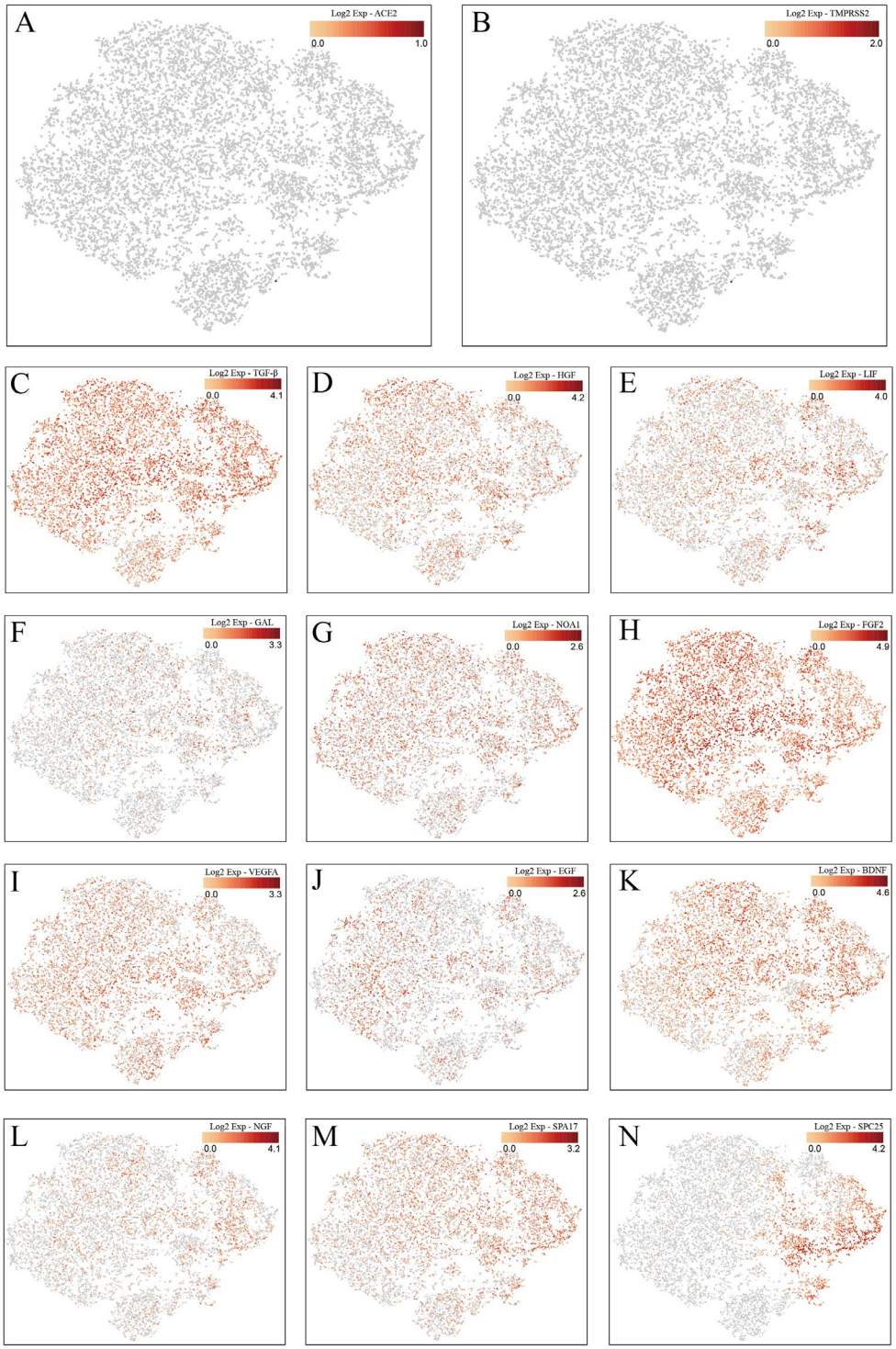

Figure 5. The 10 x RNA-seq survey of MSCs genes expression: Both ACE2 (A) and TMPRSS2 (B) were rarely expressed. TGF-β (C), HGF (D), LIF (E), GAL (F), NOA1 (G), FGF (H), VEGF (I), EGF (J), BDNF (K), and NGF (L) were highly expressed, indicating the immunomodulatory function of MSCs. SPA (M) and SPC (N) were highly expressed, indicating MSCs owned the ability to differentiate into the alveolar epithelial cells II. One point represented one cell, and red and gray color showed high expression and low expression,

respectively.

Discussion

Both the novel coronavirus and SARS-2003 could enter the host cell by binding the S protein on the viral surface to the ACE2 on the cell surface[3,5]. In addition to the lung, ACE2 is widely expressed in human tissues, including the heart, liver, kidney, and digestive organs[10]. In fact, almost all endothelial cells and smooth muscle cells in organs express ACE2, therefore once the virus enters the blood circulation, it spreads widely. All tissues and organs expressing ACE2 could be the battlefield of novel coronavirus and immune cells. This explains why not only all infected ICU patients are suffering from acute respiratory distress syndrome, but also complications such as acute myocardial injury, arrhythmia, acute kidney injury, shock, and death of multiple organ dysfunction syndrome[11](Figure 6). Moreover, the HCoV-19 is more likely to affect older males with comorbidities and can result in severe and even fatal respiratory diseases such as acute respiratory distress syndrome[21], like the critically severe case here. However, the cure of COVID-2019 is essentially dependent on the patient's own immune system. When the overactivated immune system kills the virus, it produces a large amount of inflammatory factors, leading to the severe cytokine storms[20]. It suggests that the main reason of these organs damage may be due to virus-induced cytokine storm. Older subjects may be much easier to be affected due to immunosenescence.

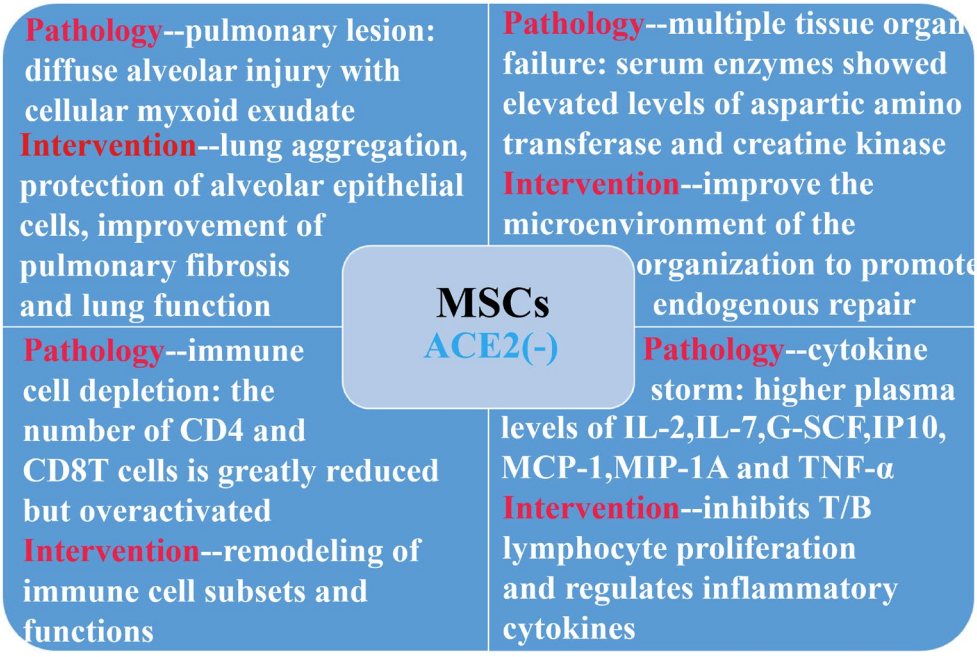

Figure 6. ACE2$^-$ MSCs benefit the COVID-19 patients via immunoregulatory function

Our 10x scRNA-seq survey shows that MSCs are ACE2$^-$ and TMPRSS2$^-$ (to the best of our knowledge, it is the first time to be reported) and secrete anti-inflammatory factors to prevent the cytokine storm. They have the natural immunity to the HCoV-19. According to the mass

cytometry streaming results, the virus infection caused a total function failure of the lymphocytes, even of the whole immune system. MSCs played the vital immune modulation roles to reverse the lymphocyte subsets mainly through dendritic cells. Our previous study showed that co-culture with MSCs could decrease the differentiation of cDC from human CD34+ cells, while increasing the differentiation of pDC through PGE2[22]. Furthermore, the induction of IL-10–dependent regulatory dendritic cells and IRF8-controlled regulatory dendritic cells from HSC were also reported in rats[23,24]. MSCs could also induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population[25]. All these interactions with different dendritic cells led to a shift of the immune system from Th1 toward Th2 responses.

Several reports also focused on lymphopenia and high levels of C-reactive protein in COVID-19 patients[20,21]. C-reactive protein is a biomarker with high-sensitivity for inflammation and host response to the production of cytokines, particularly TNFα, IL-6, MCP1 and IL-8 secreted by T cells[26]. However, most mechanistic studies suggest that C-reactive protein itself is unlikely to be a target for intervention. C-reactive protein is also a biomarker of myocardial damage[27].

MSC therapy can inhibit the overactivation of the immune system and promote endogenous repair by improving the microenvironment. After entering the human body through intravenous infusion, part of the MSCs accumulate in the lung, which could improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis and improve lung function.

As reported by Cao's team[11], the levels of serum IL-2, IL-7, G-SCF, IP10, MCP-1, MIP-1A and TNF-α in ICU patients were higher than those of normal patients. The cytokine release syndrome caused by abnormally activated immune cells deteriorated the patient's states which may cause disabled function of endothelial cells, the capillary leakage, the mucus block in lung and finally the respiratory failure. And they could cause even an inflammatory cytokine storm lead to multiple organ failure. The administration of intravenous injection of MSCs significantly improved the inflammation situation in severe COVID-19 patients. Due to its unique immunosuppression capacity, the serum levels of pro-inflammatory cytokines and chemokines were reduced dramatically which attracted less mononuclear/macrophages to fragile lung, while induced more regulatory dendric cells to the inflammatory tissue niche. Moreover, the increased IL-10 and VEGF promoted the lung's repair. Ultimately, the patients with severe COVID-19 pneumonia survived the worst condition and recovery.

Therefore, the fact that transplantation of MSCs improved the outcome of COVID-2019 patients may be through regulating inflammatory response and promoting tissue repair and regeneration.

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Conflicts of interest

We have no conflicts of interest.

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Table 1: Clinical classification of the COVID-19 released by the National Health and Health Commission of China

Mild	Common	Severe	Critically severe
Mild clinical manifestation, None Imaging Performance	Fever, respiratory symptoms, pneumonia performance on X-ray or CT	Meet any of the followings: 1. Respiratory distress, RR ≥ 30/min; 2. Oxygen saturation ≤ 93% at rest state; 3. Arterial partial pressure of oxygen (PaO_2) / Fraction of inspiration O_2 (FiO_2) ≤ 300mnHg, 1mmHg=0.133kPa	Meet any of the followings: 1. Respiratory failure needs mechanical ventilation; 2. Shock; 3. Combined with other organ failure, patients need ICU monitoring and treatment

Table 2: The general information of the enrolled patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Ctrl Patient 1	Ctrl Patient 2	Ctrl Patient 3
Gender	M	F	F	F	M	M	M	F	F	F
Age (years)	65	63	65	51	57	45	53	75	74	46
COVID-19 type	Critically severe	Severe	Severe	Common	Common	Severe	Severe	Severe	Severe	Severe
Fever (℃, baseline)	38.6	37.7	38.2	38.5	38.4	39.0	39.0	36.0	38.9	37.7
Shortness of breath	+++	+++	++	+	+	+++	+++	+++	++	+
Oxygen saturation at rest state	89%	93%	92%	95%	94%	92%	90%	91%	92%	93%
Cough, weak, poor appetite	++	+	++	+	++	++	++	+	++	+
Diarrhea	-	-	+	-	-	-	-	-	-	-
Date of diagnosed	Jan 23	Jan 27	Jan 25	Feb 3	Feb 2	Jan 27	Feb 3	Feb 3	Feb 6	Feb 5
Date of intervention (MSCs or Placebo)	Jan 31	Feb 2	Feb 4	Feb 4	Feb 4	Feb 6	Feb 6	Feb 8	Feb 6	Feb 6
Date of recovery	Feb 3	Feb 4	Feb 6 Discharged	Feb 6 Discharged	Feb 5 Discharged	Feb 7	Feb 7	Dead	ARDS	Stable

Table 3: Symptoms, signs and maximum body temperatures of the critically severe patient from Jan 21 to Feb 13, 2020. ICU: Intensive Care Unit; NA: Not Available

	Home	Hospital	Hospital	ICU	ICU	ICU	ICU	ICU	Out of ICU	Hospital	Hospital
Date	Jan 21~22	Jan 23	Jan 24~29	Jan 30	Jan 31	Feb 1	Feb 2~3	Feb 4	Feb 5~8	Feb 9~12	Feb 13
Fever (℃)	37.5	37.8	37.0~38.5	38.6	38.8	36.8	36.6~36.9	36.8	36.6~36.8	36.5~36.9	36.6
Shortness of breath	–	+	+	++	++++	++	+	–	–	–	–
Cough	+	+	+	++	++	+	+	–	–	–	–
Sputum	+	+	+	++	++	+	+	–	–	–	–
Oxygen saturation (without / with O_2 uptake)	NA / NA	NA / NA	97% / NA	91% / 95%	89% / 94%	NA / 98%	NA / 97%	NA / 96%	NA / 97%	96% / NA	97% / NA
Respiratory rate	NA	23	23	27	33	22	22	21	20~22	20~22	21
Treatment (Basics-1: Antipyretic, antiviral and supportive therapy. Basics-2: antiviral and supportive therapy)	NA	NA	Basics-1	Basics-1; Mask O_2 5L/min	Basics-1; Mask O_2 10L/min; Cell transplant	Basics-1; Mask O_2 5L/min	Basics-2; Mask O_2 5L/min	Basics-2; Mask O_2 5L/min	Basics-2; Mask O_2 5L/min	Basics-2	Basics-2
RT-PCR of the virus	NA	Positive	NA	NA	NA	NA	NA	NA	Positive (Feb 6)	NA	Negative

Table 4: The laboratory results of the critically severe patient. Red: the value was above the normal. Blue: the value was below the normal. NA: Not Available

	Reference range	Jan 24	Jan 30	Jan 31	Feb 1	Feb 2	Feb 4	Feb 6	Feb 10	Feb 13
C-reactive protein (ng/mL)	< 3.00	2.20	105.50	NA	191.00	83.40	13.60	22.70	18.30	10.10
Absolute lymphocyte count (× 10^9 per liter)	1.10-3.20	0.94	0.60	0.35	0.23	0.35	0.58	0.87	0.73	0.93
White-cell count (× 10^9 per liter)	3.50-9.50	4.91	6.35	7.90	7.08	12.16	12.57	11.26	10.65	8.90
Absolute neutrophil count (× 10^9 per liter)	1.80-6.30	3.43	5.43	7.28	6.63	11.33	11.10	9.43	9.18	7.08
Absolute monocyte count (× 10^9 per liter)	0.10-0.60	0.38	0.25	0.17	0.13	0.35	0.61	0.52	0.48	0.56
Red-cell count (× 10^{12} per liter)	4.30-5.80	4.69	4.68	4.66	4.78	4.73	4.75	5.16	4.69	4.53
Hemoglobin (g/L)	130.00-175.00	145.00	147.00	145.00	146.00	142.00	145.00	155.00	145.00	137.00
Platelet count (× 10^9 per liter)	125.00-350.00	153.00	148.00	169.00	230.00	271.00	268.00	279.00	332.00	279.00
Absolute eosinophil count (× 10^9 per liter)	0.02-0.52	0.02	0.02	0.02	0.02	0.02	0.05	0.15	0.14	0.14
Absolute basophilic count (× 10^9 per liter)	0.00-0.06	0.02	0.01	0.02	0.02	0.02	0.06	0.10	0.03	0.04
Total bilirubin (μmol/L)	5.00-21.00	7.00	23.00	21.70	19.80	14.20	15.80	16.50	12.50	8.70
Albumin (g/L)	40.00-55.00	41.70	32.30	29.70	29.90	31.60	33.00	32.20	30.10	29.10
Aspartate amino transferase (U/L)	15.00-40.00	14.00	33.00	48.00	57.00	39.00	34.00	23.00	25.00	19.00
Fibrinogen (g/L)	2.00-4.00	2.44	4.24	NA	NA	4.73	NA	3.12	3.84	3.73
Procalcitonin (ng/mL)	< 0.10	0.11	0.12	NA	NA	NA	0.10	0.18	0.15	< 0.10
Creatine kinase isoenzymes (ng/mL)	< 3.60	0.90	0.12	NA	5.67	4.24	NA	0.88	0.90	0.61
Creatine kinase (U/L)	50.00.310.00	168.00	231.00	NA	513.00	316.00	NA	47.00	83.00	40.00
Glomerular filtration rate (ml/min)	> 90.00	81.30	68.00	89.60	99.00	104.00	92.50	108.10	97.10	94.10
Potassium (mmol/L)	3.50.5.30	3.61	2.74	3.00	3.42	3.47	4.18	4.36	4.69	4.61
Sodium (mmol/L)	137.00.147.00	138.50	132.60	129.50	132.80	136.90	135.80	133.80	134.10	137.70
Myoglobin (ng/mL)	16.00.96.00	53.00	80.00	NA	138.00	77.00	NA	62.00	60.00	43.00
Troponin (ng/mL)	< 0.056	0.10	0.07	NA	0.05	0.05	NA	0.02	0.04	0.04

Supplementary Materials:

Supplementary 1: The method of Mass Cytometry of peripheral blood mononuclear cells (PBMC)

Sample preparation for mass cytometry

PBMC samples were collected from COVID-19 infected patients treated with MSCs transplantation at baseline and on Day 6, and PBMC from a healthy donor were set as the control group. All samples were cultured with 2 μM cisplatin (195-Pt, Fluidigm) for 2 minutes before quenching with CSB (Fluidigm) to identify the viability using mass cytometry analysis. A Fix-I buffer (Fluidigm) was then used to fix cells for 15 min at room temperature, followed by washing three times with phosphate buffer solution (PBS).

Mass cytometry antibody staining and CD45 barcoding

Three samples from the healthy donor, the patient at baseline and Day 6 were stained with CD45 antibodies that were labeled with different metal tags (89, 141 and 172) to minimize internal cross reaction between samples. MaxPar × 8 Polymer Kits (Fluidigm) were used to conjugate with purified antibodies (listed in Supplemental Table 1). All metal-conjugated antibodies were titrated for optimal concentrations before use. Cells were counted and diluted into 1×10^6 cells per milliliter in PBS and underwent permeabilization with 80% methanol for 15 minutes at 0°C. After triple washes in CSB, cells were cultured with antibodies in a total 50 μL CSD for 30 min in RT, triple washed in CSB and incubated with 0.125 μm intercalator in fix and perm buffer (Fluidigm) at 4 °C overnight.

Data acquisition in Helios

After cultured with intercalator, cells were washed three times with ice cold PBS and three times with deionized water. Prior to acquisition, samples were resuspended in deionized water containing 10% EQ 4 Element Beads (Fluidigm) and cell concentrations were adjusted to 1×10^6 cell/ml. Data acquisition was performed on a Helios mass cytometer (Fluidigm). The original FCS data were normalized and .fcs files for everyone were collected.

CyTOF Data Analysis

All .fcs files were uploaded into Cytobank, data cleaning and populations of single living cells were exported as .fcs files for further analysis. Files were loaded into R (http://www.rstudio.com), arcsinh transform was performed to signal intensities of all channels. PhenoGraph analysis was performed.

Supplementary 2: The method of the 10 x RNA-seq survey

Materials and reagents

All supplies and reagents were of the highest grade commercially available. The 0.20 μm-filters, dishes and tubes were purchased from Corning (NY, USA). CD105, CD90, CD44 and CD45 antibodies for the flow cytometry were purchased from Miltenyi Biotec (Bergisch gladbach, Germany). DMEM/F12, fetal bovine serum (FBS), GlutaMAX™-I, TrypLE™ Express, and penicillin and streptomycin antibiotics were purchased from Gibco (California, USA). All other reagents were analytical grade and required no further purification.

Supplemental Table 1: Antibodies used in the Mass cytometry analysis.

Antigen	Symbol and Mass	Antibody Clone	Source
CD45	89Y	HI30	Fluidigm
CD45	141Pr	HI30	Fluidigm
CD19	142Nd	HIB19	Fluidigm
CD5	143Nd	UCHT2	Fluidigm
CCR5	144Nd	NP-6G4	Fluidigm
CD4	145Nd	RPA-T4	Fluidigm
CD45RA	146N	HI100	Biolegend
CD20	147Sm	H1	Fluidigm
CD14	148Nd	RMO52	Fluidigm
CD56	149Sm	NCAM16.2	Fluidigm
CD11c	150Nd	Bu15	Biolegend
CD16	151Eu	3G8	Biolegend
TNFα	152Sm	MAb11	Fluidigm
CD62L	153Eu	DREG-56	Fluidigm
IL-1β	154Sm	Polyclonal	Abcam
CD27	155Gd	L128	Fluidigm
CXCR3	156Gd	G025H7	Fluidigm
IFN-r	158Gd	B27	Fluidigm
CCR7	159Tb	G043H7	Fluidigm
CD28	160Gd	CD28.2	Fluidigm
CD25	161Dy	BC96	biolegend
CD8	162Dy	RPA-T8	Fluidigm
TGFβ	16Dy	TW46H10	Fluidigm
CD45RO	164Dy	UCHL1	Fluidigm
IL-12	165Ho	Polyclonal	Abcam
IL-10	166Er	JES3-9D7	Fluidigm
IL-6	167Er	MQ2-13A5	Biolegend
CD206	168Er	15-2	Fluidigm
CD24	169Tm	ML5	Fluidigm
CD3	170Er	UCHT1	Fluidigm
CD68	171Yb	Y1/82A	Fluidigm
CD45	172Yb	HI30	biolegend
HLA-DR	173Yb	L243	Fluidigm
IL-4	174Yb	MP4-25D2	Biolegend
CD127	176Yb	A019D5	Fluidigm
CD11b	209Bi	ICRF44	Fluidigm

Cell culturing

The mesenchymal stem cells were cultured in DMEM/F12 medium supplemented with 2% FBS, 2%

GlutaMAX™-I, 1% antibiotics and 2 mM GlutaMAX™-I at 37°C with 5% CO_2. After three passages, MSCs were immune-phenotyped by flow cytometry for the following surface markers: CD105, CD90, CD73, CD29, HLA-DR, CD44, CD14 and CD45 (all antibodies from BD Pharmingen, San Jose, USA). And MSCs were tested for adipogenic, chondrogenic and osteogenic differentiation to identify their characters.

Cell preparation and Library construction

Cell count and viability were examined by microscope after 0.4% trypan blue coloring. When the viability was no lower than 80%, the library construction was performed. Library was constructed using the Chromium controller (10 x Genomics, Pleasanton, CA). Briefly, single cells, reagents and Gel Beads containing barcoded oligonucleotides were encapsulated into nanoliter-sized GEMs (Gel Bead in Emulsion) using the GemCode technology. Lysis and barcoded reverse transcription of polyadenylated mRNA from single cells were performed inside every GEM. Post RT-GEMs were cleaned up and cDNA were amplified. cDNA was fragmented and fragment ends were repaired, as well as A-tailing was added to the 3' end. The adaptors were ligated to fragments which were double sided SPRI selected. Another double sided SPRI selecting was carried out after sample index PCR. Quality control-pass libraries were sequenced. The final library was quantitated in two ways: determining the average molecule length using the Agilent 2100 bioanalyzer instrument; and quantifying the library by real-time quantitative PCR.

Analysis of single-cell transcriptomics data

The reads were demultiplexed by using the Cell Ranger Single Cell Software Suite (v3.1.0, 10 x Genomics) and R package Seurat (v3.1.0). The number of genes, unique molecule identifier (UMI) counts and percentage of mitochondrial genes were examined to identify outliers. Principal component analysis was used for dimensionality reduction. U-MAP was then used for two-dimensional visualization of the results. DEGs were identified with the FindConservedMarkers function in Seurat by parameters of logfc.threshold >0.25, minPct>0.25 and Padj≤0.05. KEGG pathways with FDR ≤0.05 were considered to be significantly enriched.

Supplementary 3: The detailed diagnosis and treatment procedures for the critically severe patient

On the evening of January 22, 2020, a 65-year-old man presented to the emergency department of Beijing YouAn Hospital, Beijing, with a 2-day history of cough, sputum and subjective fever. The patient wore a mask in the hospital. He disclosed to the physician that he had traveled in Wuhan, China, from December 31, 2019 to January 20, 2020 and returned to Beijing on January 20. Apart from a 10-year history of hypertension with the highest blood pressure of 180/90 mmHg ever, the patient had no other specific medical history. The physical examination showed a body temperature of 37.8, blood pressure of 138/85 mmHg, pulse of 85 beats per minute, respiratory rate of 19 breaths per minute. Lung auscultation revealed rhonchi. A blood routine examination was arranged urgently, and the result revealed that the white-cell count and absolute lymphocyte count were 4.9 × 10^9/L (reference range (3.5~9.5) × 10^9/L) and 0.94 × 10^9/L (reference range (1.1~3.2) × 10^9/L),

respectively (Table 1). According to the COVID-19 guidance released by the National Health Commission of China, the physician gave him a diagnosis of a suspected COVID-19 case and asked him to undergo medical isolation observation in the hospital. Meantime, the doctor collected his oropharyngeal swab specimen.

On January 23, 2020, the RT-PCR assay confirmed that the patient's specimen tested positive for HCoV-19. Then the patient was admitted to an airborne-isolation unit in Beijing YouAn Hospital for clinical observation. He had no dyspnea. His consciousness was clear, and the diet and sleep were normal since he became sick. A chest computed tomography (CT) was reported as showing no evidence of infiltrates or abnormalities. The admitting diagnoses were new coronary pneumonia (common type) and hypertension III. The patient received no special care except the irbesartan, which was taken all through the treatment period.

On January 24 to January 29, the patient's vital physical signs remained largely stable, apart from the development of intermittent fevers and shortness of breath. During this time, the patient received antipyretic therapy including 15 ml of ibuprofen suspension every 6 hours and 650 mg of acetaminophen every 6 hours. From January 26, the patient also received antiviral therapy including lopinavir and ritonavir twice a day, with the amount of 400 mg and 100 mg each time, respectively.

On January 30, the patient felt severe shortness of breath and appeared fatigued. The oxygen saturation values measured by pulse oximetry decreased to as low as 91% while he was breathing ambient air. Auscultation rhonchi became worse in the middle of the double sides of the lung. An urgent chest CT clearly showed evidence of pneumonia, ground-glass opacity, in the middle lobes of the right and left lung. The other positive results of laboratory tests included the C-reactive protein rise to 105.5 g/L (reference range < 3 g/L), but the absolute lymphocyte count decreased to 0.60×10^9/L. The potassium concentration went down to 2.74 mmol/L (reference range 3.5-5.5 mmol/L). The doctors decided to change the diagnosis to COVID-19 (critically severe type), and the patient was admitted to ICU unit. More treatments were conducted consisting of mask oxygen supplementation (5 liters per minute), electrocardiograph monitoring, potassium chloride sustained release tablets (oral, 500 mg per time, 3 times per day) and more glucose and amino acid injection. Finally, the discomfort was released, and the oxygen saturation increased to 95%.

On January 31, the shortness of breath even got worse under the oxygen supplementation. The doctor speeded up the oxygen airflow to 10 liters per minute. After the patient signed an agreement to perform the MSCs transplantation, 100 ml of normal saline including 6×10^7 MSCs was intravenously injected into the patient, and no adverse events were observed in association with the infusion.

On February 1 and 2, the patient did not feel better. The third chest CT revealed that the pneumonia got worse. On February 1, the levels of C-reactive protein were 191.0 g/L, and the absolute lymphocyte count decreased badly to 0.23×10^9/L. The laboratory results showed that his liver and myocardium were very likely to be affected. The electrocardiograph monitoring showed the blood pressure, heart rate, respiratory rate and oxygen saturation were 138/80 mmHg, 95 bpm, 33 bpm and 93% under the mask oxygen supplementation of 10 liters per minute. The doctors informed the

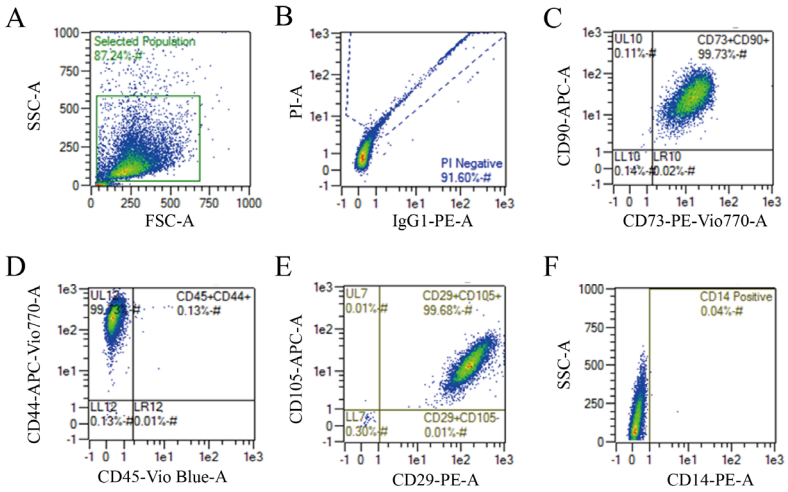
patient's families of a critical condition.

However, the patient felt better on February 3, for instance, the shortness of breath was significantly recovering. On February 4, the C-reactive protein decreased to 13.6 g/L, and the absolute lymphocyte count rose to 0.58 × 10^9/L, which indicated that the patient was recovering rapidly. The indexes of liver and myocardium function recovered. Both fever and shortness of breath disappeared on February 5. He was rolled out of ICU. On February 9, the fourth chest CT confirmed that the pneumonia was disappearing. On February 13, the C-reactive protein concentration was 10.1 g/L, and the absolute lymphocyte count was 0.93 × 10^9/L. Up to now, the patient felt much better.

Supplementary 4: More results of the 10 x RNA-seq surevey

Flow cytometry analysis

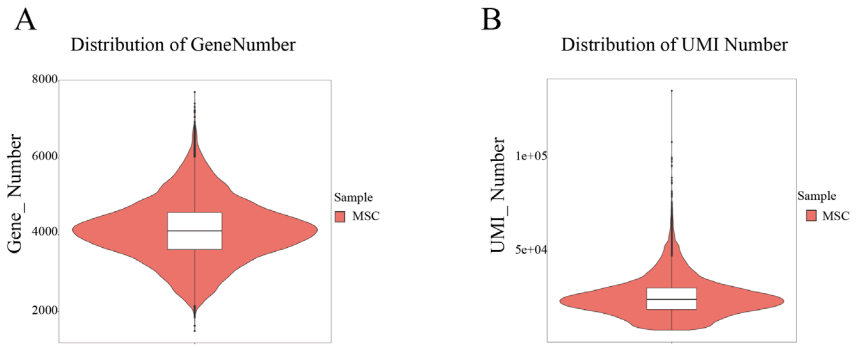
The PI staining results showed that 91.60% of the total cell population was alive, and the cells were: $CD105^+$, $CD90^+$, $CD73^+$, $CD44^+$, $CD29^+$, $CD14^-$ and $CD45^-$ (Supplemental Figure 1).



Supplemental Figure 1. Flow cytometry evaluation of transplanted MSCs (A) Single cells (87%) were gated firstly. (B) Live cells (91% of the single cells) were enrolled. (C-F) 99% of selected cells were $CD105^+$, $CD90^+$, $CD73^+$, $CD44^+$, $CD29^+$, $CD14^-$ and $CD45^-$.

The overview of the survey

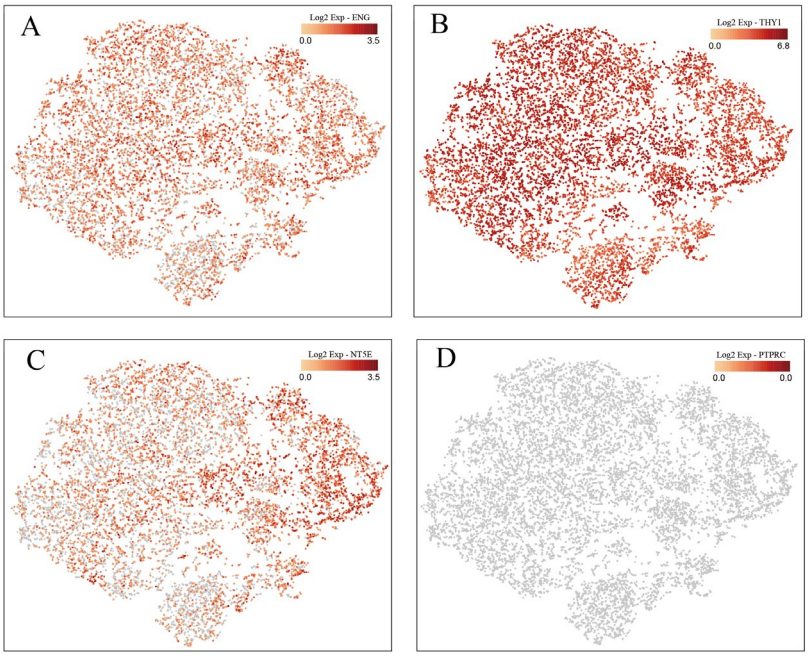
A deep transcriptional states map of MSCs and gene expression at single-cell level was generated after the performance of 10× Genomics high throughput of RNA sequencing. The 12,500 cells were acquired in the survey, leading to 881,215,280 raw reads totally. The median number of genes and UMIs detected per cell were 4,099 and 23,971, respectively (Supplemental Figure 2). The sequencing saturation rate was 72.9%, which met the scRNA-seq requirements.



Supplemental Figure 2. In the 10 x RNA-seq survey, the median number of genes and UMIs detected per cell were 4,099 (A) and 23,971 (B) as showed in the violin distribution.

MSCs marker genes expression

The scRNA-seq showed that the MSCs highly expressed ENG (CD105), THY1 (CD90), and NT5E (CD73). However, the expression of PTPRC (CD45), CD34, CD14, CD19, and HLA-DR was nearly undetected in the cells (CD45 was the only one shown in Supplemental Figure 3). The results were in accordance with the flow cytometry analysis. In Supplemental Figure 3, one point meant one cell, and red and gray color represented high expression and low expression, respectively.

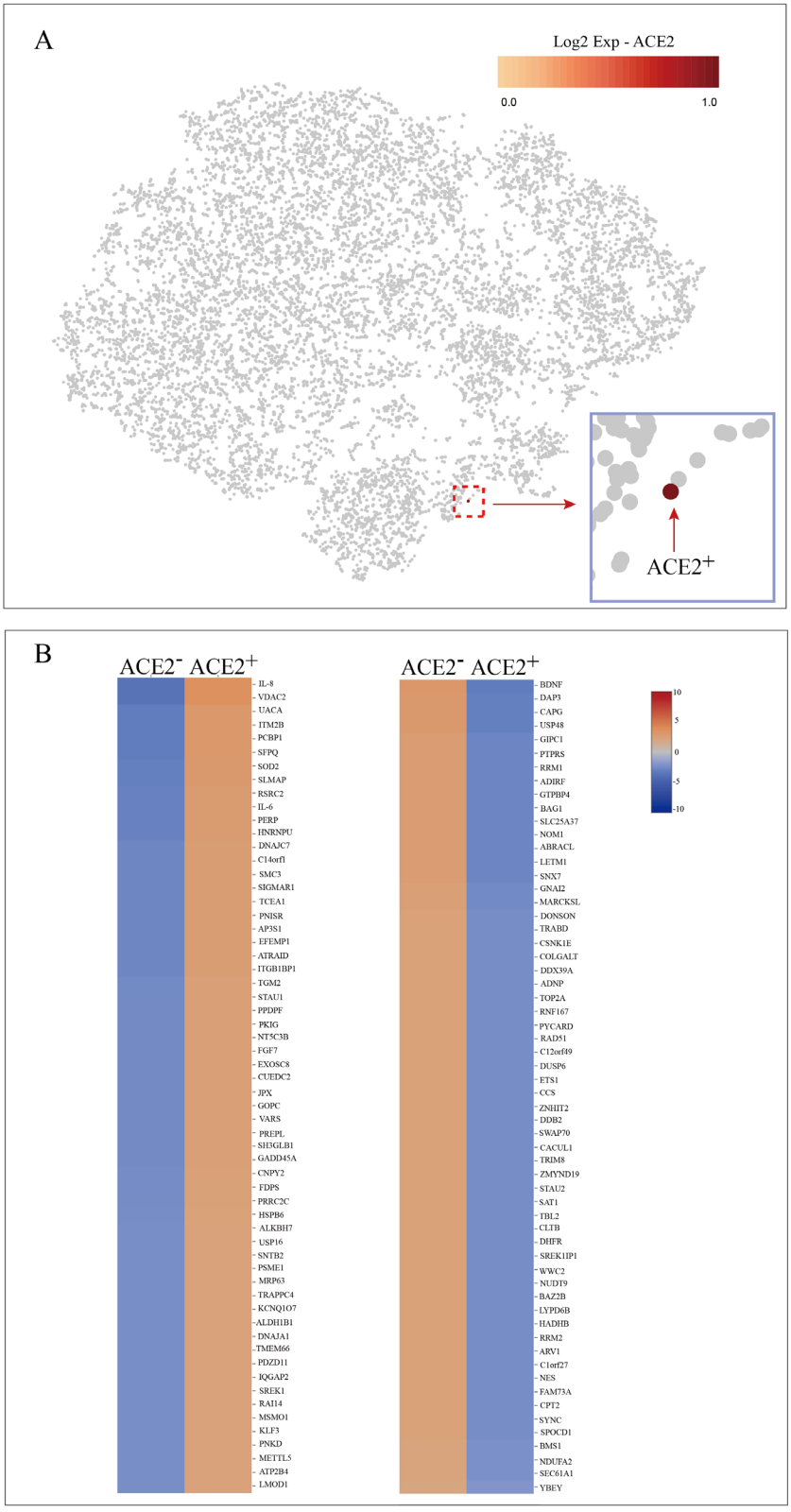


Supplemental Figure 3. MSCs marker genes expression by 10 x scRNA-seq analysis. (A) $CD105^{+}$, (B) $CD90^{+}$, (C) $CD73^{+}$, and (D) $CD45^{-}$

ACE2 gene expression and DEGs between $ACE2^{+}$ MSC and $ACE2^{-}$ MSC

Only one of the 12,500 cells was $ACE2^{+}$ as shown in Supplemental Figure 4A. Furthermore, the top 60 DEGs between the $ACE2^{+}$ MSC and the other nearby $ACE2^{-}$ MSC were shown in Supplemental Figure 4B. It is revealed that the $ACE2^{+}$ MSC tended to generate pro-inflammatory function by

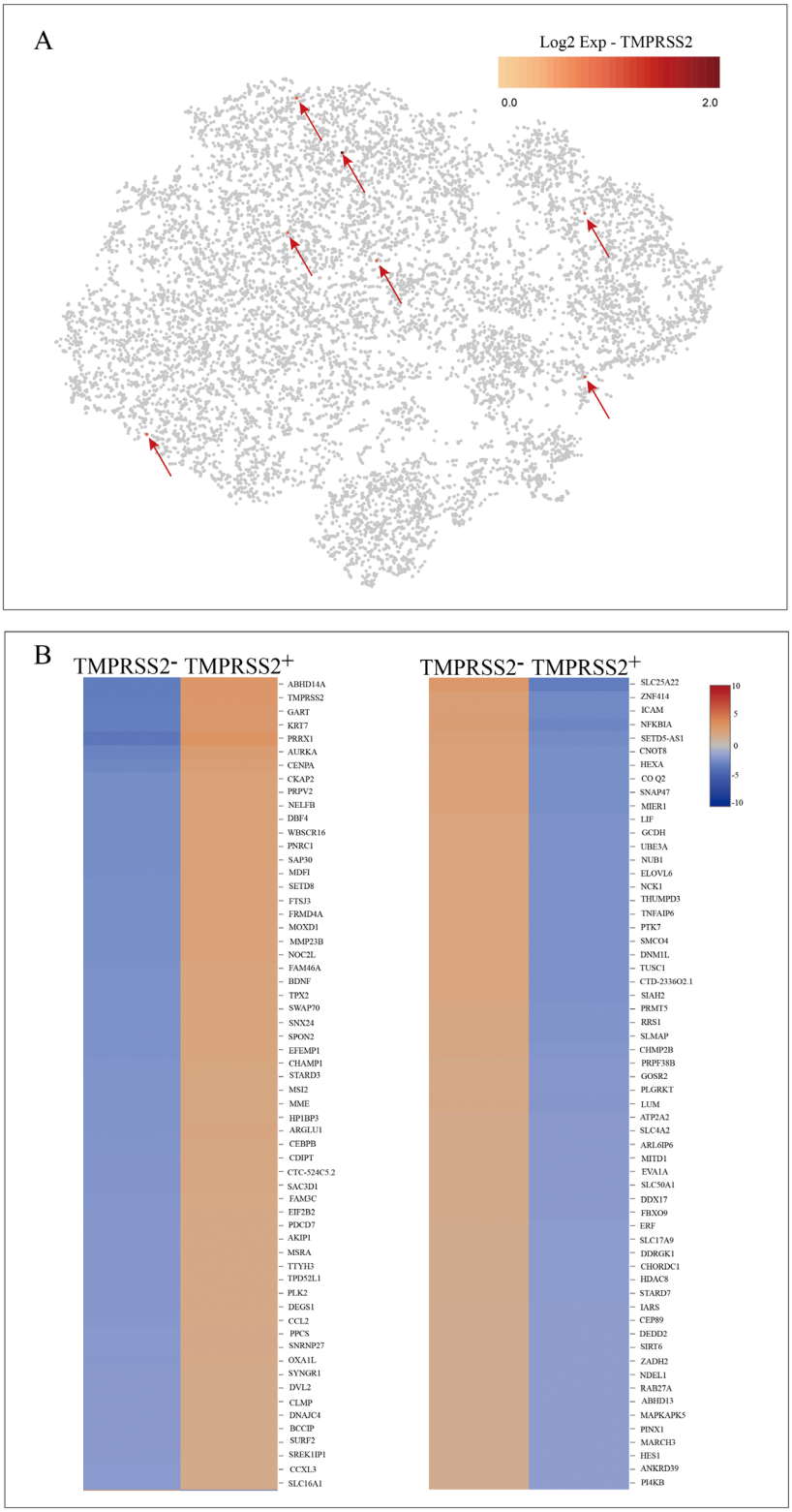
secreting IL-8, IL-6 and so on, while ACE2$^-$ MSC tended to generate anti-inflammatory effect by secreting BDNF and other factors.



Supplemental Figure 4. (A) ACE2 gene expression in MSCs. (B) top 60 DEGs between one ACE2$^+$ MSC and one ACE2$^-$ MSC

TMPRSS2 gene expression and DEGs between TMPRSS2 $^{+}$ MSC and TMPRSS2 $^{-}$ MSC

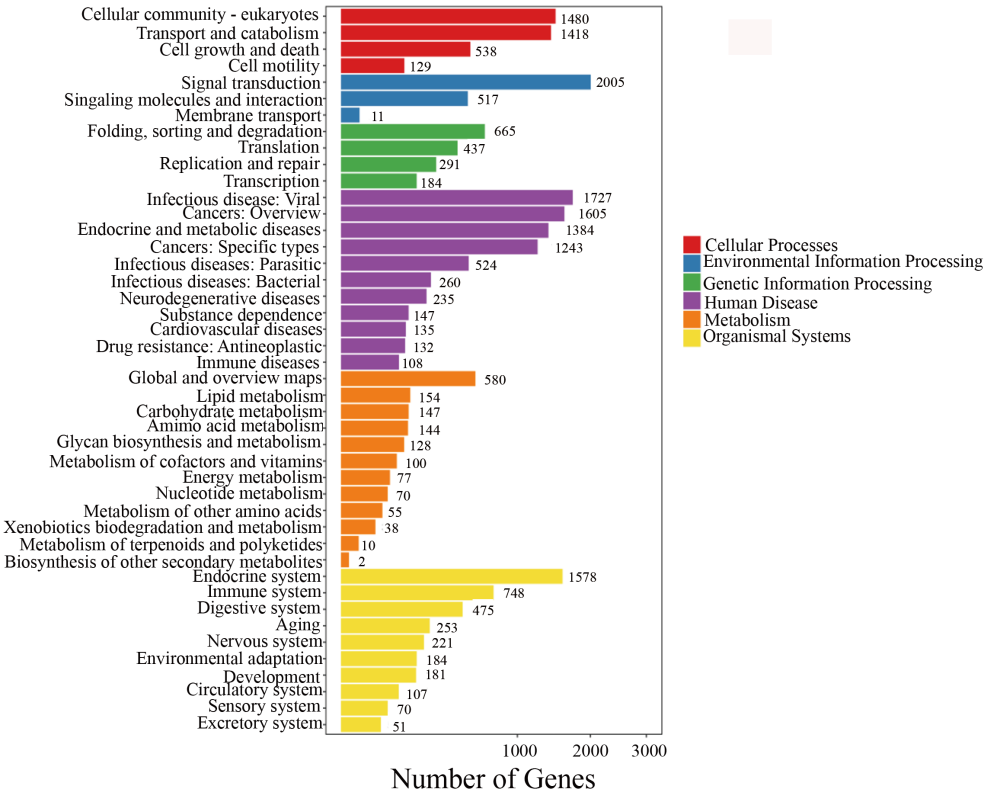
Only seven of the 12,500 cells were TMPRSS2^{+} as shown in Supplemental Figure 5A. Furthermore, the top 60 DEGs between the TMPRSS2^{+} MSC and the other seven nearby TMPRSS2^{-} MSC were shown in Supplemental Figure 5B.



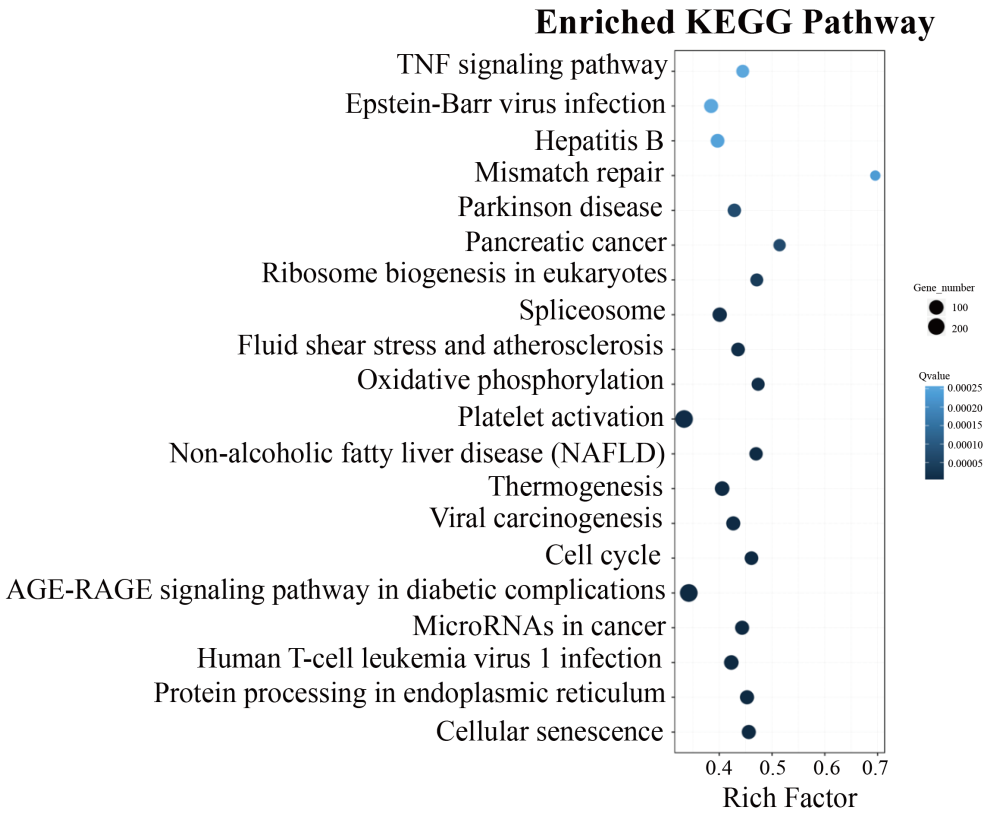
Supplemental Figure 5. (A) TMPRSS2 gene expression in MSCs. (B) top 60 DEGs between seven TMPRSS2^{+} MSCs and seven TMPRSS2^{-} MSCs

Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

KEGG pathway analysis demonstrated diseases mainly related to viral infectious diseases, cancers and endocrine and metabolic disorders (1727 genes, 1605 genes and 1384 genes, respectively). Organismal systems mainly related to endocrine and immune systems (1578 genes and 748 genes, respectively) (Supplemental Figure 6). Four enriched KEGG pathways were also involved in viral infection (Supplemental Figure 7).



Supplemental Figure 6. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that many gene expressions of MSCs were related with endocrine and immune systems.



Supplemental Figure 7. Four enriched KEGG pathways were also involved in viral infection.