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

Authors: Zikuan Leng, Rongjia Zhu, Wei Hou, Yingmei Feng, Yanlei Yang, Qin Han, Guangliang Shan, Fanyan Meng, Dongshu Du, Shihua Wang, Junfen Fan, Wenjing Wang, Luchan Deng, Hongbo Shi, Hongjun Li, Zhongjie Hu, Fengchun Zhang, Jinming Gao, Hongjian Liu, Xiaoxia Li, Yangyang Zhao, Kan Yin, Xijing He, Zhengchao Gao, Yibin Wang, Bo Yang, Ronghua Jin, Ilia Stambler, Lee Wei Lim, Huanxing Su, Alexey Moskalev, Antonio Cano, Sasanka Chakrabarti, Kyung-Jin Min, Georgina Ellison-Hughes, Calogero Caruso, Kunlin Jin, Robert Chunhua Zhao, Hongjian Liu, Ronghua Jin, Kunlin Jin, Robert Chunhua Zhao

Date: 2020-02-28T00:00:00+00:00

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 CD11bmid regulatory DC cell population dramatically increased. Meanwhile, the level TNF- 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.

Full Text

Preamble

Transplantation of ACE2- Mesenchymal Stem Cells Improves Outcomes in Patients with COVID-19 Pneumonia

Zikuan Leng[1], #, Rongjia Zhu[2]#, Wei Hou[3]#, Yingmei Feng[3]#, Yanlei Yang , Qin Han[2], Guangliang Shan[2], Fanyan Meng[1], Dongsheng Du[1], Shihua Wang[2], Junfen Fan[2], Wenjing Wang[3], Luchan Deng[2], Hongbo Shi[3], Hongjun Li[3], Zhongjie Hu[3], Fengchun Zhang , Jinming Gao , Hongjian Liu , *Xiaoxia Li , Yangyang Zhao[2], Kan Yin , Xijing He , Zhengchao Gao , Yibin Wang , Bo Yang , Ronghua Jin[3]*, Ilia Stambler ,[1] ,[11], Kunlin Jin ,[1] ,[12], *Lee Wei Lim ,[1] ,[13], Huanxing Su ,[1] ,[1] , Alexey Moskalev ,[1] ,[1] , Antonio Cano ,[1] ,[1] , Sasanka Chakrabarti ,[1] ,[1] , Armand Keating ,[1] ,[1] , Kyung-Jin Min ,[1] ,[1] , Georgina Ellison-Hughes ,[1] ,[2] , Calogero Caruso ,[1] ,[21], Robert Chunhua Zhao[1],[2], ,[1]*

[1]School of Life Sciences, Shanghai University, Shanghai, 200444, China
[2]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine, Peking Union Medical College, Beijing, 100005, China
[3]Beijing You' an Hospital, Capital Medical University, Beijing, China
Department of Medicine, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China
Department of Orthopaedics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
Institute of Stem Cell and Regeneration Medicine, School of Basic Medicine, Qingdao University, Qingdao, Shandong, China
Department of Orthopaedics, the Second Affiliated Hospital of Xi' an Jiaotong University, Xi' an, China
Department of Neurosurgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
The Executive Committee on Anti-aging and Disease Prevention in the framework of Science and Technology, Pharmacology and Medicine Themes under an Interactive Atlas along the Silk Roads, UNESCO, Paris, France
[1] International Society on Aging and Disease (ISOAD), Fort Worth, Texas, USA
[11]The Geriatric Medical Center "Shmuel Harofe" , Beer Yaakov, affiliated to Sackler School of Medicine, Tel-Aviv University, Tel-Aviv, Israel
[12]University of North Texas Health Science Center, Fort Worth, TX, USA
[13]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, University of

Hong Kong, Hong Kong, China
[1] Institute of Chinese Medical Science, University of Macau, Taipa, Macau, China
[1] Laboratory of Geroprotective and Radioprotective Technologies, Institute of Biology, Komi Science Center of Russian Academy of Sciences, Syktyvkar, Russia
[1] Department of Pediatrics, Obstetrics and Gynecology, University of Valencia, Valencia, Spain
[1] Department of Biochemistry, Maharishi Markandeshwar University, Kolkata, India
[1] Institute of Medical Science, University of Toronto, Toronto, Canada
[1] Department of Biological Sciences, Inha University, Incheon, South Korea
[2] Centre of Human & Aerospace Physiological Sciences & Centre for Stem Cells and Regenerative Medicine, Faculty of Life Sciences & Medicine, King' s College London, London, UK
[21]Laboratory of Immunopathology and Immunosenescence, Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Palermo, Italy

#These authors contributed equally to this work.

*Corresponding authors:

Robert Chunhua Zhao, M.D. & Ph.D., Professor, School of Life Sciences, Shanghai University, Shanghai 200444, China. Email: zhaochunhua@vip.163.com
Kunlin Jin, M.D. & Ph.D., Professor, University of North Texas Health Science Center, Fort Worth, TX, 76107, USA. Email: kunlin.Jin@unthsc.edu
Ronghua Jin, M.D. & Ph.D., Professor, You' an Hospital, Capital Medical University, Beijing, China. Email: jin_eagle@sina.com
Hongjian Liu, M.D. & Ph.D., Professor, Department of Orthopaedics, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China. Email: hongjianmd@126.com

Abstract

A novel coronavirus (HCoV-19) has caused the COVID-19 outbreak in Wuhan, China. Preventing and reversing the cytokine storm may be the key to saving patients with severe COVID-19 pneumonia. Mesenchymal stem cells (MSCs) have been shown to possess comprehensive and powerful immunomodulatory functions. This study aimed to investigate whether MSC transplantation could improve outcomes in seven enrolled patients with COVID-19 pneumonia at Beijing YouAn Hospital, China, from January 23, 2020, to February 16, 2020. We assessed clinical outcomes, inflammatory and immune function markers, and adverse effects for 14 days following MSC injection. MSCs cured or significantly improved functional outcomes in all seven patients with COVID-19 pneumonia within 14 days without any observed adverse effects. Pulmonary function and symptoms improved significantly within two days after MSC transplanta-

tion. Among these patients, two with common-type and one with severe disease recovered and were discharged within ten days after treatment. Following treatment, peripheral lymphocytes increased, while overactivated cytokine-secreting immune cells—including CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells—disappeared within 3–6 days. Concurrently, a population of CD14+CD11c+CD11bmid regulatory dendritic cells increased dramatically. Meanwhile, TNF- levels decreased significantly while IL-10 levels increased in the MSC treatment group compared to the placebo control group. Furthermore, gene expression profiling revealed that MSCs were ACE2- and TMPRSS2-, indicating that MSCs are free from COVID-19 infection. Thus, intravenous transplantation of MSCs was safe and effective for treating patients with COVID-19 pneumonia, particularly those in critically severe condition.

Keywords: COVID-19, ACE2 negative, mesenchymal stem cells, cell transplantation, immunomodulatory, functional recovery

Introduction

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

Several reports have demonstrated that the first step in HCoV-19 pathogenesis involves the virus specifically recognizing the angiotensin-converting enzyme 2 (ACE2) receptor via its spike protein [2-4]. ACE2-positive cells are infected by HCoV-19, similar to SARS-2003 [5,6]. Additionally, a German research team revealed that the cellular serine protease TMPRSS2, which primes the HCoV-19 spike protein, is also essential for host cell entry and viral spread [7], as with other coronaviruses such as SARS-2003 [8,9]. Unfortunately, the ACE2 receptor is widely distributed on human cell surfaces, particularly on alveolar type II cells (AT2) and capillary endothelium [10], and AT2 cells highly express TMPRSS2 [9]. However, in bone marrow, lymph nodes, thymus, and spleen, immune cells such as T and B lymphocytes and macrophages consistently lack ACE2 expression [10]. These findings suggest that immunological therapy may be used to treat infected patients. However, the immunomodulatory capacity may not be strong enough if only one or two immune factors are used, as the virus can stimulate a severe cytokine storm in the lung involving IL-2, IL-6, IL-7, GSCF, IP10, MCP1, MIP1A, and TNF , followed by edema, air exchange dysfunction, acute respiratory distress syndrome, acute cardiac injury, and secondary infection [11], which can lead to death.

Therefore, preventing the cytokine storm may be key to treating HCoV-19-infected patients. MSCs, owing to their powerful immunomodulatory abilities,

may have beneficial effects in preventing or attenuating the cytokine storm. MSCs have been widely used in cell-based therapy, from basic research to clinical trials [12,13]. Their safety and effectiveness have been clearly documented in many clinical trials, particularly for immune-mediated inflammatory diseases such as graft-versus-host disease (GVHD) [14] and systemic lupus erythematosus (SLE) [15]. MSCs exert positive effects mainly through two mechanisms: immunomodulatory effects and differentiation capacity [16]. MSCs can secrete various cytokines via paracrine secretion or through direct interactions with immune cells, leading to immunomodulation [17]. The immunomodulatory effects of MSCs are further triggered by activation of TLR receptors in MSCs, which are stimulated by pathogen-associated molecules such as LPS or double-stranded RNA from viruses [18,19], including HCoV-19.

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

Materials and Methods

Study Design

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

Patients

Patients were enrolled from January 23, 2020, to January 31, 2020. All enrolled patients were confirmed positive for HCoV-19 RNA by real-time reverse transcription polymerase chain reaction (RT-PCR) assay at the Chinese Center for Disease Control and Prevention using previously described protocols [11,20]. The primer sequences were as follows: forward primer 5 -TCAGAATGCCAATCTCCCCAAC-3 ; reverse primer 5 -AAAGGTCCACCCGATACATTGA-3 ; probe 5 -CY5-CTAGTTACACTAGCCATCCTTACTGC-3 -BHQ1.

We initially enrolled patients with COVID-19 (age 18–95 years) according to guidance from the National Health Commission of China (Table 1). If no improvement was observed under standard treatment, patients were offered MSC transplantation. Patients were ineligible if diagnosed with any cancer or if declared by a physician to be in critically severe condition. We excluded patients

participating in other clinical trials or who had participated in other trials within three months.

Cell Preparation and Transplantation

Clinical-grade MSCs were supplied free of charge 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 was certified by the National Institutes for Food and Drug Control of China (authorization numbers: 2004L04792, 2006L01037, CXSB1900004). Before intravenous infusion, MSCs were suspended in 100 ml of normal saline, with a total transplanted cell count of 1×10 cells per kilogram of body weight.

The window period for cell transplantation was defined as the time when symptoms and/or signs continued to worsen despite expectant treatment being administered. The injection was performed over approximately forty minutes at a rate of ~40 drops per minute.

Patients were assessed by investigators through a 14-day observation period after receiving the investigational product. Clinical, laboratory, and radiological outcomes were recorded and verified by a trained group of physicians. Detailed records included primary safety data (infusion-related and allergic reactions, secondary infection, and life-threatening adverse events) and primary efficacy data (cytokine level variations, plasma C-reactive protein levels, and oxygen saturation). Secondary efficacy outcomes included total lymphocyte count and subpopulations, chest CT, respiratory rate, and patient symptoms (particularly fever and shortness of breath). Additionally, therapeutic measures (e.g., antiviral medication and respiratory support) and outcomes were examined.

Statistical Analysis

MIMICS 21.0 (Interactive Medical Image Control System, Materialise, Belgium) was used to evaluate chest CT data. Mass cytometry analysis of peripheral blood mononuclear cells is described in Supplementary Material 1. The 10× RNA-seq survey analysis is described in Supplementary Material 2. Data were analyzed using 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 January 23, 2020, to February 16, 2020. Seven confirmed COVID-19 patients were enrolled, including one critically severe (patient 1), four severe (patients 2, 3, 6, 7), and two common-type (patients 4, 5)

cases. The timing of MSC transplantation for each patient is shown in Figure 1 [Figure 1: see original paper]. General patient information is listed in Table 1.

To date, the critically severe patient had completed MSC treatment. This patient had a 10-year history of hypertension with a recorded maximum blood pressure of 180/90 mmHg. All treatment information for the patients was collected.

Primary Safety Outcomes

Before MSC transplantation, patients exhibited symptoms of high fever (38.5℃ ± 0.5℃), weakness, shortness of breath, and low oxygen saturation. However, 2–4 days after transplantation, all symptoms disappeared in all patients, and oxygen saturation increased to 95% at rest, with or without oxygen uptake (5 liters per minute). Additionally, 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.

Detailed diagnosis and treatment procedures for the critically severe patient are shown in Supplementary Material 3. Main symptoms and signs are presented in Table 3 .

Efficacy Outcomes

The immunomodulatory function of MSCs contributed to the primary efficacy outcomes, and MSC transplantation demonstrated impressive positive results (Table 3). For the primary outcome in the critically severe patient (patient 1), plasma C-reactive protein levels decreased from 105.5 g/L (January 30) to 10.1 g/L (February 13), after reaching a peak of 191.0 g/L on February 1, indicating rapid alleviation of inflammatory status. Oxygen saturation without supplementary oxygen increased from 89% (January 31) to 98% (February 13), suggesting that pulmonary alveoli regained air-exchange function.

Secondary outcomes also improved (Table 4). In the critically severe patient, lymphopenia improved significantly after cell transplantation. The patient was isolated in the hospital ward with a history of hypertension reaching grade 3 levels. On February 1, blood tests showed that aspartate aminotransferase, creatine kinase activity, and myoglobin increased sharply to 57 U/L, 513 U/L, and 138 ng/ml, respectively, indicating severe liver and myocardial damage. However, these functional biochemical indicators decreased to normal reference values within 2–4 days after treatment (Table 4). By February 13, all indices reached normal levels: 19 U/L, 40 U/L, and 43 ng/ml, respectively. Respiratory rate decreased to the normal range on the fourth day after MSC transplantation. Both fever and shortness of breath disappeared on the fourth day after MSC transplantation. Chest CT imaging showed that ground-glass opacity and pneumonia infiltration had largely resolved by the ninth day after MSC transplantation (Figure 2 [Figure 2: see original paper]).

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, HCoV-19 nucleic acid was positive before transplantation (January 23) and six days after transplantation (February 6), but turned negative 13 days after transplantation (February 13). Patients 3, 4, and 5 also showed negative HCoV-19 nucleic acid results by the report date.

Mass Cytometry (CyTOF) Analysis of Patient Peripheral Blood

To investigate immune system composition during MSC transplantation, we performed CyTOF analysis of immune cells in patient peripheral blood before and after transplantation. CyTOF revealed virtually no increase in regulatory T cells (CXCR3-) or dendritic cells (DC, CXCR3-) in the two common-type patients (patients 4 and 5). However, in severe patients, both regulatory T cells and DCs increased after cell therapy, particularly in the critically severe patient. Notably, no significant enhancement of CXCR3- DCs was observed after placebo treatment in three severe control patients. Moreover, in the critically severe patient before MSC transplantation, the percentages of overactivated CXCR3+CD4+ T cells, CXCR3+CD8+ T cells, and CXCR3+ NK cells in PBMCs were remarkably increased compared to healthy controls, contributing to the inflammatory cytokine storm. However, six days after MSC transplantation, these overactivated T cells and NK cells nearly disappeared, and other cell subpopulations were almost restored to normal levels, particularly the CD14+CD11c+CD11bmid regulatory dendritic cell population (Figure 3 [Figure 3: see original paper]).

Serum Cytokine/Chemokine/Growth Factor Analysis

After intravenous MSC injection, the decrease ratio of pro-inflammatory cytokine TNF- in serum was significant before and after MSC treatment ($p<0.05$). Meanwhile, the increase ratio of anti-inflammatory IL-10 was also remarkable in the MSC treatment group ($p<0.05$). Serum levels of chemokines such as IP-10 and growth factor VEGF both increased, though not significantly (Figure 4 [Figure 4: see original paper]).

10× RNA-seq Analysis of Transplanted MSCs

To further elucidate the mechanisms underlying MSC-mediated protection in COVID-19-infected patients, we performed 10× RNA-seq analysis of transplanted MSCs. The survey captured 12,500 MSCs, which were sequenced with a total of 881,215,280 raw reads (Supplementary Material 4). Results revealed that MSCs are ACE2- and TMPRSS2-negative, indicating that MSCs are resistant to COVID-19 infection. Moreover, anti-inflammatory and trophic factors such as TGF- , HGF, LIF, GAL, NOA1, FGF, VEGF, EGF, BDNF, and NGF were highly expressed in MSCs, further demonstrating their immunomodulatory

function. Additionally, SPA and SPC were highly expressed in MSCs, suggesting that MSCs might differentiate into AT2 cells (Figure 5 [Figure 5: see original paper]). KEGG pathway analysis showed that MSCs were closely involved in antiviral pathways (Supplementary Material 4).

Discussion

Both the novel coronavirus and SARS-2003 can enter host cells by binding the S protein on the viral surface to 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 bloodstream, it spreads widely. All tissues and organs expressing ACE2 can become battlegrounds between the novel coronavirus and immune cells. This explains why all infected ICU patients suffer not only from acute respiratory distress syndrome but also from complications such as acute myocardial injury, arrhythmia, acute kidney injury, shock, and death from multiple organ dysfunction syndrome [11] (Figure 6 [Figure 6: see original paper]). Moreover, HCoV-19 more likely affects older males with comorbidities and can result in severe and even fatal respiratory diseases such as acute respiratory distress syndrome [21], as seen in the critically severe case reported here.

However, cure of COVID-19 essentially depends on the patient' s own immune system. When the overactivated immune system attacks the virus, it produces large amounts of inflammatory factors, leading to severe cytokine storms [20]. This suggests that organ damage may be primarily due to virus-induced cytokine storms, and older subjects may be more susceptible due to immunosenescence.

Our 10× scRNA-seq survey shows that MSCs are ACE2- and TMPRSS2- (to the best of our knowledge, this is the first such report) and secrete anti-inflammatory factors to prevent cytokine storms. They possess natural immunity to HCoV-19. According to mass cytometry results, viral infection caused total functional failure of lymphocytes and even the entire immune system. MSCs played vital immune-modulating roles in reversing lymphocyte subsets, primarily through dendritic cells. Our previous study showed that co-culture with MSCs could decrease differentiation of conventional DCs from human CD34+ cells while increasing plasmacytoid DC differentiation through PGE2 [22]. Furthermore, induction of IL-10–dependent regulatory dendritic cells and IRF8-controlled regulatory dendritic cells from hematopoietic stem cells has also been 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 have focused on lymphopenia and high C-reactive protein levels in COVID-19 patients [20,21]. C-reactive protein is a highly sensitive biomarker for inflammation and host response to cytokine production, particularly TNF ,

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 overactivation of the immune system and promote endogenous repair by improving the microenvironment. After entering the human body through intravenous infusion, some MSCs accumulate in the lung, where they can improve the pulmonary microenvironment, protect alveolar epithelial cells, prevent pulmonary fibrosis, and improve lung function.

As reported by Cao' s team [11], serum levels of IL-2, IL-7, G-SCF, IP10, MCP-1, MIP-1A, and TNF- in ICU patients were higher than in non-ICU patients. The cytokine release syndrome caused by abnormally activated immune cells deteriorated patient conditions, potentially causing endothelial cell dysfunction, capillary leakage, mucus blockage in the lung, and ultimately respiratory failure. These factors could even trigger an inflammatory cytokine storm leading to multiple organ failure. Intravenous MSC administration significantly improved the inflammatory situation in severe COVID-19 patients. Due to their unique immunosuppressive capacity, serum levels of pro-inflammatory cytokines and chemokines were dramatically reduced, attracting fewer mononuclear/macrophages to the fragile lung while inducing more regulatory dendritic cells to the inflammatory tissue niche. Moreover, increased IL-10 and VEGF promoted lung repair. Ultimately, patients with severe COVID-19 pneumonia survived the worst conditions and recovered.

Therefore, the improvement in outcomes of COVID-19 patients after MSC transplantation may occur through regulation of inflammatory responses and promotion of tissue repair and regeneration.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2016YFA0101000, 2018YFE0114200), CAMS Innovation Fund for Medical Sciences (2017-I2M-3-007) and the 111 Project (B18007), National Natural Science Foundation of China (81971324, 81672313, 81700782, 81972523, 81771349).

Conflicts of Interest

We have no conflicts of interest to declare.

References

1. Munster VJ, Koopmans M, van Doremalen N, van Riel D, de Wit E (2020). A Novel Coronavirus Emerging in China –Key Questions for Impact Assessment. New England Journal of Medicine.

2. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. SCIENCE CHINA Life Sciences.

3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. (2020). Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet (London, England).

4. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, et al. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature.

5. Kuba K, Imai Y, Rao SA, Gao H, Guo F, Guan B, et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nature Medicine, 11:875-879.

6. Ge X-Y, Li J-L, Yang X-L, Chmura AA, Zhu G, Epstein JH, et al. (2013). Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature, 503:535-538.

7. Hoffmann M, Kleine-Weber H, Krüger N, Müller M, Drosten C, Pöhlmann S (2020). The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. bioRxiv:2020.2001.2031.929042.

8. Glowacka I, Bertram S, Mueller MA, Allen P, Soilleux E, Pfefferle S, et al. (2011). Evidence that TMPRSS2 Activates the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Membrane Fusion and Reduces Viral Control by the Humoral Immune Response. Journal of Virology, 85:4122-4134.

9. Iwata-Yoshikawa N, Okamura T, Shimizu Y, Hasegawa H, Takeda M, Nagata N (2019). TMPRSS2 Contributes to Virus Spread and Immunopathology in the Airways of Murine Models after Coronavirus Infection. Journal of Virology, 93.

10. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. Journal of Pathology, 203:631-637.

11. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet.

12. Connick P, Kolappan M, Crawley C, Webber DJ, Patani R, Michell AW, et al. (2012). Autologous mesenchymal stem cells for the treatment of secondary progressive multiple sclerosis: an open-label phase 2a proof-of-concept study. Lancet Neurology, 11:150-156.

13. Wilson JG, Liu KD, Zhuo NJ, Caballero L, McMillan M, Fang XH, et al. (2015). Mesenchymal stem (stromal) cells for treatment of ARDS: a phase 1 clinical trial. Lancet Respiratory Medicine, 3:24-32.

14. Hashmi S, Ahmed M, Murad MH, Litzow MR, Adams RH, Ball LM, et al. (2016). Survival after mesenchymal stromal cell therapy in steroid-refractory acute graft-versus-host disease: systematic review and meta-analysis. Lancet Haematology, 3:E45-E52.

15. Kamen DL, Nietert PJ, Wang H, Duke T, Cloud C, Robinson A, et al. (2018). CT-04 Safety and efficacy of allogeneic umbilical cord-derived mesenchymal stem cells (MSCs) in patients with systemic lupus erythematosus: results of an open-label phase I study. Lupus Science & Medicine, 5:A46-A47.

16. Galipeau J, Sensebe L (2018). Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. Cell Stem Cell, 22:824-833.

17. Bernardo ME, Fibbe WE (2013). Mesenchymal Stromal Cells: Sensors and Switchers of Inflammation. Cell Stem Cell, 13:392-402.

18. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010). A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. PLoS One, 5:e10088.

19. Li W, Ren G, Huang Y, Su J, Han Y, Li J, et al. (2012). Mesenchymal stem cells: a double-edged sword in regulating immune responses. Cell Death Differ, 19:1505-1513.

20. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA.

21. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet (London, England).

22. Chen L, Zhang W, Yue H, Han Q, Chen B, Shi M, et al. (2007). Effects of human mesenchymal stem cells on the differentiation of dendritic cells from CD34(+) cells. Stem Cells and Development, 16:719-731.

23. Liu X, Qu X, Chen Y, Liao L, Cheng K, Shao C, et al. (2012). Mesenchymal Stem/Stromal Cells Induce the Generation of Novel IL-10-Dependent Regulatory Dendritic Cells by SOCS3 Activation. Journal of Immunology, 189:1182-1192.

24. Liu X, Ren S, Ge C, Cheng K, Zenke M, Keating A, et al. (2015). Sca-1(+)Lin(-)CD117(-) Mesenchymal Stem/Stromal Cells Induce the Genera-

tion of Novel IRF8-Controlled Regulatory Dendritic Cells through Notch-RBP-J Signaling. Journal of Immunology, 194:4298-4308.

25. Zhang B, Liu R, Shi D, Liu X, Chen Y, Dou X, et al. (2009). Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population. Blood, 113:46-57.

26. Sproston NR, Ashworth JJ (2018). Role of C-Reactive Protein at Sites of Inflammation and Infection. Frontiers in Immunology, 9.

27. Bisoendial RJ, Boekholdt SM, Vergeer M, Stroes ESG, Kastelein JJP (2010). C-reactive protein is a mediator of cardiovascular disease. European Heart Journal, 31:2087-U1505.

Table 1: Clinical Classification of COVID-19 Released by the National Health Commission of China

Category	Criteria
Common Type	Clinical manifestation: Fever, respiratory symptoms, pneumonia performance on X-ray or CT
Severe Type	Meets any of the following: 1. Respiratory distress, respiratory rate 30 breaths/min 2. Oxygen saturation 93% at rest 3. Arterial partial pressure of oxygen (PaO)/Fraction of inspired O (FiO) 300 mmHg (1 mmHg = 0.133 kPa)
Critically Severe Type	Meets any of the following: 1. Respiratory failure requiring mechanical ventilation 2. Shock 3. Combined with other organ failure requiring ICU monitoring and treatment

Table 2 : General Information of Enrolled Patients

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Ctrl 1	Ctrl 2	Ctrl 3
Gender										
Age (years)										

Parameter	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Ctrl 1	Ctrl 2	Ctrl 3
COVID-19 Type	Critically severe	Severe	Severe	Common	Common	Severe	Severe	Severe	Severe	Severe
Fever (℃, baseline)										
Shortness of breath										
Oxygen saturation at rest										
Cough, weakness, poor appetite										
Diarrhea										
Date diagnosed	Jan 23	Jan 27	Jan 25							
Date of intervention (MSCs or Placebo)	Jan 31	Feb 2								
Date of recovery	Feb 3	Feb 4	Feb 4	Feb 6	Feb 3	Feb 4	Feb 2	Feb 4	Feb 6	Feb 5

Table 3: Symptoms, Signs, and Maximum Body Temperatures of the Critically Severe Patient from January 21 to February 13, 2020

Date	Fever (℃)	Shortness of breath	Cough	Sputum	Oxygen saturation (without/with O uptake)	Respiratory rate	Treatment
Jan 21-22	37.0-38.5	+	+	+	97% / NA	20-22	Basics-1: Antipyretic, antiviral and supportive therapy
Jan 23	36.6-36.9	+	+	+	NA / NA	20-22	Basics-1; Mask O 5L/min
Jan 24-29	36.6-36.8	+	+	+	NA / NA	20-22	Basics-1; Mask O 5L/min
Jan 30	36.5-36.9	++	+	+	91% / 95%	20-22	Basics-1; Basics-2; Mask O 5L/min
Jan 31	NA	++	+	+	89% / 94%	20-22	Basics-2; O 10L/min; Mask O 5L/min; **Cell transplant**

Date	Fever (℃)	Shortness of breath	Cough	Sputum	Oxygen saturation (without/with O uptake)	Respiratory rate	Treatment
Feb 1	NA	++	+	+	NA / 98%	33	Basics-2; Mask O 5L/min
Feb 2-3	NA	+	+	+	NA / 97%	20-22	Basics-2; Mask O 5L/min
Feb 4	NA	-	+	+	NA / 96%	20-22	Basics-2; Mask O 5L/min
Feb 5-8	NA	-	+	-	NA / 97%	20-22	Basics-2
Feb 9-12	NA	-	-	-	96% / NA	20-22	Basics-2
Feb 13	NA	-	-	-	97% / NA	20-22	Basics-2

Notes: ICU = Intensive Care Unit; NA = Not Available; Basics-1 = Antipyretic, antiviral and supportive therapy; Basics-2 = Antiviral and supportive therapy

Table 4: Laboratory Results of the Critically Severe Patient

Note: Red indicates values above normal range; Blue indicates values below normal range; NA = Not Available

Test	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		**105.50**	NA	**191.00**		**13.60**			**10.10**

Test	Reference Range	Jan 24	Jan 30	Jan 31	Feb 1	Feb 2	Feb 4	Feb 6	Feb 10	Feb 13
Absolute lymphocyte count (×10 /L)	(1.1-3.2)		**0.60**		**0.23**		**0.58**			**0.93**
White cell count (×10 /L)	(3.5-9.5)									
Absolute neutrophil count (×10 /L)										
Absolute monocyte count (×10 /L)										
Red-cell count (×10^{12}/L)										
Hemoglobin (g/L)										
Platelet count (×10 /L)										
Absolute eosinophil count (×10 /L)										
Absolute basophilic count (×10 /L)										
Total bilirubin (mol/L)										

Test	Reference Range	Jan 24	Jan 30	Jan 31	Feb 1	Feb 2	Feb 4	Feb 6	Feb 10	Feb 13
Albumin (g/L)										
Aspartate amino-trans-ferase (U/L)				**57**		**19**			**19**	
Fibrinogen (g/L)										
Procalcitonin (ng/mL)	<0.10									
Creatine ki-nase isoen-zymes (ng/mL)	<3.60									
Creatine ki-nase (U/L)				**513**	**316**				**40**	
Glomerular fil-tra-tion rate (ml/min)	>90.00									
Potassium (mmol/L)	3.5–5.5	**2.74**								
Sodium (mmol/L)										
Myoglobin (ng/mL)				**138**					**43**	
Troponin (ng/mL)	<0.056									

Supplementary Materials

Supplementary 1: Mass Cytometry Method for Peripheral Blood Mononuclear Cells (PBMC)

Sample Preparation for Mass Cytometry

PBMC samples were collected from COVID-19-infected patients treated with MSC transplantation at baseline and on Day 6, with PBMCs from a healthy donor serving as the control group. All samples were cultured with 2 M cisplatin (195-Pt, Fluidigm) for 2 minutes before quenching with CSB (Fluidigm) to assess viability using mass cytometry analysis. Cells were then fixed using Fix-I buffer (Fluidigm) for 15 minutes at room temperature, followed by three washes 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 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 purified antibodies (listed in Supplemental Table 1). All metal-conjugated antibodies were titrated for optimal concentrations before use. Cells were counted and diluted to 1×10 cells per milliliter in PBS, then permeabilized with 80% methanol for 15 minutes at 0°C. After triple washes in CSB, cells were cultured with antibodies in a total volume of 50 L CSD for 30 minutes at room temperature, triple-washed in CSB, and incubated with 0.125 m intercalator in fix and perm buffer (Fluidigm) at 4°C overnight.

Data Acquisition on Helios

After incubation 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 cells/ml. Data acquisition was performed on a Helios mass cytometer (Fluidigm). Original FCS data were normalized, and .fcs files were collected for each sample.

CyTOF Data Analysis

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

Supplementary 2: 10× RNA-seq Survey Method

Materials and Reagents

All supplies and reagents were of the highest grade commercially available. 0.20 m filters, dishes, and tubes were purchased from Corning (NY, USA). CD105, CD90, CD44, and CD45 antibodies for flow cytometry were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). DMEM/F12, fetal bovine serum (FBS), GlutaMAX™-I, TrypLE™ Express, and penicillin-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 Mass Cytometry Analysis

Antigen	Symbol and Mass	Antibody Clone	Source
CD45RA	141Pr	HIB19	Fluidigm
CD11c	142Nd	UCHT2	Fluidigm
CD62L	143Nd	NP-6G4	Fluidigm
IL-1	144Nd	RPA-T4	Fluidigm
CXCR3	145Nd	HI100	Fluidigm
IFN-	147Sm	RMO52	Fluidigm
CD45RO	148Nd	NCAM16.2	Fluidigm
IL-12	149Sm	MAb11	Fluidigm
IL-10	150Nd	DREG-56	Fluidigm
CD206	151Eu	Polyclonal	Fluidigm
HLA-DR	152Sm	G025H7	Biolegend
CD127	153Eu	G043H7	Biolegend
CD11b	154Sm	CD28.2	Fluidigm
CD4	155Gd	RPA-T8	Fluidigm
CD8	156Gd	TW46H10	Fluidigm
CD3	158Gd	UCHL1	Fluidigm
CD19	159Tb	Polyclonal	Fluidigm
CD56	160Gd	JES3-9D7	Fluidigm
CD14	161Dy	MQ2-13A5	Fluidigm
CD16	162Dy	UCHT1	Fluidigm
CD33	164Dy	Y1/82A	Fluidigm
HLA-ABC	165Ho	MP4-25D2	Fluidigm
CD123	166Er	A019D5	Fluidigm
CD1c	167Er	ICRF44	Fluidigm
CD141	168Er	Fluidigm	
CD45	169Tm	Polyclonal	Fluidigm
CD86	170Er	J.119-1134	Fluidigm
CD274	171Yb	MIH1	Biolegend
CD163	172Yb	GHI/61	Fluidigm
CD273	173Yb	MIH18	Fluidigm
CD40	174Yb	5C3	Fluidigm
Viability	209Bi	Cisplatin	Fluidigm

Cell Culture

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 . After three passages, MSCs were immunophenotyped 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). MSCs were also tested for adipogenic, chondrogenic, and osteogenic differentiation to confirm their characteristics.

Cell Preparation and Library Construction

Cell count and viability were examined by microscopy after 0.4% trypan blue staining. When viability was 80%, library construction was performed. Libraries were constructed using the Chromium Controller (10× Genomics, Pleasanton, CA). Briefly, single cells, reagents, and gel beads containing barcoded oligonucleotides were encapsulated into nanoliter-sized GEMs (Gel Bead in Emulsion) using GemCode technology. Lysis and barcoded reverse transcription of polyadenylated mRNA from single cells were performed within each GEM. Post-RT GEMs were cleaned up, and cDNA was amplified. cDNA was fragmented, fragment ends were repaired, and A-tailing was added to the 3 end. Adaptors were ligated to fragments, which underwent double-sided SPRI selection. Another double-sided SPRI selection was performed after sample index PCR. Quality control-passed libraries were sequenced. The final library was quantified by determining average molecule length using the Agilent 2100 Bioanalyzer and by real-time quantitative PCR.

Analysis of Single-Cell Transcriptomics Data

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

Supplementary 3: 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 with a 2-day history of cough, sputum production, and subjective fever. The patient wore a mask in the hospital. He disclosed traveling in Wuhan, China, from December 31, 2019, to January 20, 2020, and returning to Beijing on January 20. Apart from a 10-year history of hypertension with a maximum recorded blood pressure of 180/90 mmHg, the patient had no other significant medical history. Physical examination revealed a body temperature of 37.8°C, blood pressure of 138/85 mmHg, pulse of 85 beats per minute, and respiratory rate of 19 breaths per minute. Lung auscultation revealed rhonchi. Urgent blood tests showed white-cell count and absolute lymphocyte count of 4.9 × 10 /L (reference range: 3.5–9.5 × 10 /L) and 0.94 × 10 /L (reference range: 1.1–3.2 × 10 /L), respectively (Table 1). According to COVID-19 guidance from the National Health Commission of China, the

physician diagnosed suspected COVID-19 and admitted the patient for medical isolation observation. An oropharyngeal swab specimen was collected.

On January 23, 2020, RT-PCR assay confirmed the patient' s specimen tested positive for HCoV-19. The patient was admitted to an airborne isolation unit for clinical observation. He had no dyspnea, remained conscious, and maintained normal diet and sleep since symptom onset. Chest CT showed no evidence of infiltrates or abnormalities. Admission diagnoses were novel coronavirus pneumonia (common type) and grade III hypertension. The patient received no special care except irbesartan, which was continued throughout treatment.

From January 24 to 29, the patient' s vital signs remained largely stable, apart from intermittent fevers and shortness of breath. He received antipyretic therapy including 15 ml ibuprofen suspension every 6 hours and 650 mg acetaminophen every 6 hours. From January 26, he also received antiviral therapy with lopinavir (400 mg) and ritonavir (100 mg) twice daily.

On January 30, the patient experienced severe shortness of breath and fatigue. Oxygen saturation measured by pulse oximetry decreased to 91% while breathing ambient air. Auscultation revealed worsening rhonchi in both lungs. Urgent chest CT showed pneumonia with ground-glass opacity in middle lobes of both lungs. Laboratory tests showed C-reactive protein elevated to 105.5 g/L (reference: <3 g/L) and absolute lymphocyte count decreased to 0.60 × 10 /L. Potassium concentration decreased to 2.74 mmol/L (reference: 3.5–5.5 mmol/L). The diagnosis was changed to COVID-19 (critically severe type), and the patient was admitted to the ICU. Additional treatments included mask oxygen supplementation (5 liters per minute), electrocardiograph monitoring, potassium chloride sustained-release tablets (oral, 500 mg three times daily), and glucose/amino acid injections. Symptoms improved, and oxygen saturation increased to 95%.

On January 31, shortness of breath worsened despite oxygen supplementation. Oxygen flow was increased to 10 liters per minute. After obtaining informed consent for MSC transplantation, 100 ml of normal saline containing 6 × 10 MSCs was intravenously injected over 40 minutes at ~40 drops per minute, with no adverse events observed.

On February 1–2, the patient did not feel better. The third chest CT showed worsening pneumonia. On February 1, C-reactive protein reached 191.0 g/L, and absolute lymphocyte count decreased severely to 0.23 × 10 /L. Laboratory results suggested liver and myocardial involvement. Electrocardiograph monitoring showed blood pressure 138/80 mmHg, heart rate 95 bpm, respiratory rate 33 bpm, and oxygen saturation 93% with mask oxygen at 10 liters per minute. The family was informed of critical condition.

However, on February 3, the patient felt better, with significant improvement in shortness of breath. On February 4, C-reactive protein decreased to 13.6 g/L, and absolute lymphocyte count increased to 0.58 × 10 /L, indicating rapid recovery. Liver and myocardial function indices normalized. Fever and shortness of breath disappeared on February 5, and he was transferred out of the ICU. On

February 9, the fourth chest CT confirmed resolving pneumonia. On February 13, C-reactive protein was 10.1 g/L, and absolute lymphocyte count was 0.93 × 10 /L. The patient felt much better.

Supplementary 4: Additional 10× RNA-seq Survey Results

Flow Cytometry Analysis

PI staining showed that 91.60% of the total cell population was viable, 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 first. (B) Live cells (91% of single cells) were selected. (C-F) 99% of selected cells were CD105+, CD90+, CD73+, CD44+, CD29+, CD14-, and CD45-.

Survey Overview

A deep transcriptional state map of MSCs and gene expression at single-cell level was generated after 10× Genomics high-throughput RNA sequencing. The survey acquired 12,500 cells, yielding 881,215,280 raw reads total. 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%, meeting scRNA-seq requirements.

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

MSCs Marker Gene Expression

scRNA-seq showed that MSCs highly expressed ENG (CD105), THY1 (CD90), and NT5E (CD73). However, expression of PTPRC (CD45), CD34, CD14, CD19, and HLA-DR was nearly undetectable (CD45 shown in Supplemental Figure 3). These results were consistent with flow cytometry analysis. In Supplemental Figure 3, each point represents one cell, with red and gray indicating high and low expression, respectively.

Supplemental Figure 3. MSCs Marker Gene Expression by 10× scRNA-seq Analysis

(A) CD105+, (B) CD90+, (C) CD73+, and (D) CD45-.

ACE2 Gene Expression and DEGs Between ACE2+ and ACE2- MSCs

Only one of 12,500 cells was ACE2+ (Supplemental Figure 4A). The top 60 DEGs between this ACE2+ MSC and nearby ACE2- MSCs are shown in Supplemental Figure 4B. The ACE2+ MSC tended to generate pro-inflammatory

function by secreting IL-8, IL-6, etc., while ACE2- MSCs tended to produce anti-inflammatory effects 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+ and TMPRSS2- MSCs

Only seven of 12,500 cells were TMPRSS2+ (Supplemental Figure 5A). The top 60 DEGs between these seven TMPRSS2+ MSCs and seven nearby TMPRSS2- MSCs are 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 that diseases were mainly related to viral infectious diseases, cancers, and endocrine/metabolic disorders (1,727 genes, 1,605 genes, and 1,384 genes, respectively). Organismal systems were mainly related to endocrine and immune systems (1,578 genes and 748 genes, respectively) (Supplemental Figure 6). Four enriched KEGG pathways were also involved in viral infection (Supplemental Figure 7 [Figure 7: see original paper]).

Supplemental Figure 6. KEGG analysis revealed that many MSC gene expressions were related to endocrine and immune systems.

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

Note: Figure translations are in progress. See original paper for figures.