Mesenchymal stem cell research progress for the treatment of COVID-19

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Abstract
At the end of 2019, novel coronavirus (COVID-19) infection was detected in Wuhan City, Hubei Province, China. The COVID-19 infection characteristics include a long incubation period, strong infectivity, and high fatality rate, and it negatively affects human health and social development. COVID-19 has become a common problem in the global medical and health system. It is essentially an acute self-limiting disease. Patients with severe COVID-19 infection usually progress to acute respiratory distress syndrome, sepsis, metabolic acidosis that is difficult to correct, coagulation dysfunction, multiple organ failure, and even death within a short period after onset. There remains a lack of effective drugs for such patients clinically. Mesenchymal stem cells (MSCs) are expected to reduce the risk of complications and death in patients because they have strong anti-inflammatory and immunomodulatory capabilities, which can improve the microenvironment, promote neovascularization, and enhance tissue repair capabilities. China is currently conducting several clinical trials on MSCs for the treatment of COVID-19. Here, we review the research progress related to using stem cells to treat patients with COVID-19.

Keywords
Severe Acute Respiratory Syndrome-related Virus 2, mesenchymal stem cells, cytokine storm, immunoregulation, neovascularization, tissue repair

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Introduction
At the end of 2019, a novel coronavirus infection was diagnosed for the first time in patients in Wuhan City, Hubei Province. Since the outbreak of the disease, the number of confirmed cases at home and abroad has increased dramatically within a
short period.\textsuperscript{1–3} As of 14 April 2020, the World Health Organization (WHO) has reported more than 1.2 million confirmed cases worldwide, with more than 110,000 deaths, and the death rate of hospitalized patients in China is about 2.3\% to 4.3\%.\textsuperscript{4,5} The International Virus Classification Committee named it Severe Acute Respiratory Syndrome-related Virus 2 (SARS-CoV-2), and the WHO named the novel coronavirus caused by SARS-CoV-2 Coronavirus Pneumonia 2019 (COVID-19). SARS-CoV-2 is similar to Severe Acute Respiratory Syndrome-related Coronavirus (SARS-CoV) that was first diagnosed in 2003 in China, but SARS-CoV-2 has a longer incubation period and stronger infectivity than SARS-CoV. A study showed that nearly 4.8 million people are at risk of infection in the United States alone, among whom more than 1.9 million patients could need to be admitted to the intensive care unit (ICU), and about 960,000 patients may need mechanical ventilation-assisted respiratory therapy. However, there are less than 100,000 ICU beds and 200,000 ventilators in the United States, and thus, the limited medical resources are facing great challenges.\textsuperscript{6} Currently, China has incorporated COVID-19 into Class B infectious diseases as stipulated in the Law of the People’s Republic of China on Prevention and Control of Infectious Diseases, and it is being managed in accordance with Class A infectious diseases.\textsuperscript{7}

COVID-19 is an acute self-limiting disease. The clinical manifestations of patients are complex and varied from no obvious symptoms to severe respiratory failure that mechanical ventilation support is required. Patients with severe COVID-19 are also prone to acute respiratory distress syndrome (ARDS), coagulation dysfunction, metabolic acidosis difficult to correct, septic shock, and multiple organ failure (MOD).\textsuperscript{5,8–11} Huang et al.\textsuperscript{12} were the first to report the clinical situation of patients in our country. One-third of 41 patients (n = 13) required admittance to the ICU, and 15\% of the patients (n = 6) died. Arentz et al.\textsuperscript{13} analyzed 21 critically ill patients with COVID-19 in Washington state, USA. The initial symptoms were chest tightness and shortness of breath (76\%), high fever (52\%), and cough (48\%), and most patients (86\%, n = 18) had basic chronic diseases when they were admitted to hospital, mostly with kidney disease or heart failure. Additionally, 81\% (n = 17) of patients needed to be transferred to the ICU within 24 hours after admission. All 15 patients with ARDS required mechanical ventilation-assisted respiratory therapy, among whom eight (53\%) patients progress to severe ARDS within 72 hours. Medical staff around the world soon realized that there was, and currently is, no specific treatment for COVID-19. The treatment strategy for severe patients was to prevent and treat complications on the basis of symptomatic treatment while actively treating basic diseases and preventing secondary infection. Blood purification, artificial membrane lung, and serum perfusion of convalescent patients lacked sufficient effectiveness for disease treatment, and targeted vaccines cannot be developed in the short term. As a sudden major global public health event, researchers are urgently needed to develop safe and effective treatment methods.\textsuperscript{14–18}

Currently, cell-based therapy has been formally incorporated into the diagnosis and treatment guidelines or consensus for diseases including lung, cardiovascular, liver, and kidney diseases,\textsuperscript{19,20} and there are also multi-center successful clinical cases of umbilical cord stem cell therapy for critically ill patients in China.\textsuperscript{3,21} On 15 February 2020, Professor Zhang Xinmin, director of the China Biological Center of the Ministry of Science and Technology, held a press conference at the joint defense and control mechanism of the State Council. He reported that
mesenchymal stem cell (MSC) technology can improve microcirculation, promote endogenous repair, and relieve ARDS symptoms by inhibiting the over-activation of immune system in patients with COVID-19, and he affirmed its effect in the treatment of severe patients. This article reviews the research progress on the mechanism of COVID-19 virus at home and abroad, and the clinical research status of MSC technology in the treatment of patients with COVID-19 to provide reference for frontline clinical and scientific researchers.

**SARS-CoV-2 pathogenesis**

Some studies have shown that SARS-CoV-2 pathogenesis occurs through the specific recognition of its spike protein (S protein) and angiotensin I converting enzyme-2 receptor (ACE-2). Cells with positive ACE-2 expression are more susceptible (such as SARS-2003 virus, but its affinity for ACE-2 is only 5% to 10% of SARS-CoV-2). Another study from Germany showed that the intracellular serine protease TMPRSS2 has the ability to specifically recognize and bind with the S protein of SARS-CoV-2, which plays an important role in the process of virus infection and transmission. ACE-2 is widely distributed on the surface of human cells except for bone marrow, lymph nodes, thymus, spleen, and immune cells, while TMPRSS2 is highly expressed in alveolar type II epithelial cells and capillary endothelial cells. Therefore, COVID-19 pathogens entering the patient’s blood circulation can be widely spread within a short period and lead to organ damage in addition to the lung such as acute kidney injury, myocardial injury, shock, and MOD.

**Research progress on COVID-19 therapy**

Both chloroquine and hydroxychloroquine have been shown to have certain effects on the treatment of patients with COVID-19. A study showed that hydroxychloroquine has better effects in the early treatment of severe patients (<5 days). Some scholars claimed that remdesivir (RDV), which is an antiviral nucleoside analogue drug, can add virus RNA-dependent RNA polymerase (RdRp) through competitive inhibition of natural nucleoside triphosphate (NTP) to prevent virus RNA synthesis and, thus, inhibit virus replication. RDV has shown good anti-MERS-CoV, SARS-CoV, and SARS-CoV-2 activity in *in vitro* studies and animal models, indicating that RDV can be used as a potential anti-COVID-19 drug, but its safety and effectiveness still need to be verified by phase II and phase III clinical trials.

Pharmaceutical companies and epidemic prevention agencies worldwide have begun to develop a COVID-19 vaccine, but because the vaccine must have a sufficient scientific basis and sufficient safety, its research and development cycle may take months to years. On the basis of the drug research and development experience of MERS-CoV and SARS-CoV, searching for new potential drugs using existing drugs has become the current main strategy owing to its low research cost and short research and development cycle. A recombinant protein vaccine has just been approved to start clinical trials, an inactivated vaccine is in the stage of establishing animal infection models, a nucleic acid vaccine has undergone clinical trials (NCT04283461), a recombinant virus vector vaccine is undergoing adenovirus vector vaccine clinical trials (NCT04313127), and two lentivirus vector vaccine clinical trials (NCT04299724, NCT04276896) are being conducted. However, owing to the lack of animal models that can effectively evaluate *in vivo* efficacy and the diversity and mutability of coronaviruses, there are still challenges in the research and development of vaccines and new drugs.
**MSC therapy for COVID-19**

Stem cell therapy usually refers to the process of extracorporeal separation, culture, subculture, proliferation, and differentiation of exogenously obtained stem cells, which are then transplanted into patients for immune regulation and microenvironment repair. Currently, MSCs and natural killer (NK) cells are mainly divided into two types on the basis of their cell types, among which the former is widely used because it has advantages of a widely available source, convenient material acquisition, strong immune regulation, and microenvironment effects. The safety and effectiveness of MSCs have been shown in a number of basic studies and clinical trials. MSCs have been widely used in the treatment of inflammatory diseases in the field of immunology, such as in graft versus host disease (GVHD) and systemic lupus erythematosus (SLE). Some studies have shown that MSCs have definite efficacy in improving cardiovascular, kidney, liver, and other diseases.38–41

**Potential mechanism of MSCs therapy for COVID-19**

**MSC regulation of the immune system.** Previous studies have shown that MSCs can regulate immune cells and inflammatory factors when exposed to an inflammatory environment, which can eventually affect specific or nonspecific immune responses in the human body. This regulation is related to exosomes or various cytokines that are secreted by MSCs, such as prostaglandin (PG)E-2, interleukin (IL)-10, and transforming growth factor (TGF)-β.42,43 MSCs regulate T cell function in many ways, including T cell proliferation, which is controlled by inflammatory stimulation. A study on cell cycle analysis revealed that T cell subsets can be blocked at the G0/G1 phase. Additionally, MSCs can regulate T cell function through cytokines, such as up-regulating the FoxP3 gene by releasing TGF-β, inhibiting the immune activity of Th17 cells, inducing their transformation into T regulatory cell (Treg) cells, or secreting hepatocyte growth factor (HGF) to regulate the Th17/Treg cell balance.44 MSCs also play a regulatory role in the proliferation, differentiation, and antibody secretion of B cells. MSCs can affect the G0/G1 phase transition of B cells and regulate the antibody secretion ability of B cells through various transcription pathways.45 For cell phenotype, MSCs increase the number of regulatory B cells that express IL-10 by producing EBI3, and MSCs also activate T cells to release interferon. Suppression of activated B cells in follicular and marginal areas indirectly regulates the immune function of B cells, and MSCs can also affect innate immune cells (such as macrophages and dendritic cells) to realize immune regulation. Under inflammatory conditions, MSCs regulate macrophage function in a negative feedback manner.46 When macrophages of the pro-inflammatory type (M1) release inflammatory factors, activated MSCs can up-regulate the cyclooxygenase (COX)-2 signal and increase PGE2 secretion, thereby promoting the transformation of macrophages from classic activated pro-inflammatory type to selectively activated anti-inflammatory type (M2). MSCs secreting the anti-inflammatory factor TSG-6 combined with CD44 of macrophages will destroy the interaction between CD44 and toll-like receptor (TLR)2, inhibit the nuclear factor (NF)-κB signal downstream, and reduce the inflammatory response.47 For dendritic cells, MSCs can secrete HGF under endotoxin stimulation to induce differentiation into regulatory dendritic cells and alleviate acute lung injury.48

**MSCs inhibit cytokine storm in severe patients.** Cytokine storm in patients with severe COVID-19 can lead to the release of nitric oxide, which affects the normal systolic and
diastolic function of blood vessels, thereby causing hypotension and multi-organ hypoxia. Research has found that there are a large number of inflammatory factors in blood of patients with COVID-19, such as interferon (INF)-γ, interferon-inducible protein-10 (IP-10), and monocyte chemoattractant protein 1 (MCP-1). Research has also shown that the concentration of granulocyte colony-stimulating factor (G-CSF), MCP-1, tumor necrosis factor (TNF)-α, and other inflammatory factors in ICU patients is significantly higher than that in non-ICU patients. The severity of cytokine storm is positively correlated with the clinical manifestations of COVID-19.

COVID-19 can change from a mild disease to a severe disease. Although this partially results from complications, cytokine storm also has certain damaging effects. IL-6 levels in severe patients are ten-times higher than those in non-severe patients. In addition, the IL-6 level is closely related to the serum SARS-CoV-2 virus content and vital signs of patients with COVID-19. Some scholars have now shown that sufficient use of tozumab (anti-IL-6 receptor) can prevent worsening of the disease. MSCs that are derived from the human umbilical cord can also inhibit monocyte activation and IL-6 production to inhibit the development of cytokine storm and improve the patient’s prognosis. Under stimulation by high IL-6 levels, MSCs can adaptively produce cytokines and exosomes that are enriched with mirR-455-3p, thus relieving cytokine storm and treating acute inflammatory liver injury. However, the effect of MSCs on cytokine storm in patients with COVID-19 still needs further research and confirmation.

MSCs inhibit pulmonary fibrosis and ARDS aggravation. Studies have shown that after intravenous MSC infusion, MSCs can return to the lungs and improve the microenvironment of the lungs, protect alveolar epithelial cells, promote neovascularization, and prevent pulmonary fibrosis. MSCs achieve their repair function through a variety of cytokines, especially keratinocyte growth factor (KGF). KGF promotes alveolar fluid clearance and alleviates acute lung injury that is induced by endotoxin by up-regulating α1 subunit of ACE-2. KGF can also up-regulate the activity of sodium potassium ATP enzyme in alveolar cells, improve alveolar fluid transport, and play a therapeutic role in ARDS and lung injury that is induced by bacterial pneumonia.

The role of MSCs in bacteriostasis. Previously, some scholars questioned whether the virus could cause MSCs to lose their function when the MSCs are invaded by bacteria. However, a clinical trial in Beijing, which included seven patients with severe COVID-19 and three controls, showed that the COVID-19 virus could not infect umbilical cord MSCs that were infused intravenously. Current research has confirmed that MSCs can exert their anti-COVID-19 virus effect through direct and indirect mechanisms. MSCs can produce a direct anti-virus effect by secreting antibacterial peptides and proteins (AMPs), indoleamine 2,3-dioxygenase (IDO), IL-17, and other molecules, and unlike somatic cells that produce interferon during virus invasion and then activate hundreds of genes that resist virus infection, MSCs can continuously activate a large number of anti-virus genes independent of interferon, such as the IFITM gene, which can encode protein structures that prevent viruses from invading cells. MSCs can also exert an indirect antiviral effect by regulating the dynamic coordination of pro-inflammatory and anti-inflammatory elements of the patient’s immune system and promoting the activity of phagocytes. Researchers have also confirmed the immunoregulation and antibacterial and antiviral values of MSCs.
using an in vitro sepsis model, ARDS model, and alveolar epithelial fibrosis model. MSCs have been found to secrete at least four AMPs, which are antibacterial peptide LL-37 (Cathelicidin LL-37), human defensin 2 (Human defensin-2), hepcidin, and lipocalin-2. These AMPs mediate the cell killing process by killing cells, inhibiting the synthesis of essential proteins, DNA, and RNA of infected cells, interacting with certain targets in infected cells, and playing an active regulatory role in the infection and inflammatory progress of patients with COVID-19.

**MDC clinical studies**

The results of two recent clinical trials showed that MSCs have a curative effect in the treatment of COVID-19. In the first clinical trial, human umbilical cord MSCs were used in three intravenous infusions that were administered to patients with COVID-19. After the second intravenous infusion, the neutrophil levels in the subjects decreased significantly, lymphocytes increased, CD4+ T and CD8+ T cells returned to normal level, and most vital signs were improved. The second clinical trial showed that transplantation of MSCs can improve the prognosis of patients with COVID-19. This clinical trial recruited seven patients with COVID-19 (two mild cases, four severe cases, and one critical case) to receive one intravenous MSC transplantation each. Two to 4 days after MSC transplantation, the patient’s regulatory dendritic cell population increased, the level of the pro-inflammatory factor TNF-α decreased, and the level of anti-inflammatory factor IL-10 increased. The above evidence shows the beneficial effect of MSCs on the treatment of severe patients. However, more clinical data are still needed to confirm its effectiveness.

**Selection of different MSC sources**

In accordance with the current US Food and Drug Administration (FDA) regulations, autologous bone marrow MSCs are widely used for clinical treatment of pain that is caused by an abnormal musculoskeletal system in patients. This approach was shown to be safe and effective by many groups. The content of MSC in adult bone marrow is relatively low, and it can be injected into joints, the spine, and other places, but it is not enough for patients with systemic diseases caused by COVID-19. Other MSCs that are available for clinical use include adipose MSC, amniotic membrane MSCs, and umbilical cord MSCs, among which umbilical cord MSCs have the best clinical promotion value for the reasons that are described below.

Compared with bone marrow, the umbilical cord has a wider source and a higher concentration of MSCs. Systemic multi-system injury of patients with COVID-19 is serious, the demand for MSCs infusion is large, and the expansion speed of umbilical cord MSCs is extremely fast, which can efficiently complete this process under laboratory conditions. Umbilical cord MSCs can be extracted using a non-invasive method, unlike bone marrow or fat MSCs. Compared with bone marrow or adipose MSCs, umbilical cord MSCs have a gene profile that is similar to embryonic MSCs, which means that it has a faster amplification speed, stronger plasticity, and no tumorigenic effect, and thus, it has better clinical efficacy. However, in contrast to the value of embryonic MSCs, umbilical cord MSCs are a type of tissue that is formed after birth that was previously considered to have no effect. Extraction of umbilical cord MSCs will not cause damage to the human body.
complex (MHC) class I molecule expression, and their cell surface expresses few MHC class II molecules, and thus, it has good immune escape. For these reasons, MSCs that were derived from the umbilical cord have also been used in the clinical trials of MSC therapy for patients with COVID-19 in China.

**Route of administration**

To date, research results have shown that intravenous infusion is still the most preferred route of administration that has been used in the current relevant studies in China. Compared with arterial infusion and intra-tissue injection, intravenous infusion has the characteristics of less trauma, safety, and reliability. Most MSCs will eventually settle in lung tissue after systemic circulation, and the lung happens to be one of the target organs that is most seriously infected by COVID-19. Previous studies also showed that MSCs that settled in lung can act on distant damaged organs through direct secretion or in a paracrine manner. Some studies have shown that MSC administration to tracheal intubation patients via the airway also has certain clinical effects.

**Safety of MSC treatment**

The most important thing is the quality of the MSCs, which should be obtained from authorized laboratories that meet the standards of China’s Health and Safety Commission. Researchers should strictly screen umbilical cord MSC donors and ensure sterility at every step from the collection of umbilical cord tissue to the final preparation of reagents for clinical application. The quality assurance staff should analyze cell activity, and they must meet the clinical requirements. Clinically, sufficient preventive measures should be taken for possible complications such as pulmonary embolism when MSCs are infused into patients. Because most of the injected MSCs come from allogeneic tissues, we should always be alert for allergic reactions in patients. There have been reports of serious complications that were caused by improper administration of MSCs in the past. First-line doctors should formulate individualized treatment plans based on the patient’s situation, such as cell dosage, cell suspension concentration, and infusion speed, to ensure the maximum curative effect.

**Discussion**

COVID-19 enters host cells by binding S protein on its surface via ACE-2 that are located on the surface of human cells. ACE-2 is widely expressed in human lungs, heart, liver, kidney, and various digestive organs. Almost all endothelial cells and smooth muscle cells in the human body express some ACE-2. Therefore, once the COVID-19 virus enters the blood circulation, it will rapidly spread to multiple organs and tissues, which can also explain why ICU patients often have complications such as acute myocardial injury, arrhythmia, acute kidney injury, shock, MOD, and other complications besides ARDS.

Epidemiological data show that COVID-19 has a greater impact on elderly men who have underlying diseases, and they are more prone to fatal respiratory diseases such as ARDS. The current treatment and rehabilitation for critically ill patients still mainly depends on the patient’s own immune mechanism. When an over-activated immune system kills virus pathogens, it also produces a large number of inflammatory factors. Some patients progress into life-threatening inflammatory factor storms, while the elderly are more affected by the decline of the immune system. Abnormal activated immune cells
cause a large amount of cytokines to be released, leading to endothelial cell dysfunction, capillary leakage, accumulation of mucus in the lungs, and eventually respiratory failure in patients. Cao et al.\textsuperscript{12} reported that serum IL-2, IL-7, G-CSF, IP10, MCP-1, MIP-1A, and TNF-\textgreek{a} levels in ICU patients were higher than those in ward patients. A study showed that MSCs can secrete anti-inflammatory factors to inhibit the progression of the disease.\textsuperscript{3} Flow cytometric analysis results showed that COVID-19 virus causes the failure of lymphocytes and whole immune system function through infection. MSCs can regulate or even reverse this process by inducing mature dendritic cells to enter the Jagged-2-dependent dendritic cell population, prevent monocyte migration to the lung, and up-regulate IL-10 and vascular endothelial growth factor (VEGF) expression and that other factors to promote lung repair. Therefore, MSCs have some therapeutic value for severe COVID-19 patients.\textsuperscript{76,77}

Conclusion and future prospects
COVID-19 has become a common problem that is facing the global medical system. It is characterized by a long incubation period, strong infectivity, and high mortality rate, which seriously endangers human health and social development. However, there is still a lack of effective treatment for severe patients. SARS-CoV-2 can cause inflammatory factor storm through the patient's excessive immune response, which can lead to ARDS, sepsis, MOD, and even death. MSCs have obvious two-way immunoregulation ability. On the one hand, MSCs regulate the balance of patients' immune system by secreting factors that inhibit inflammation. On the other hand, MSCs accumulate in damaged tissues by homing, and MSCs also have direct antiviral ability by secreting various growth factors to improve the microenvironment, repair damaged cells, and stimulate cardiovascular formation. Studies have shown that intravenous infusion of the umbilical cord MSCs can reduce lung injury and prevent or reduce ARDS and other serious complications that have occurred in patients. MSCs also show efficacy in reducing the mortality rate of COVID-19 patients. Currently, China is conducting several clinical trials related to MSC treatment of COVID-19 at different stages, which have initially shown the safety and effectiveness of MSCs, but the complete mechanism of action requires further research.

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Dr. Wei Shixiong collected data and wrote the first draft of the article. All authors read and approved the final manuscript.

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