

Review

Potential roles of mesenchymal stem cells and their exosomes in the treatment of COVID-19

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1. Abstract

Background: Corona Virus Disease 2019 (COVID-19) is an acute respiratory infectious disease caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2). The primary pathogenesis is over-activation of the immune system. SARS-CoV-2 continues to mutate and spread rapidly and no effective treatment options are yet available. Mesenchymal stem cells (MSCs) are known to induce anti-inflammatory macrophages, regulatory T cells and dendritic cells. There are a rapidly increasing number of clinical investigations of cell-based therapy approaches for COVID-19. **Objective:** To summarize the pathogenic mechanism of SARS-CoV-2, and systematically formulated the immunomodulation of COVID-19 by MSCs and their exosomes, as well as research progress. **Method:** Searching PubMed, clinical-trials.gov and Chictr.cn for eligible studies to be published or registered by May 2021. Main keywords and search

strategies were as follows: ((Mesenchymal stem cells) OR (MSCs)) AND (COVID-19). **Results:** MSCs regulate the immune system to prevent cytokine release syndrome (CRS) and to promote endogenous repair by releasing various paracrine factors and exosomes. **Conclusions:** MSC therapy is thus a promising candidate for COVID-19.

2. Introduction

According to real-time WHO network data, the worldwide number of confirmed COVID-19 cases to April 22, 2021 was 143,488,236 and 3,055,587 deaths, posing an unprecedented threat to the global economy and to human health [1]. The International Committee on the Taxonomy of Viruses named the COVID-19 pathogen as SARS-CoV-2. This virus gains phagocytic entry into AT2 via interaction with angiotensin-converting enzyme 2 (ACE2) (See Fig. 1). It increases Angiotensin-2 (Ang-2) levels, lead-

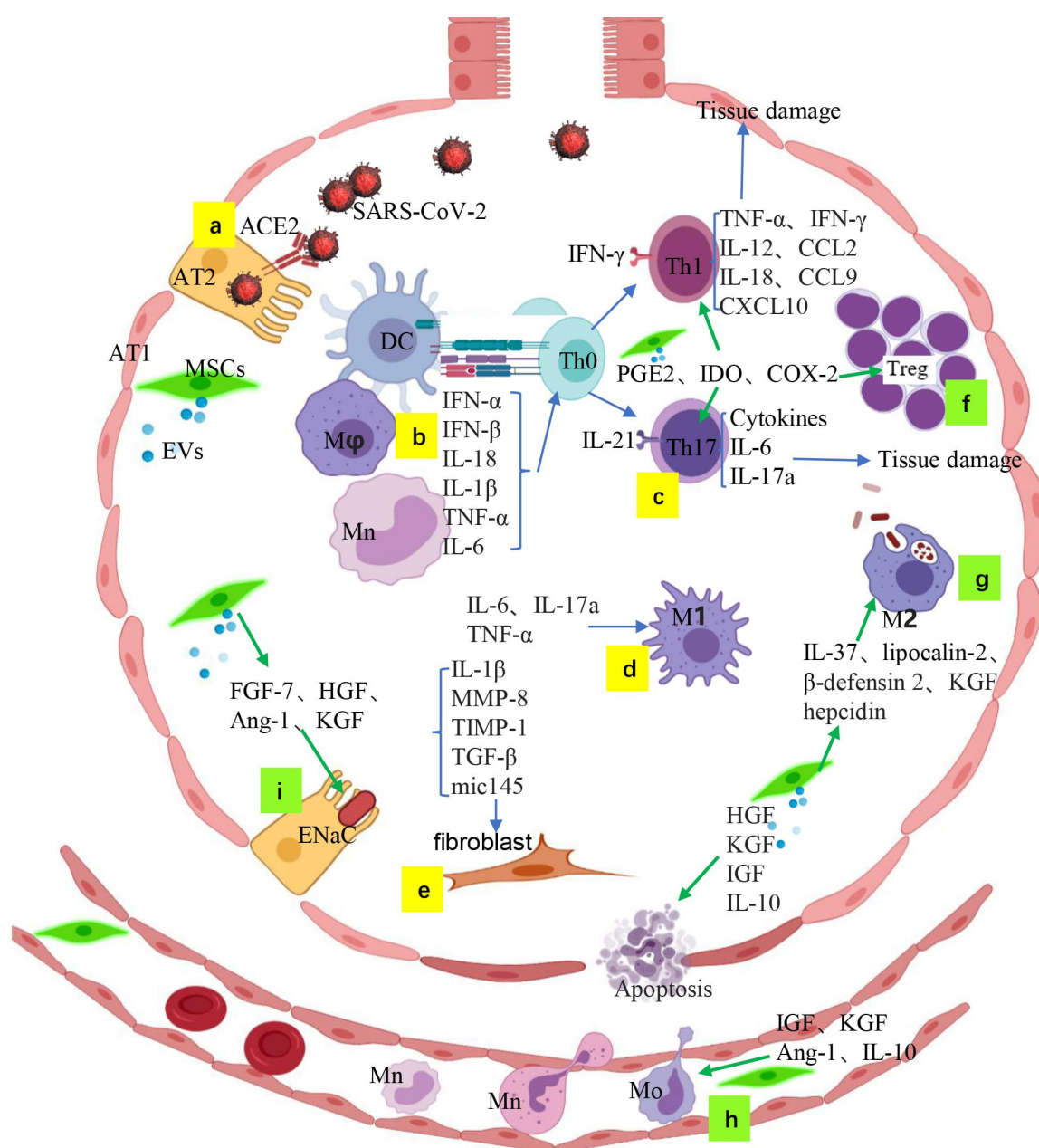


Fig. 1. The pathological processes of COVID-19 (blue arrows) and multiple therapeutic mechanisms of MSCs and their exosomes in COVID-19 (green arrows). (a) SARS-CoV-2 gains entry into AT2s via ACE2. (b) Immune cells recognize SARS-CoV-2 via TLRs. (c) T cells are polarized into pro-inflammatory phenotypes. (d) Excessive activation of M1 causes CRS. (e) Inflammation and oxidative damage cause lung fibrosis and remodeling. MSCs reduce fibrosis by various paracrine factors. (f) MSCs force T cells to polarize into Tregs. (g) MSCs and MSC-exosomes increase the number of anti-inflammatory M2 macrophages. (h) MSCs restore microvascular permeability. (i) MSCs activate the Na⁺/K⁺ pump to remove lung fluid and reduce pulmonary edema. Abbreviations: ECM, extracellular matrix; PGE 2, Prostaglandin E 2; COX, Cyclooxygenase; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor.

ing to long-term and intense activation of pro-inflammatory Ras-related pathways. A high concentration of Ang-2 in the lung interstitium promotes cell apoptosis, releases pro-inflammatory cytokines and triggers the inflammatory response, thereby causing immune-induced tissue damage and increased vascular permeability [2, 3]. In patients with severe disease, the development of CRS is an abnormal systemic inflammatory response that manifests clinically as a

rapid and sharp rise in the level of cytokines. These include C-X-C Motif Chemokine Ligand 10 (CXCL10), monocyte chemo-attractant protein-1 (MCP-1/CCL2), macrophage inflammatory protein-1 (MIP-1), platelet-derived growth factor (PDGF), tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor (VEGF) [4]. This mechanism results in an imbalance between tissue damage and repair, leading to respiratory failure. Patients may eventu-

Table 1. The potential roles and mechanisms of MSC-exosomes in various diseases.

Functions	Involved genes/factors/pathways	Diseases	References
(+) Immunomodulatory activities (+) re-pair (+) angiogenesis (-) fibrosis	miR-21, miR-24, miR-124, miR-145, miR-122, KGF, VEGF, HGF, VE-cadherin, Occludin-1, Claudin-1, PCNA, Cyclin D1, IDO, Wnt/ β -catenin signalling, TGF- β 1/Smad 2	COVID-19	[17–22]
(-) EMT	miR-182-5p, miR-23a-3p (-) NF- κ B, Hedgehog	LPS induced lung injury	[23]
(-) Inflammation, proliferation	miR-34a, miR-122, miR-124, miR-127	IPAH	[24]
(-) Leukocyte infiltration	ICAM-1	ARDS	[25]
Anti-viral	COX-2, PGE2	Influenza	[26]
(-) Airway hyperresponsiveness	miR-146a-5p	Allergic airway inflammation	[27]
(-) Autophagy, apoptosis	miR-125b, Bcl-xL, Bcl2, and BIRC8 (-) Caspase1, Caspase 8, lymphotoxin α	Myocardial infarction, acute kidney injury.	[28, 29]
(-) Fibrosis	(-) Col-I, Col-III, TGF- β 1/Smad	Liver fibrosis	[30, 31]
(+) M2 macrophage polarization	miR-1260b (-) Wnt5a/RANKL	Periodontitis, Tendon healing	[32, 33]
(+) Treg cell induction, T-cell apoptosis	FASL/FAS	Acute colitis	[34]
(-) Dendritic cell maturation	a blockade in G0/G1 phase of the cell cycle	LPS intervention	[35]
(+) Autophagy	AMPK/ mTOR, Akt/mTOR	Myocardial I/R	[36]

(+) indicates stimulation. (-) indicates inhibition.

ally die from multiple organ failure. Disruption of matrix metalloproteinases (MMPs) during the inflammatory stage causes complex damage to the alveolar epithelium and to the pulmonary vascular endothelium [5]. Moreover, persistent stimulation of epithelial cells results in senescence-related phenotypes. Consequently, abnormal interactions between fibroblasts and epithelial cells generate irreversible damage and fibrosis [6].

Although the efficacy of vaccines in preventing COVID-19 ranges from 50% to 95%, an increasing number of COVID-19 cases still require effective treatment options [7, 8]. There is currently no standard drug therapy for COVID-19. Antiviral, anti-malarial and anti-inflammatory drugs are unable to repair or regenerate lung tissues, especially in patients with severe complications such as acute respiratory distress syndrome (ARDS) [9–11]. Since the outbreak of COVID-19, a number of studies have been carried out on MSCs and MSC-exosome therapy for this disease. MSCs have good safety for the treatment of COVID-19 and show particular clinical efficacy in shortening the course of disease, alleviating lung injury and reducing the level of inflammatory factors [6, 12, 13]. This is expected to provide a new approach for the treatment of severe and critical COVID-19. MSC-exosomes also show promise as a cell-free substitute for COVID-19 [14–16] (Table 1, Ref. [17–36]). The potential mechanisms of action of MSCs and MSC-exosome therapy are shown in Figs. 1,2.

3. MSCs regulate immune cells in COVID-19

3.1 MSCs interfere with the differentiation, maturation, and function of antigen-presenting cells (APCs) in COVID-19

Once SARS-CoV-2 infects the human lung epithelium and is internalized (Fig. 1a), SARS-CoV-2 RNA acti-

vates the intracellular receptors TLR3/7 and membrane receptors TLR2/4. TLR3 in dendritic cells (DCs) specifically recognize dsRNA, an intermediate product of viral replication, thereby activating nuclear factor kappa-B (NF- κ B) and interferons (IFNs). TLR2 and TLR4 located on the surface of macrophages and DCs can activate interferon regulatory factors (IRFs) and NF- κ B, resulting in the production of different cytokines and chemical activators. TLR2 can mediate DCs to express interleukin-8 (IL-8) and IL-23 (Fig. 2b), while TLR4 mainly mediates DCs to produce IL-12 and IFN- γ -induced protein 10 (IP-10). IP-10 in turn stimulates T cells to secrete IFN and promotes the differentiation of T helper-type (Th) cells into Th1 cells (Fig. 1c) [24].

MSCs can interfere with the antigen-presenting function, differentiation and maturation of DCs by paracrine IFN- γ , indoleamine 2,3-dioxygenase (IDO), transforming growth factor- β (TGF- β), IL-10 and prostaglandin E2 (PGE2) [37]. Consequently, this reduces the activation of DCs and their pro-inflammatory secretions [38, 39]. Leng *et al.* [40] observed a significant increase in the number of CD14+CD11c+CD11^{mid} low-activity phenotypic DCs on day 6 after MSC transplantation. This prevented the excess proliferation of T cells in COVID-19 patients. The interaction between MSCs and DCs also leads to an indirect conversion of pro-inflammatory Th1 to anti-inflammatory Th2 immunity [41].

3.2 MSCs regulate the polarization of macrophages in COVID-19: from M1 to M2

Pro-inflammatory macrophages were reportedly more abundant in the bronchoalveolar lavage fluid from severe compared to mild COVID-19 cases [42]. Zhang *et al.* [43] proposed that CRS in severe COVID-19 is mainly a virus-triggered macrophage activation syndrome. Viral RNA stimulates macrophages to produce various soluble

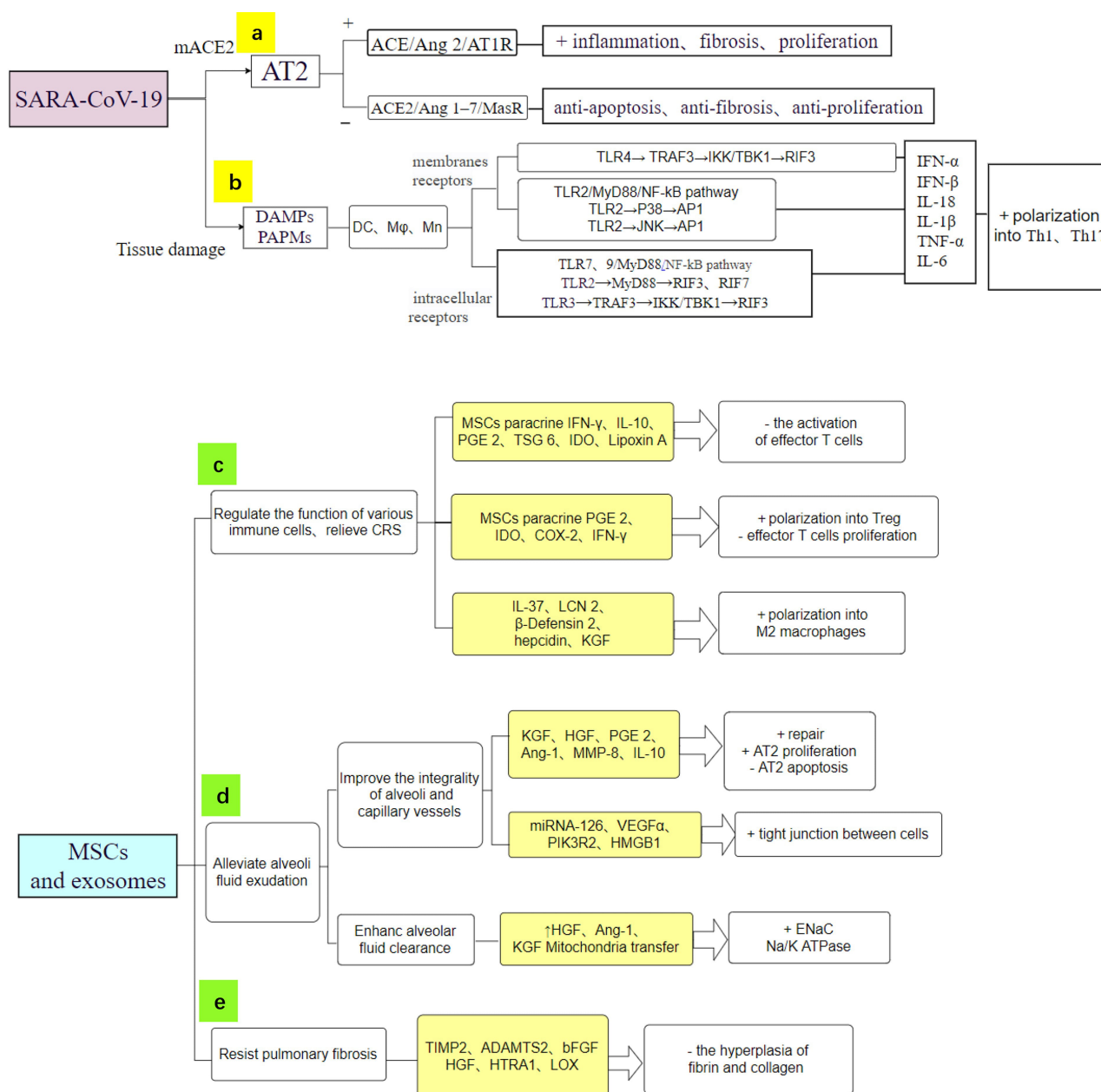


Fig. 2. MSCs act on COVID-19 through multiple mechanisms and specific secretory products (light yellow squares). (a) SARS-CoV-2 causes immune-induced tissue damage via pro-inflammatory Ras-related pathways. (b) TLRs of immune cells recognize SARS-CoV-2, leading to CRS. (c) MSCs have powerful anti-inflammatory and immunomodulatory functions. (d) MSCs repair microvascular permeability and alleviate pulmonary edema. (e) MSCs secrete substances to inhibit the hyperplasia of fibrin and collagen, thereby alleviating PF. + indicates stimulation. – indicates inhibition.

factors via the activation of TLRs. These factors include IFN- γ , IP10, MCP1, MIP10, granulocyte colony stimulating factor (G-CSF), IL-2, IL-6, IL-7 and TNF- α . In particular, IL-6 and TNF- α cause macrophages to differentiate into M1 (Fig. 1d), thus causing an imbalance in M1/M2. In addition to direct stimulation by viral RNA, ATP is released by the dead cells as DAMPs and binds to the P2X7 receptor (P2X7R). This in turn activates NOD-like receptor protein 3 (NLRP3) inflammasomes and increases macrophage-derived IL-1 β and IL-18 [37]. The loss of alveolar macrophages is a major underlying cause of

refractory respiratory failure in COVID-19 and it has been reported they are almost entirely depleted in severely infected patients [44].

MSCs regulate macrophage polarization to limit inflammation and to promote tissue healing following injury. Anti-inflammatory soluble factors and two main inhibitory molecules secreted or expressed by MSCs trigger the immune system's inhibitory response. MSCs are stimulated to produce IL-10, thyrotropin-releasing hormone-6 (TRH-6), human leukocyte antigen (HLA), human growth factor (HGF), heme oxygenase-1 (HO-1) [45], superoxide

dismutase (SOD), cyclooxygenase-2 (COX-2), PGE2 and IDO [46]. Meanwhile, MSCs are induced by IFN- γ to up-regulate the expression of other inhibitory molecules, including PD-1/PDL-1 and FAS-FASL [47–49]. These soluble factors and molecules inhibit pro-inflammatory pathways such as the NF- κ B signaling pathway and also polarize M1 macrophages into the anti-inflammatory M2 phenotype [50, 51]. In this way, MSCs reduce the production of inflammatory cytokines by macrophages in COVID-19 [52].

3.3 MSCs regulate lymphocyte subsets and apoptosis in COVID-19

Flow cytometry analysis has shown that the number of CD4+ and CD8+T cells in the peripheral blood of COVID-19 patients was significantly reduced, with the degree of reduction being related to the severity of COVID-19 [53]. This phenomenon may be related to the recruitment of T cells from peripheral blood to lung tissue, and to the apoptosis of T cells induced by the virus [54]. COVID-19 patients present with lymphocyte deficiency and over-activation of T cells. These effector T cells are stimulated by pro-inflammatory mediators produced by DCs, macrophages and neutrophils [55, 56]. A significant rise in HLA-DR+CD38+ cell levels can manifest in the over-activation of T cells. The proportion of highly pro-inflammatory CCR4+CCR6+ Th17 cells amongst CD4+ T cells then increases [57]. High expression of IL-17A in Th17 induces the migration of inflammatory white blood cells, leading to inflammatory infiltration and destruction of lung tissue. Additionally, the major histocompatibility complex 1 (MHC-1) of infected cells presents viral antigens, thus activating CTLs to produce high levels of cytotoxic granules such as perforin and granzymes. This implies that over-activation of T cells and the elevated cytotoxicity of CD8+T cells leads to an excessive immune response. T cell-derived cytokines and chemokines such as TNF- α , IFN- γ , IL-2, IL-12, CCL2, IL-18, CCL9, CXCL10, IL-6 and IL-17 are released in large quantities and damage the lung tissue [58]. When the T cell count falls to its lowest level, the concentrations of serum IL10, IL2, IL4, TNF- α and IFN- γ reach their peak on days 4–6. Moreover, the levels of IL-6, IL-7, G-CSF, IP-10, monocyte chemotactic protein-1 (MCP-1) and MIP1a increase significantly, thus causing CRS [59].

Leng *et al.* [40] reported that on day 4 after MSC transplantation, the absolute lymphocyte count increased to $0.58 \times 10^9/L$ and lymphocytopenia improved significantly. On days 3 to 6 after transplantation, the level of TNF- α decreased whereas that of IL-10 increased. Similar reversals were reported in another study [60]. T cell counts were also analyzed in a non-randomized, open-label cohort study of COVID-19 patients [18]. This indicated that MSC-exosome therapy significantly improved the absolute neutrophil count by a mean of 32% [p value < 0.001] in patients

with severe COVID-19. Moreover, the mean CD3+, CD4+ and CD8+ lymphocyte counts increased by 46% ($p < 0.05$), 45% ($p < 0.05$) and 46% ($p < 0.001$), respectively.

The mechanism of action of MSCs in reversing lymphocytopenia and reducing inflammatory mediators in COVID-19 is mainly attributed to the more than 30 soluble paracrine factors such as PEG2, IDO and COX-2 [61]. These have been shown to inhibit the proliferation of CD4+ Th1 and Th17 cells as well as CD8+T cells, and to induce Foxp3+ Treg differentiation (Fig. 1f). They also indirectly inhibit excessive T cell proliferation by interacting with APCs and other immune cells. IL-10 is a critical negative regulator of T cell responses and directly inhibits the ability of T cells to produce pro-inflammatory mediators. IL-10 also reduces the antigen presenting capacities and co-stimulation of macrophages and DCs, thereby decreasing T cell-derived IL-6 and TNF- α , which is one of the essential mechanisms by which MSCs alleviate inflammation [62, 63]. This was demonstrated in a recent clinical report by Meng *et al.* [64] which showed that patients who received MSCs had lower IL-6 levels than those who received placebo [65].

Through their expression of PD-L1 and FasL, MSCs can inhibit abnormally activated Th1 cells, thus inhibiting IL- γ release from Th1. This prevents further macrophage activation in a vicious loop and restores Th1/Th2 balance. Long-term FasL interaction can induce apoptosis of cytotoxic T cells [66].

At the same time, MSCs release TGF- β which promotes the proliferation of CD4(+) CD25(+) FoxP3(+) Treg, CD3(+) CD8(+) CD28(–) T-suppressor cells (Ts), and IL-10-producing B cells [67]. MSCs also up-regulate IDO and PGE2, which have synergistic inhibitory effects on NK and Th17 cells. MSCs inhibit B cells through cell cycle stagnation in the G0/G1 phase rather than by inducing apoptosis. With regard to cellular immunity, MSCs reduce the production of immunoglobulin M (IgM), IgG, and IgA, and down-regulate the expression of CXCR4, CXCR5 and CXCR7 in B cells, thereby altering their chemotactic properties [68].

4. MSCs improve ARDS in COVID-19

ACE2 is down-regulated following the entry of SARS-CoV-2 into alveolar epithelial cells, resulting in an imbalance of ACE/Ang II/AT1R and ACE2/Ang 1-7/MASR. Elevated Ang-2 levels then lead to cell apoptosis and trigger inflammatory responses, giving rise to immune-induced tissue damage and increased vascular permeability [69–71] (Fig. 2b).

In COVID-19 patients the average time from symptom onset to dyspnea is 5 days, the average hospital stay is 7 days, and the average time for onset of ARDS is 8 to 9 days [72]. By day 8 to 14 of disease onset, the overexpression of cytokines such as IL-2, IL-6, IL-7, IP10, MCP1, MIP1A and TNF α causes activation of lympho-

cytes and macrophages, leading to an excessive inflammatory response [73]. The integrity of alveolar walls and pulmonary capillaries are destroyed, resulting in edema that impairs oxygen exchange and respiration and inevitably develops into ARDS [74, 75].

MSCs and MSC-exosomes can effectively alleviate COVID-19-induced ARDS in a dose-dependent manner by increasing alveolar fluid clearance and by improving airway and hemodynamic parameters [76]. MSC-exosomes have been used as intravenous infusion therapy for ALI and pulmonary fibrosis (PF) [23, 77, 78]. An earlier study showed the exosomes release keratinocyte growth factor (KGF) and Lipoxin A4 which act to prevent long-term lung damage caused by COVID-19 and to promote tissue repair by activating Na⁺/K⁺ pumps [79] (Fig. 2d). Importantly, MSCs have been shown to restore epithelial protein permeability, stabilize endothelial fluid leakage, and maintain alveolar-capillary barrier function by secreting Ang-1 [80–82]. In addition, MSCs can inhibit cellular signaling pathways mediated by TLRs or PRRs, as well as reducing local immune cell recruitment (Fig. 2c). miRNA-126, VEGF- α , phosphoinositide-3-kinase regulatory subunit 2 (PIK3R2) and high mobility group box chromosomal protein 1 (HMGB1) can each restore the vascular endothelial cadherin-catenin (VE-Cadherin) complex and reduce endothelial barrier permeability to relieve ARDS.

5. MSCs promote lung regeneration and reverse PF in COVID-19

PF is a refractory lung disease that develops due to persistent alveolar injury, repeated destruction, repair, reconstruction, and excessive deposition of extracellular matrix (ECM) [83]. Current studies have determined that only 1% of AT2 cells can regenerate following SARS-CoV-2-induced lung injury [84, 85].

High expression of IL-17A by Th17 in COVID-19 can induce the migration of inflammatory leukocytes, leading to inflammatory infiltration and the destruction of lung tissue [86]. High levels of TNF- α induce the recruitment of immune cells and reduce antioxidant molecules in parenchymal and endothelial cells, causing lung fibrosis and remodeling (Fig. 1e). MSCs improve angiogenesis mainly through paracrine release of pro-angiogenic/anti-apoptotic agents such as Ang, IL-3, MMP-1 and VEGF [87]. They also secrete ECM regulators such as fibroblast growth factor, HGF and MMPs to regenerate damaged tissues [88]. Moreover, MSCs express or secrete ADAM, metalloproteinase with thrombospondin Type 1 Motif 2 (ADAMTS2), basic fibroblast growth factor (bFGF), collagen 15A1 (COL15A1), COL16A2, COL18A1, HGF, high temperature requirement A1 (HTRA1), lipoxigenase (LOX), and tissue inhibitor of metalloproteinases 2 (TIMP2). These regulate the expression of collagen, fibronectin and elastin fibrilaments in lung tissue, thereby

alleviating PF [89]. MSCs can also reverse PF by over-expressing MMP-P1 and decreasing collagen-1 (COL-I) production during TGF- β 1-induced fibrosis. In conclusion, MSCs can promote angiogenesis and the regeneration of alveolar epithelial cells, prevent the apoptosis of endothelial cells, reduce the levels of TGF- β , TNF- α , COL-I, COL-III, Hydroxyproline and serum ceruloplasmin, inhibit myofibroblast growth, and thereby alleviate or reverse PF (Fig. 2e).

Human embryonic stem cells (hESCs) derived from immune and stromal regulatory cells (IMRCs) have been used to treat lung injury and fibrosis *in vivo*. IMRCs have superior efficacy to FDA-approved pirfenidone [14] and show excellent efficacy and safety in both mice and monkeys [90].

6. MSC-exosomes for the treatment of COVID-19

MSC-exosomes are able to transfer cargoes such as mRNA, miRNA, proteins, lipids and even mitochondria to target cells and tissues, resulting in changes to gene expression and in the behavior of target cells. Hence, MSC-exosomes could have a therapeutic role in COVID-19 [91, 92]. Preclinical studies have confirmed that MSC-exosomes are able to serve as acellular alternatives [78].

6.1 Comparison of MSC-exosomes and MSCs

6.1.1 Can MSC-exosomes effectively replace MSCs?

A growing number of studies have established that the healing, nutritional, immunoregulation, and anti-inflammatory effects of administered MSCs are due to the exosomes they release. These effects of MSCs have been observed *in vitro* after the addition of MSC-exosomes [37]. MSCs were cleared from the circulation within 24 hours, but MSC-exosomes were detected in lung parenchymal cells and macrophages just 1 hour after injection and remained there for up to 7 days [93]. The efficacy and safety of a single intravenous injection of MSC-exosomes were recently assessed in 24 COVID-19 patients who presented with moderate to severe ARDS. The clinical symptoms, oxygenation, serum markers of acute inflammation, neutrophil and lymphocyte counts all improved in patients who received MSC-exosomes, with no side effects reported [18].

The immunomodulatory effects of MSC-exosomes have also been attributed to their anti-inflammatory cargo, such as IDO, HLA-G, PD-L1, galectin-1, IL-10, TGF- β 1 and HGF. These factors inhibit IL-1, IL-6, NK cells, effector and cytotoxic T cells, while activating M2 macrophages and Treg to further suppress the over-activated immune system [94, 95]. Importantly, MSC-exosomes also transfer miRNAs that play a role in COVID-19, including miRNA-145, miRNA-126, miRNA-199a, miRNA-221, miRNA-27 and Let-7f [88, 96, 97]. In coronavirus pneumonia and influenza, mi-RNAs carried by

MSC-exosomes inactivate cytoplasmic mRNA encoding proteins and change nuclear DNA through methylation [26]. Thus by changing the expression of cell receptors and directly preventing the entry of RNA viruses. In addition, MSCs have comparable immunoregulatory activity.

MSC-exosome-transferred miRNAs cause APCs to produce fewer Ag/MHC molecules on their surface, thus resulting in reduced activation of effector T cells. miRNAs carried by MSC-exosomes also mediate the function of macrophages, NK cells, T cells and B cells to inhibit infection [98].

6.1.2 MSC-exosomes are safer than MSCs for COVID-19 treatment

In the context of COVID-19, MSCs are known to aggregate in the peripheral microvasculature and exacerbate vascular clots, causing central or peripheral vascular dysfunction. This is probably because MSCs express procoagulant tissue factor (TF/CD142) on their surface [99, 100].

The small size and low immunogenic effect of MSC-exosomes allows them to pass through small blood capillaries without triggering a blood clot [101]. Because of their strong ability for self-replication and differentiation, the carcinogenicity of MSCs is also another clinical challenge. MSC-exosomes cannot replicate and hence there is no risk of endogenous tumor formation [102].

6.2 Unique advantages of MSC-exosomes over MSCs for COVID-19 treatment

6.2.1 Advantages of MSC-exosomes for COVID-19: practical considerations

The challenges surrounding the use of MSCs for COVID-19 that still need to be overcome include their immuno-compatibility, stability, heterogeneity, differentiation and migration. The low homing rate of MSCs is also the focus of current research. Although Xiao *et al.* raised the possibility that CD90 binding to the specific integrins b3 and b5 could to some extent promote MSC homing [103], MSC-exosomes have an important advantage in their homing ability. Due to their nanosized dimension, intravenously injected MSC-exosomes accumulate in COVID-19-damaged organs through blood circulation [104]. MSC-exosomes from allogenic sources can also be used immediately after thawing and without washing. In addition, MSC-exosomes are easier to use routinely in hospitals compared to MSCs. Finally, the cost of using MSC-exosomes is much lower than that of MSCs.

6.2.2 MSC-exosome as a drug and miRNA delivery system for COVID-19

Designing miRNAs that specifically bind to the SARS-CoV-2 genome could allow disruption of SARS-CoV-2 without any side effects on human gene expression [105]. Thus, MSC-exosomes that carry miRNAs may be a promising new approach to COVID-19 therapy. MSC-

exosomes can be loaded with miRNAs either by direct insertion of the nucleic acids, or by collecting the exosomes from genetically-modified MSCs [106]. For example, miR-32, the first miRNA found to target viral RNA, binds to retrovirus PFV-1 transcripts to reduce viral replication [107], while miR-146a has been shown to specifically inhibit COX-2 in lung epithelial cells. miR-375 inhibits the trans-differentiation of myofibroblasts and their synthesis of collagen by blocking P38 [108].

MSC-exosomes are thus a novel intervention tool for COVID-19 treatment that can successfully deliver exogenous miRNAs to exert antiviral function. When combined with antiviral drugs such as Remdesivir, MSC-exosomes can therefore serve as an effective drug delivery system [109].

6.2.3 Potential of MSC-exosomes for vaccine development

Spike protein is one of the structural proteins of SARS-CoV-2 that facilitates viral entry into the host cells. Therefore, spike protein is a good target for the development of anti-SARS coronavirus vaccines. Seraphin *et al.* showed that MSC-exosome-based vaccines containing the SARS-CoV-2 spike protein could induce high levels of neutralizing antibodies [17, 110–112].

7. Clinical trials of MSCs and MSC-exosome therapy for COVID-19

Current treatment trials for COVID-19 include corticosteroids, PD-1/PD-L1 checkpoint inhibitors, cytokine absorption devices, convalescent plasma [113] and anti-malarial and antiviral drugs [114]. Definite clinical benefits from these treatments have yet to be established and their safety and efficacy still need to be validated through Phase II and III clinical trials.

However, clinical trials have shown that MSC therapy and its derivatives are promising candidates for COVID-19 with known safety and efficacy. The United States FDA has approved MSCs for severe COVID-19 patients as compassionate use and progress has been made in this field. A study from Spain involving 13 COVID-19 patients requiring mechanical ventilation reported that no treatment-related adverse events (TRAEs) were observed [115]. After the first intervention with MSCs, clinical improvements were observed in 9 patients (70%) after a median follow-up of 16 days. Seven patients were extubated and discharged, while 4 patients continued intubation (2 with improved ventilation and radiological parameters, and 2 with stable conditions). The research team compared the clinical progress and mortality rates of their study cohort with similar cases in the intensive care unit (ICU). The mortality rate of patients who received MSC therapy dropped from 70–85% to 15% (2/13). Only 2 patients died during the study, one from massive gastrointestinal bleeding unrelated to the MSC treatment, and the other from secondary

Table 2. Completed clinical trials of MSCs and MSC-exosomes for the treatment of COVID-19.

NCT Number	Phase	Interventions	Outcome measures	Enrollment	Allocation
NCT04713878	NA	Biological: MSCs	Change of clinical symptoms as respiratory distress or need for oxygen support	21	Randomized
			Change of cytokine storm parameters		Parallel Assignment
			Change of pulmonary functions		Open Label
			Change of clinical symptoms		Primary Purpose: Treatment
NCT04288102	2	Biological: UC-MSCs	Change in lesion proportion (%) of full lung volume from baseline to day 28	100	Randomized
		Biological: Saline containing 1% Human serum albumin	Change in ground-glass lesion proportion (%) of full lung volume		Parallel Assignment
			Time to clinical improvement in 28 days		Masking: Quadruple
			Oxygenation index		Primary Purpose: Treatment
NCT04573270	1	Biological: PrimePro	Survival Rates	40	Single Group Assignment
		Other: Placebo	Contraction Rates		Masking: Triple Primary Purpose: Treatment
NCT04355728	1/2	Biological: UC-MSCs + Heparin along with best supportive care.	Incidence of pre-specified infusion associated adverse events	24	Randomized
		Other: Vehicle + Heparin along with best supportive care	Incidence of Severe Adverse Events		Parallel Assignment
			Survival rate after 90 days post first infusion		Masking: Triple
			Ventilator-Free Days		Primary Purpose: Treatment
			Change in Oxygenation Index (OI) C-Reactive Protein levels		
NCT04522986	1	Biological: MSCs	Safety: Adverse Event	6	Single Group Assignment Open Label Primary Purpose: Treatment
NCT04535856	1	Drug: allogeneic MSCs	Incidence of TEAE in Treatment group	9	Randomized
		Other: Placebo	Survival rate		Parallel Assignment
			Duration of hospitalization		Masking: Quadruple
			Clinical improvement Ordinal scale		Primary Purpose: Treatment
			Clinical improvement Oxygenation index Inflammation markers change		
NCT04276987	1	Biological: MSCs-derived exosomes	Adverse reaction (AE) and severe adverse reaction (SAE)	24	Single Group Assignment
			Time to clinical improvement (TTIC)		Open Label Primary Purpose: Treatment
			Number of patients weaning from mechanical ventilation		
			Duration (days) of ICU monitoring		
			Duration (days) of vasoactive agents usage Rate of mortality		
NCT04492501	NA	Procedure: Therapeutic Plasma exchange	Survival	600	Non-Randomized
		Biological: Convalescent Plasma	Duration of hospitalization		Factorial Assignment
		Drug: Tocilizumab	Time to resolution of cytokine release storm		Masking: Open Label
		Drug: Remdesivir	Time of viral clearance		Primary Purpose: Treatment
NCT04491240	1/2	Biological: MSCs	Complications	30	Randomized
		Drug: EXO 1 inhalation	Adverse Events		Parallel Assignment
		Drug: EXO 2 inhalation	Time to Clinical Recovery (TTCR)		Masking: Double
		Drug: Placebo inhalation	SpO2 Concentration		Primary Purpose: Treatment
			LDH		

NA, Not Applicable.

Table 3. Clinical trials of MSCs and MSC-exosomes for Covid-19 registered in Chictr.cn

ChiCTR number	Biological	Interventions	Phase	Enrollment	Registration date
ChiCTR2000031430	umbilical cord	MSCs + Routine treatment	2	200	2020/2/5
		Routine treatment			
ChiCTR2000030835	umbilical cord	High dose group: routine treatment + MSCs (2×10^6 /kg/time)	NA	20	2020/3/15
		Low dose group: routine treatment + MSCs (1×10^6 /kg/time)			
ChiCTR2000030866	umbilical cord	MSCs based on conventional treatments	NA	30	2020/3/16
ChiCTR2000030261	exosomes	Aerosol inhalation of exosomes	NA	13	2020/2/26
		Blank		13	
ChiCTR2000030088	Wharton's Jelly	Iv injection of Wharton's Jelly MSCs (1×10^6 /kg)	NA	40	2020/2/22
		saline			
ChiCTR2000030020	MSCs	MSCs therapy	NA	20	2020/2/20
ChiCTR2000029580	Ruxolitinib combined with MSCs	Ruxolitinib combined with MSCs	NA	70	2020/2/5
		Routine treatment			
ChiCTR2000029990	MSCs	MSCs therapy	1–2	60	2020/2/18
		saline		60	
ChiCTR2000030116	MSCs	MSCs in dose 1	NA	8	2020/2/1
		MSCs in dose 2		8	
ChiCTR2000030138	UC-MSCs	UC-MSCs	NA	30	2020/2/24
		Routine treatment + placebo		30	
ChiCTR2000030173	UC-MSCs	UC-MSCs	NA	30	2020/2/17
		Routine treatment		30	
ChiCTR2000030484	HUMSCs	HUMSCs: intravenous infusion, 5×10^7 cells/time, once/week, twice/course	NA	30	2020/2/2
		HUMSCs: intravenous infusion, 5×10^7 cells/time, 1 time/week, 2 times/course, a total of 2 courses; Exosomes: intravenous administration, 180 mg/time, 1 time/day, 7 days/course, 2 courses in total		30	
		The same amount of placebo (stem cell solvent)		30	
ChiCTR2000030944	NK cells and MSCs	NK cells and MSCs + Routine treatment	1	10	2020/9/1
		Routine treatment + placebo		10	
ChiCTR2000031319	Human dental pulp stem cells	Human dental pulp stem cells + Routine treatment	1	10	2020/4/1
		Routine treatment + placebo		10	
ChiCTR2000031430	MSCs	MSCs + Routine treatment	2	100	2020/3/20
		Routine treatment		100	
ChiCTR2000031494	MSCs	MSCs + Routine treatment	1	18	2020/2/1
		Routine treatment		18	

NA, Not Applicable.

fungal pneumonia caused by *Candida* spp. We searched for “COVID-19”, AND “exosome” OR “extracellular vesicles” OR “mesenchymal stem cells” up to April 22, 2021. Clinicaltrial.gov had 83 registered trials for the clinical use of MSCs, MSC-exosomes or MSC-exosome. Of these, 38 are ongoing and are recruiting patients. Nine trials had been completed to that date (Table 2). Using similar methodology, 16 registered clinical trials of MSCs for the treatment of COVID-19 were found in the Chinese Clinical Trial Register (Chictr).cn (Table 3). One clinical trial enrolled 101 patients with severe COVID-19 lung injury. Patients received human umbilical cord MSCs (HU-MSCs) (4×10^7 cells per infusion) on days 0, 3 and 6 [116]. In this phase 1 trial (NCT 04252118), the researchers demonstrated that intravenous HU-MSCs are safe and well tolerated in patients with moderate and severe COVID-19. Compared

to placebo, UC-MSCs reduced the volume of lung lesions (median difference: -13.31% , 95% CI: -29.14% , 2.13% , $p = 0.080$).

Compared with MSCs, MSC-exosomes have the ability to transmit and exchange intracellular chemical information. However, MSC-exosomes have received less attention than MSCs in COVID-19 research to date. At April 22, 2021, only two clinical trials using MSC-exosomes to treat COVID-19 had been completed (NCT04276987 and NCT04491240; see Table 2). Preliminary results of NCT04491240 released on 21 October 2020 showed that compared to placebo, the clinical recovery time, C-reaction protein (CRP) and layered double hydroxide (LDH) levels were lower for 10 consecutive days after inhalation of a solution containing $0.5\text{--}2 \times 10^{10}$ nanoparticles (MSC-exosomes) twice daily. These effects may have been me-

diated by the contents released from the MSC-exosomes, which included for example miRNA-126, miRNA-290, miRNA-21, miRNA-30b-3p, let-7, miRNA-200, miRNA-145, miRNA-27a-3p, Syndecan-1, HGF and Ang-1 [117–119]. Clearly, the application of MSC-exosomes instead of MSC therapy offers significant advantages [120], including more manageable dosing, easier storage, more readily available sources, better stability and lower immunogenicity [121–123]. Moreover, its noninvasive administration via inhalation avoids the side effects and pain that are commonly associated with parenteral therapy.

For these reasons, MSC-exosomes are a highly promising, cell-free therapy for COVID-19 [124, 125]. The U.S. Food and Drug Administration has in fact allowed the expanded use of MSC-exosome preparations for the treatment of COVID-19 [126]. These include aerosol inhalation of MSC-exosomes, targeted drug delivery based on MSC-exosomes, and the development of MSC-exosome-based vaccines [127, 128]. However, a phase 3 trial is needed to further evaluate the effects of MSC-exosomes on mortality and long-term lung dysfunction from COVID-19.

8. Conclusions

COVID-19 treatment is currently very challenging, especially because of its complications and sequelae. Intravenous MSC administration or inhalation of MSC-exosomes can improve the overall prognosis for COVID-19 by a variety of mechanisms: (1) through their immune regulation, (2) by promoting tissue repair and regeneration, (3) through their anti-fibrosis effect, and (4) by resuming normal vascular permeability. All these mechanisms can interact to strengthen lung repair and to protect the organs from damage caused by the excessive immune response. Despite the readily available sources, high proliferation rate, minimally invasive or noninvasive administration, and no ethical concerns, several challenges remain to be addressed with MSC and MSC-exosomes therapy. In particular, the dosing and timing of MSC and MSC-exosome therapy require careful consideration, since improper use may aggravate immunosuppression and lead to an unfavorable prognosis for COVID-19.

9. Author contributions

JM contributed to the conception of the study and led to the submission. XC performed the tables and wrote the manuscript; LL helped perform the figures with constructive discussions; MJ contributed significantly to manuscript preparation; All authors approved the final version.

10. Ethics approval and consent to participate

Not applicable.

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13. Conflict of interest

The authors declare no conflict of interest.

14. References

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Abbreviations: ACE2, angiotensin-converting enzyme 2; ADAMTS2, ADAM Metalloproteinase With Thrombospondin Type 1 Motif 2; Ang-2, Angiopoietin-2; APCs, Antigen presenting cells; ARDS, acute respiratory distress syndrome; AZD1222, Oxford-AstraZeneca 1,2's Cha-

dox1 nCoV-19; bFGF, basic fibroblast growth factor; COL15A1, Collagen 15A1; COL-I, collagen-1; COVID-19, Corona Virus Disease 2019; COX-2, cyclooxygenase-2; CRS, cytokine release syndrome; CRP, C-reaction protein; CXCL10, C-X-C Motif Chemokine Ligand 10; DC, Dendritic cells; ECM, extracellular matrix; FDA, Food and Drug Administration; G-CSF, granulocyte colony stimulating factor; hESCs, Human embryonic stem cells; HGF, human growth factor; HMGB1, High mobility group box chromosomal protein 1; HLA, human leukocyte antigen; HO-1, heme oxygenase-1; HTRA1, high temperature requirement A1; HU-MSCs, human umbilical cord mesenchymal stem cells; ICU, intensive care unit; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IgM, immunoglobulin M; IL, interleukin; IMRCs, immune and stromal regulatory cells; IP-10, interferon gamma induced protein-10; KGF, keratinocyte growth factor; LOX, lipoxygenase; LDH, layered double hydroxide; MCP-1/CCL2, Monocyte chemoattractant protein-1; MHC-1, major histocompatibility complex 1; MIP-1, Macrophage inflammatory protein-1; MMPs, matrix metalloproteinases; mRNA, messenger RNA; MSCs, Mesenchymal stem cells; MSC-EVs, MSC-derived extracellular vesicles; NLRP3, NOD-like receptor protein 3; PGE2, prostaglandin E2; PIK3R2, phosphoinositide-3-kinase regulatory subunit 2; SARS-COV-2, severe respiratory syndrome coronavirus 2; SOD, superoxide dismutase; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; Th, T helper type; TIMP2, tissue inhibitor of metalloproteinases 2; TRH-6, thyrotropin-releasing hormone-6; TRAEs, Treatment-related adverse events; VEGF, Vascular endothelial growth factor.

Keywords: COVID-19; SARS-CoV-2; Mesenchymal stem cell; Exosome; Immunoregulation

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