



Mechanism and Potential of Extracellular Vesicles Derived From Mesenchymal Stem Cells for the Treatment of Infectious Diseases

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Extracellular vesicles (EVs) are nano-sized membrane vesicles secreted by cells. EVs serve as a mediator for cell-to-cell communication by regulating the exchange of genetic materials and proteins between the donor and surrounding cells. Current studies have explored the therapeutic value of mesenchymal stem cells-derived EVs (MSC-EVs) for the treatment of infectious diseases extensively. MSC-EVs can eliminate the pathogen, regulate immunity, and repair tissue injury in contagious diseases through the secretion of antimicrobial factors, inhibiting the replication of pathogens and activating the phagocytic function of macrophages. MSC-EVs can also repair tissue damage associated with the infection by upregulating the levels of anti-inflammatory factors, downregulating the pro-inflammatory factors, and participating in the regulation of cellular biological behaviors. The purpose of this mini-review is to discuss in detail the various mechanisms of MSC-EV treatment for infectious diseases including respiratory infections, sepsis, and intestinal infections, as well as challenges for implementing MSC-EVs from bench to bedside.

Keywords: mesenchymal stem cells, exosome, extracellular vesicles, acute lung injury, COVID-19, sepsis, infectious diseases

INTRODUCTION

Infectious diseases have been a significant cause of morbidity and mortality worldwide; respiratory infections and pneumonia are among the major causes of global death (Sharma et al., 2021b). With the increasing number of outbreaks of new infectious diseases and the lack of effective treatments, it is crucial to identify new therapeutic strategies to combat infections and restore infection-related organ and tissue damage.

Mesenchymal stem cells (MSCs) are among the most commonly employed cell types in tissue repair and homeostasis, which have become an attractive therapeutic option for treating infectious diseases and disease-related tissue injury (Kashte et al., 2018; Kotas and Matthay, 2018). The effects of MSCs include anti-inflammatory properties, immunomodulatory capabilities, and regeneration

(Fu et al., 2019). The efficacy of MSCs is mainly coming from the paracrine effect mediated by secreted growth factors, cytokines, and extracellular vesicles (EVs) (Liang et al., 2014; Paliwal et al., 2018).

MSC-derived extracellular vesicles (MSC-EVs) are identified to be the main components responsible for the paracrine effect. They transfer functional molecules, such as messenger RNA (mRNA), microRNA (miRNA), lipid, and protein, into tissue-specific cells that request repair (Taverna et al., 2017). Compared with MSCs, MSC-EVs possess hypoinmunogenic properties, have low tumorigenesis, and are more stable (Trounson and McDonald, 2015). In this mini-review, we briefly summarize the function of exosomes and discuss their potential role in therapeutic regimens in infectious diseases, including respiratory infections, sepsis, and intestinal infections in recent years.

EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS

Almost all cells, including MSCs, can secrete EVs due to intracellular vesicle sorting (Kourembanas, 2015). EVs are nano-sized spherical bio-membrane structures, which were previously divided into three main categories based on their size and biosynthesis: smaller-sized exosomes (30–100 nm) from the endocytic pathway, medium-sized microvesicles (MVs) (100–1,000 nm) from the cell plasma membrane shedding, and larger-sized apoptotic bodies (1,000–5,000 nm) from the apoptosis (Raposo and Stoorvogel, 2013). The endocytosis of the cell membrane may form early endosomes, which then develop into late endosomes, namely, multivesicular bodies (MVBs). MVBs either combine with lysosomes or be released as exosomes through exocytosis (Joo et al., 2020). In terms of MVs, they can be secreted directly by budding from the plasma membrane (Abbaszadeh et al., 2020) (**Figure 1**).

Assigning an EV to a particular biogenesis pathway remains extraordinarily difficult because of the absence of specific surface markers for three EV categories and the overlap in their physical size (Carnino et al., 2021). Therefore, guidelines set by the International Society for Extracellular Vesicles (ISEV) suggest considering the use of operational terms for EV subtypes that are based on: (a) physical characteristics of EVs, such as size [“small EVs” (< 200 nm) and “medium/large EVs” (> 200 nm)] or density (low, middle, high, with each range defined); (b) biochemical composition (CD63⁺/CD81⁺-EVs, Annexin A5-stained EVs, etc.); or (c) descriptions of conditions or cell of origin (podocyte EVs, hypoxic EVs, large oncosomes, apoptotic bodies) (Théry et al., 2018).

Over 80% of researchers chose differential ultracentrifugation for EVs isolation (Tkach and Théry, 2016). Traditional identification ways for EVs usually involve nanoparticle tracking analysis (NTA) for size information, transmission electron microscope (TEM) for morphological details, and Western blotting for membrane protein makers (Théry et al., 2018). Kim et al. (2019) recently developed an atomic force microscope-infrared spectroscopy (AFM-IR) approach to probe the structural composition of a single EV. Their protocol involves incubating

the EV sample on a suitable substrate and setting up the AFM-IR instrument, as well as collecting nano-IR spectra and nano-IR images. Recorded IR spectra for EVs showed characteristic peaks at specific wavenumbers; it is possible to determine the presence of DNA (1,050–1,290 cm⁻¹), RNA (1,250–1,380 cm⁻¹), proteins (1,500–1,700 cm⁻¹), and phospholipids (1,000–1,250 cm⁻¹, 1,730–1,750 cm⁻¹, 2,800–3,000 cm⁻¹) (Kim et al., 2019) that may contribute to the understanding of EV biology and the development of EV therapies. This method could improve the understanding of EV biology and the development of EV therapies.

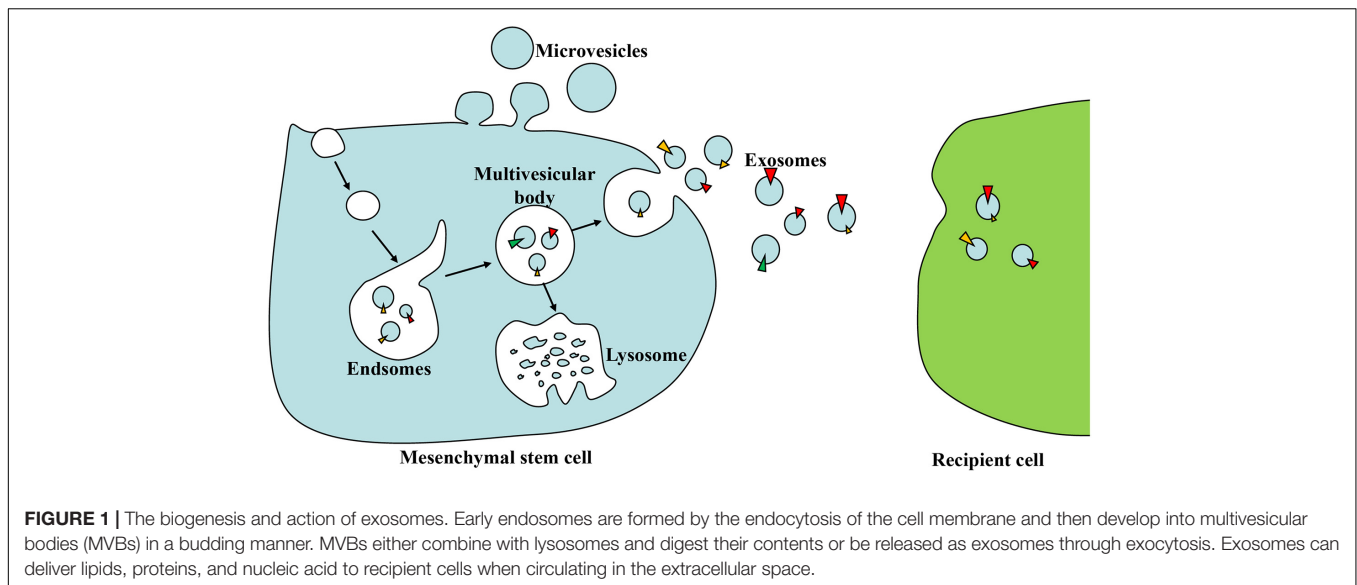
EVs secreted from MSCs can deliver many functional molecules such as mRNA, miRNA, lipids, and protein into recipient cells (Yin et al., 2019). These biological components are considered stable and can modulate cell behaviors in recipient cells. EVs use specific receptors or membrane fusion to enter recipient cells. Once EVs are absorbed, the biomolecules of EVs can regulate gene expression, essential enzyme reactions, signal cascade pathways, or other mechanisms in recipient cells (Ranghino et al., 2017). Thus, MSC-EVs can promote tissue regeneration by reprogramming several pathophysiological pathways such as immunomodulation, proliferation, apoptosis, angiogenesis, and oxidative (Grange et al., 2019a,b).

THE THERAPEUTIC APPLICATION OF MESENCHYMAL STEM CELL-EXTRACELLULAR VESICLES IN INFECTIOUS DISEASES

The function of EVs is mainly dependent on their source cells (Keshtkar et al., 2018). The therapeutic use of MSCs was reported in lung injury, sepsis, and necrotizing enterocolitis (NEC) caused by bacteria or viruses (Krasnodembskaya et al., 2010; Sung et al., 2016; Rodrigues et al., 2019). MSC-EVs have similar functions to their parental cells, such as antimicrobial effects, immunomodulation property, and damage tissue repairability. Compared with MSCs, MSC-EVs keep the biological function of MSCs and are more stable and less easy to tumorigenesis, making them a promising candidate for the treatment of infectious diseases (Thirabanjasak et al., 2010).

For Respiratory Infection

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a heterogeneous syndrome characterized by diffuse epithelial and endothelial damage and a robust inflammatory response (Thompson et al., 2017). The most common risk factors of ARDS are infectious pneumonia caused by bacteria and viruses (Muraca et al., 2020; Meyer et al., 2021). Respiratory infections take more than 1.5 million lives a year. The number of deaths and disabled people is devastating in epidemic and pandemic outbreaks, such as the severe acute respiratory syndrome (SARS) outbreak in 2002, H1N1 flu in 2009, Middle East respiratory syndrome (MERS) outbreak in 2012, and coronavirus disease 2019 (COVID-19) outbreak in 2020 (Sharma et al., 2021b).



Cell-based therapy with MSCs has been promising in ALI/ARDS in pre-clinical models for their immunomodulation and tissue repair properties (Laffey and Matthay, 2017). However, there were higher mean scores of Acute Physiology and Chronic Health Evaluation III (APACHE III) in models treated with MSCs than in those treated with placebo, but without difference of their 28-day mortality (Matthay et al., 2019). Since MSCs have limited engraftment and differentiation efficacy, high risk of tumorigenicity, and unstable ability (Eggenhofer et al., 2014), researchers paid more attention to MSC-EVs as a new candidate cell-free treatment for ALI/ARDS. Both other researchers and we demonstrated that intratracheal administration of MSC-EVs showed therapeutic effects in hyperoxia-induced lung injury, revealing that MSC-EVs could ameliorate alveolarization in both short-term and long-term bronchopulmonary dysplasia (BPD) models and activate M2 macrophages (Porzionato et al., 2019, 2021; You et al., 2020). The anti-inflammatory and pro-regenerative properties of MSC-EVs are well established and have been exploited in a large number of studies (Phinney and Pittenger, 2017).

The application of MSC-EVs on ALI/ARDS and severe pneumonia has been investigated in some pre-clinical studies. MSC-EVs' main effects on ALI/ARDS are reducing inflammation, promoting alveolar epithelial regeneration, and enhancing pulmonary endothelial repair (Shah et al., 2019). As a result, pro-inflammatory cytokine production was decreased, and alveolar fluid clearance was improved in ALI/ARDS models.

Two clinical trials are undergoing to determine the effects of MSC-EVs on COVID-19, a pandemic that lacks specific antiviral medicine. MSC-EVs will be administrated intravenously (NCT04798716) or by inhalation (NCT04276987). A prospective non-randomized open-label cohort study showed that allogeneic bone marrow MSC-derived exosomes (ExoFloTM) could be safe and effective in severe COVID-19 patients, which could restore oxygenation, downregulate cytokine storm, and reconstitute immunity (Sengupta et al., 2020). However, it is premature to

draw any conclusion based on a single study, and it should be emphasized that there are no approved MSC-EV therapies for COVID-19 to date. The specific and scientific rationale for administering MSC-EV treatment in COVID-19 patients needs to be better understood and justified (Börger et al., 2020). In the meantime, the prevention and control of urgent COVID-19 should make efforts to test existing approved vaccines, antiviral therapeutics, and monoclonal antibodies (Sharma et al., 2021a).

miRNA, protein, mRNA, and mitochondria in MSC-EVs play vital roles in modulating immune responses and repairing lung damage of ALI/ARDS. miR-21-5p plays an essential role in alleviating ALI by reducing pro-inflammatory cytokine secretion and enhancing M2 polarization (Li et al., 2019). MSC-EVs are reported to ameliorate ALI *via* transferring miR-27a-3p to alveolar macrophages inhibiting NF- κ B expression and inducing M2 polarization (Wang et al., 2020). MiR-145 mediated the antimicrobial effect of MSC-EV by suppressing the expression of multidrug resistance-associated protein 1 (MRP1) and increasing the levels of leukotriene B₄ (LTB₄) (Hao et al., 2019), a chemoattractant for immune cells including T cells, macrophages, and neutrophils, with the role of facilitating pathogen elimination (Saeki and Yokomizo, 2017).

EVs from interferon (IFN)- γ -primed MSCs more effectively attenuated *Escherichia coli*-induced lung injury *via* enhancing phagocytosis and killing of bacteria in macrophage (Varkouhi et al., 2019). MSC-EVs decreased the lipopolysaccharide (LPS)-induced permeability of microvascular endothelial cells partly through the presence of hepatocyte growth factor (HGF) (Wang et al., 2017). The expression of keratinocyte growth factor (KGF) (Zhu et al., 2014) and angiopoietin-1 (Ang1) (Tang et al., 2017) mRNA enclosed in EVs partly mediated the anti-inflammatory effects on *E. coli* endotoxin-induced ALI in mice models. The effectiveness of MSC-EVs has also been demonstrated in large animals and found that EVs from swine bone marrow-derived MSCs had anti-influenza and anti-inflammatory effects in influenza virus-induced pig ALI (Khatri et al., 2018).

TABLE 1 | The related exosomal cargo and mechanisms of mesenchymal stem cell-derived extracellular vesicles treatment in infectious diseases.

Related exosomal cargo	Disease model	Exosome source	MSC-EV isolation	Experimental outcome and related mechanism
miR-27a-3p (Wang et al., 2020)	LPS-induced ALI in mouse	hADMSCs	UC	Elevated miR-27-3a levels in alveolar macrophages, induced M2 polarization, and decreased alveolar macrophage expression of NF- κ B
miR-145 (Hao et al., 2019)	<i>E. coli</i> -induced ALI in mouse	hBMSCs	UC	Suppressed MRP1 activity through transfer of miR-145, thereby resulting in enhanced LTB ₄ production and antimicrobial activity through LTB ₄ /BLT1 signaling
Unknown (Varkouhi et al., 2019)	<i>E. coli</i> -induced ALI in rat	IFN- γ -primed hUCMSCs	UC	Enhanced macrophage phagocytosis and killing of <i>E. coli</i>
HGF (Wang et al., 2017)	<i>In vitro</i> LPS treatment of endothelial cells	mBMSCs	UC	Increased the expression of VE-cadherin and occluding, decreased endothelial apoptosis, induced endothelial cell proliferation
KGF (Zhu et al., 2014), Ang-1 (Tang et al., 2017)	<i>E. coli</i> /LPS-induced ALI in mouse	hBMSCs	UC	Demonstrated a reduction in pulmonary edema, lung protein permeability, and inflammation
RNAs (Khatiri et al., 2018)	Influenza virus-induced ALI in pig	sBMSCs	UC	Reduced virus shedding in the nasal swabs, influenza virus replication, and pro-inflammatory cytokines in the lungs
miR-146a (Song et al., 2017), miR-21 (Yao et al., 2021)	CLP-induced sepsis in mouse	IL-1 β primed hUCMSCs	UC	Exosomal miR-146a/miR-21 was transferred to macrophages, resulted in M2 polarization by modulating IRAK1, TRAF6, and IRF 5 signaling, or inhibited the effects of PDCD4.
miR-223 (Wang et al., 2015)	CLP-induced sepsis in mouse	mBMSCs	UC	Exosomal miR-223 was transferred to cardiomyocytes, inhibited the expression of Sema3A and Stat3, and reduced inflammation and cell death.
Unknown (Rager et al., 2016; McCulloh et al., 2018)	Premature and hypercaloric feeds-induced NEC in rat	rAFMSCs, rBMSCs, and mBMSCs	UC	Reduced the incidence and severity of experimental NEC and protected the intestines from NEC
miR-200b (Sun et al., 2020b)	<i>In vitro</i> TNF- α treatment of endothelial cells	HO-1-modified rBMSCs	Exosome separation kits	Targeted HMGB3 in intestinal epithelial cells to alleviate inflammatory injury
Let-7f, miR-145, miR-199a, and miR-221 (Qian et al., 2016)	<i>In vitro</i> HCV treatment of human hepatoma-7 cells	hBMSCs	UC	Suppression of HCV RNA replication, combined with INF- α or telaprevir, enhanced their anti-HCV ability
Unknown (Gu et al., 2020)	<i>In vitro</i> D-GalN/LPS treatment of hepatocytes	BMSCs	UC	Decreased the expression levels of the pro-apoptotic proteins Bax and cleaved caspase-3, upregulated the anti-apoptotic protein Bcl-2, reduced hepatocyte apoptosis
Unknown (Sun et al., 2020a)	CVB3-induced myocarditis in mouse	hBMSCs	UC	Activated AMPK/mTOR-mediated autophagy flux pathway to attenuate cardiomyocyte apoptosis

MSC-EV, mesenchymal stem cell-derived extracellular vesicle; hADMSCs, human adipose-derived MSCs; hBMSCs, human bone marrow-derived MSCs; hUCMSCs, human umbilical cord-derived MSCs; mBMSCs, mouse bone marrow-derived MSCs; sBMSCs, swine bone marrow-derived MSCs; rAFMSCs, rat amniotic fluid-derived MSCs; rBMSCs, rat bone marrow-derived MSCs; HCV, hepatitis C virus; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; Ang-1, angiopoietin-1; LPS, lipopolysaccharide; ALI, acute lung injury; *E. coli*, *Escherichia coli*; CLP, cecal ligation and puncture; NEC, necrotizing enterocolitis; D-GalN, D-galactosamine hydrochloride; CVB3, coxsackievirus B3; HO-1, heme oxygenase-1; IL-1 β , interleukin-1 β ; TNF- α , tumor necrosis factor- α ; UC, ultracentrifugation; NF- κ B, nuclear factor kappa B subunit 1; MRP1, multidrug resistance-associated protein 1; LTB₄, leukotriene (LT) B₄; HMGB3, high mobility group box 3.

For Sepsis

Sepsis is a systemic inflammatory response to infection that leads to multiple organ dysfunction, and one out of four sepsis patients died during their hospital stay (Iskander et al., 2013; Fleischmann-Struzek et al., 2020). Sepsis is caused by the accumulation of various pro-inflammatory factors in the process of inflammatory response and immune dysfunction (Prescott and Angus, 2018). Even with the continuous development of intensive care and advances in the antibiotic application, the mortality of sepsis in intensive care units remains high (Angus and van der Poll, 2013). Therefore, a new therapy is urgent to improve the clinical outcomes.

Patients with sepsis had severe immunosuppression, leading to macrophage dysfunction and poor wound healing

(Davis et al., 2019). Therefore, the new therapy strategy could be related to the immunoregulation of macrophages. Several studies have proven that MSC-EVs can improve the outcomes of sepsis in animal models. MiRNAs in MSC-EVs have been considered as a critical substance to exert efficacy in sepsis. For example, miRNA-146a was found to be strongly upregulated in MSC-EVs primed with interleukin-1 β (IL-1 β), which could more effectively induce M2 polarization by modulating IRAK1, TRAF6, and IRF5 signaling (Song et al., 2017). MiR-21 in MSC-EVs was abundantly upregulated in IL-1 β -stimulated MSCs, which induced M2 polarization of macrophages *in vitro* and *in vivo* sepsis by inhibiting the effects of PDCD4, which can participate in multiple cellular biological behaviors, including apoptosis and transcription (Yao et al., 2021). Both studies supported

that pretreated MSCs with pro-inflammatory cytokines could enhance their immunomodulatory function of MSCs. The exosomal miR-223 was reported to contribute to MSC-mediated cardioprotection in sepsis by downregulation of Sema3A and STAT3 (Wang et al., 2015).

For Intestinal Infection

The balance between beneficial and harmful bacteria plays an important role in neonatal intestinal health (Rhoads et al., 2018). Bacterial infection is one of the most significant risk factors in NEC pathogenesis, a life-threatening disease in premature infants, with mortality as high as 30% (Neu and Walker, 2011; Markel et al., 2020). Full-thickness destruction of the intestine is the character of NEC, and inflammatory response is increased in infants affected by this disease, leading to intestinal perforation, peritonitis, bacterial invasion of the bloodstream, and systemic infection (Neu, 2014; Neu and Pammi, 2018). Survivors are faced with severe sequelae, including short gut syndrome and neurodevelopmental retardation (Neu, 2014). Despite decades of research on the pathophysiology of NEC, the treatment remains inadequate and supportive and desired a novel preventive and therapeutic intervention.

MSCs have great potential in NEC treatment, decreasing NEC incidence in rat models (Augustine et al., 2017; Thébaud, 2019). EVs from MSCs carry important biological components and can be utilized in disease prevention and treatment (Baglio et al., 2015). EVs from bone marrow-derived MSCs, heparin-binding EGF-like growth factor (HB-EGF) primed MSCs, and human umbilical cord MSCs have been reported to protect the integrity of the intestinal barrier and reduce the severity and incidence of NEC in an experimental model (Rager et al., 2016; McCulloh et al., 2018). Both miR-34 and miR-29 improved the intestinal epithelial barrier through the Snail/Claudins signaling pathway (Li et al., 2020). MiR-200b in heme oxygenase-1 (HO-1)-modified bone marrow MSCs-derived EVs was reported to target high mobility group box 3 (HMGB3) gene in intestinal epithelial cells to alleviate its inflammatory response (Sun et al., 2020b).

For Other Infectious Diseases

Qian et al. (2016) revealed that miRNAs, especially let-7f, miR-145, miR-199, and miR-221 from MSC-EVs, inhibited viral replication in hepatitis C virus (HCV)-treated cells. Hepatocyte injury model caused by D-galactosamine (D-GaIN) and LPS could be ameliorated by MSC-EVs through inducing autophagy and inhibiting apoptosis (Zhao et al., 2019). In addition, MSC-EVs had therapeutic effects on coxsackievirus (CVB3)-induced myocarditis in the mice model, which can shrink the production of pro-inflammatory cytokines and improve cardiac function *via* activating the AMPK/mTOR-mediated autophagy flux pathway to attenuate apoptosis (Gu et al., 2020).

CONCLUSION

MSC-EVs had outstanding prospects in treating infectious diseases, such as respiratory infections, sepsis, and intestinal infections. The therapeutic mechanisms included direct

antimicrobial effects, immunomodulation, and tissue repair. MSC-EVs exert their effect through the transfer of mRNAs, miRNAs, and proteins (Table 1). MiRNA containing EV may be a new target for the development of new therapeutic drugs. The use of MSC-EVs has several benefits, namely, (a) small vesicles, readily circulating and penetrating biological barriers, like blood-brain; (b) low tumorigenesis; and (c) stable properties, MSC-EVs may achieve a higher “dose” than MSCs due to the poor viability and considerable death of engrafted MSCs in target tissues (Barbash et al., 2003). Importantly, EVs can maintain high activities at low temperatures. All the profits make MSC-EVs a promising agent in infectious diseases.

Despite the promising progress that has been made in the treatment of MSC-EVs on infectious diseases, several challenges are faced by the field in clinical translation: (a) there is wide variability of MSC-EVs preparations in the whole process (Börger et al., 2020), such as the different productions of cell sources, purification, and identification of the final product. Careful consideration of the optimal purity and rational clinical trial design of MSC-EVs is necessary to advance large-scale clinical trials (Muraca et al., 2018). Furthermore, lacking standardized quality parameters caused discrepancies and controversies about the biology and function of MSC-EVs. Members of four societies (SOCRATES, ISCT, ISEV, and ISBT) identified potential metrics of MSC-EVs to facilitate data sharing and comparison of MSC-EVs among different studies, including biological activity, vesicle integrity, the concentration of membrane lipid vesicles, the ratio of specific lipids, the ratio of membrane lipids to protein, and the ratio of MSC to non-MSC surface antigens (Witwer et al., 2019). Each metric needs to be quantified and validated in further studies. (b) How to determine reproducible and robust parameters to predict the therapeutic potency of MSC-EVs is unsolved. The therapeutic efficacy of MSC-EVs depends not only on the cell, such as the cell source and status of MSCs, delivery dose and route (Sun et al., 2020a), and half-life and *in vivo* biodistribution of MSC-EVs, but also on the disease condition, such as the disease microenvironment and the time window for intervention. (c) MSC-EVs from different sources have been reported to be efficacious in various kinds of infectious diseases; the therapeutic mechanism may be different and specific for each source and disease condition. To better understand the therapeutic activity, the mode of action needs to be studied further, trying to find out the key components in MSC-EVs, target cells in injured tissues, and the involved molecular signaling cascade.

AUTHOR CONTRIBUTIONS

LZ: conceptualization and review. ZF: supervision. JY: writing and editing. All authors contributed to the article and approved the submitted version.

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