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Concise Review: Therapeutic Potential of the Mesenchymal Stem Cell Derived Secretome and Extracellular Vesicles for Radiation-Induced Lung Injury: Progress and Hypotheses

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Received February 22, 2018; accepted for publication November 27, 2018; first published January 7, 2019.

http://dx.doi.org/10.1002/sctm.18-0038

Key Words. Mesenchymal stem cells • Secretome • Exosome • Radiation pneumonitis • Lung fibrosis

ABSTRACT

Radiation-induced lung injury (RILI) is a common complication in radiotherapy of thoracic tumors and limits the therapeutic dose of radiation that can be given to effectively control tumors. RILI develops through a complex pathological process, resulting in induction and activation of various cytokines, infiltration by inflammatory cells, cytokine-induced activation of fibroblasts, and subsequent tissue remodeling by activated fibroblasts, ultimately leading to impaired lung function and respiratory failure. Increasing evidence shows that mesenchymal stem cells (MSCs) may play a main role in modulating inflammation and immune responses, promoting survival and repair of damaged resident cells and enhancing regeneration of damaged tissue through soluble paracrine factors and therapeutic extracellular vesicles. Therefore, the use of the MSC-derived secretome and exosomes holds promising potential for RILI therapy. Here, we review recent progress on the potential mechanisms of MSC therapy for RILI, with an emphasis on soluble paracrine factors of MSCs. Hypotheses on how MSC derived exosomes or MSC-released exosomal miRNAs could attenuate RILI are also proposed. Problems and translational challenges of the therapies based on the MSC-derived secretome and exosomes are further summarized and underline the need for caution on rapid clinical translation. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:344–354*

SIGNIFICANCE STATEMENT

Although it has been reported that soluble cytokines based on MSC therapy that could attenuate RILI, the mechanism of MSC-based secretome therapy for RILI is still not fully understood. This review summarized the recent progress regarding the potential mechanisms of MSCs therapy for RILI, with an emphasis on MSC-secreted cytokines and miRNAs as a safe and, effective cell-free therapy, which may be helpful to accelerate the strategy from bench to bedside.

INTRODUCTION

Radiotherapy is an effective and important strategy for cancer treatment that may extend the survival time of patients by improving localized inhibition of tumor development [1]. However, radiation-induced lung injury (RILI) is a common adverse effect, with a lethality of up to 15%, and limits the therapeutic dose of radiation that can be administered to control tumors [2]. RILI is a complex pathological process, resulting in an early radiation pneumonitis (RP) and late radiation-induced lung fibrosis (RILF) [2]. Symptomatic RP occurs in ~5%–50%, ~5%–10%, and ~1%–5% of patients irradiated for cancers of the lung, mediastinal lymphatics, and breast, respectively [3, 4]. Pneumonitis is characterized by shortness of breath, cough, and fever; however, patients with severe RP have almost 50% mortality [5]. RLIF is a chronic, progressive, and fatal interstitial pulmonary disease with a poor prognosis, and poor response to available medical therapies [6]. The rate of RLIF, which can continue to evolve around 1 year after radiotherapy, is reportedly up to 70%–80% in regions that use high-dose radiotherapy [7]. Therefore, RILI has become a focus of prevention and treatment in biomedical research.

Currently, RP can be treated with steroids but abrupt withdrawal may activate latent injury to the lung [8]. Amifostine (WR-2721) remains

the only agent currently in clinical use as a radioprotector, which can scavenge free radicals, protect DNA, and accelerate repair [9]. However, the radioprotective effects of chemical compounds, including amifostine, are short-term, and associated with side effects such as nausea, vomiting, diarrhea, and hypotension [10, 11], thereby limiting their clinical use. The biological growth factors and cytokines such as IL-7, IL-11, granulocyte-colony stimulating factor, macrophage-colony stimulating factor, and keratinocyte growth factor have been used to alleviate radiation-induced damage. However, success with these compounds has also been limited [11]. Lycopene, as a naturally occurring dietary carotenoid, can protect against γ-radiation induced DNA damage and antioxidant status in rats [12]. However, there are still key considerations that need to be addressed in evaluating a potential antioxidant. Similarly, the signaling inhibitors, including TLR agonists CBLB502 and the STAT3 signaling inhibitor WP1066, can alleviate RP, but their toxicity and side effects still need to be considered before clinical application [9, 13]. In addition, although lung transplantation is the most useful intervention for treating RILF, it is limited by the lack of available donated lungs and transplantation-related complications [14, 15].

Therefore, a new and more effective therapeutic strategy based on the pathological mechanisms of RILI is urgently needed. Mesenchymal stem cells (MSCs), as a population of multipotent cells, can modulate the inflammation response, promote survival and repair of damaged resident cells, and enhance regeneration of damaged tissue [16], and thus show potential for clinical utility. Moreover, their advantages include convenient isolation and culture, low immunogenicity, regenerative and multiple differentiation abilities, and potent immunosuppressive effects [17–22]. These beneficial properties make MSC therapy a promising candidate for the treatment of RILI. Although preclinical studies showed therapeutic effects of MSCs for RILI therapy [23], many hurdles still exist for translating the therapeutic promise of MSCs in preclinical studies to the clinical setting [24, 25]. To overcome those concerns of MSC-based therapy, several studies showed that MSC-derived conditioned medium (CM) recapitulated many of the therapeutic properties of the parent cells, suggesting that the development of cell-free strategies based on using components of MSC-derived CM, such as soluble factors, and extracellular vesicles, such as exosomes, merits further investigation [25, 26]. Indeed, accumulating evidence shows that the therapeutic effects of MSCs are due to their capacity to secrete paracrine factors [26–28]. In this review, we have summarized the potential mechanisms underlying MSC therapy for radiation-induced pulmonary events in the lungs, with an emphasis on the importance of specific secreted soluble paracrine cytokines. Additionally, based on currently published data, we have predicted a potential therapeutic role for miRNAs shuttled by exosomes from MSCs. These data may help to support the therapeutic strategy of using the MSC-derived secretome and exosomes to treat RILI and to accelerate this strategy from bench to bedside.

The Potential of MSC Therapy for RILI Based on the Secretion of Soluble Factors

Modulation of Expression Levels of Inflammatory and Fibrotic Cytokines

For decades, the biologic response to radiation has been reported to start with the generation of reactive oxygen species (ROS) [29]. Similarly, RILI, as a radiation-associated pulmonary complication, is different from other types of acute lung injury because it starts with radiation-induced energy deposition and the generation of ROS, followed by a series of biologic responses, including a cascade of subsequent inflammatory events, angiogenesis, programmed cell death, autophagy, production of extracellular matrix, and crosstalk of activated signal transduction pathways, ultimately leading to lung fibrosis and respiratory failure [3, 30, 31]. In this process, the release of various cytokines is considered to play a major role in the pathogenesis of RILI [30, 32, 33], including proinflammatory cytokines such as interleukin-1α (IL-1α), IL-1β, IL-6, and profibrogenic cytokines such as transforming growth factor β1 (TGF-β1). Indeed, MSCs have been reported to inhibit ROS and reduce oxidative stress, playing an antifibrotic role and reducing proinflammatory responses by regulating the release of various cytokines in many experimental models [34–37]. Moreover, increasing evidence suggests that gene-modified MSCs or MSC-expressed cytokines play a key role in activating anti-inflammatory and antifibrotic signaling or neutralizing proinflammatory and profibrotic cytokines via secreting paracrine cytokines, superoxide dismutases, or soluble inflammatory cytokine receptors. These activities may aid in repairing damaged lung tissue and in tissue regeneration following RILI.

Inhibition of Lung Myofibroblasts by MSC Secretion of Hepatocyte Growth Factor and Prostaglandin E2

Radiation can stimulate lung fibroblasts to secrete cytokines, undergo hyperplasia, and differentiate into myofibroblasts [38–40]. Myofibroblasts can further promote the synthesis of additional collagens, leading to excessive deposition and abnormal remodeling of the extracellular matrix, which is a hallmark of RILI [39–41]. Zhang et al. reported that human umbilical cord MSCs (hucMSCs) can attenuate RILI by inhibiting myofibroblastic differentiation of human lung fibroblasts [42]. However, whether the inhibition involves the secretome of MSCs was unclear. Dong et al. further showed that human adipose tissue-derived MSCs can downregulate levels of TNF-α and TGF-β1 by stimulating secretion of hepatocyte growth factor (HGF) and prostaglandin E2 (PGE2) [43].

Endogenous HGF/c-Met signaling plays important roles in tissue repair [44], and the expression of HGF and c-Met can be upregulated by exogenous stimuli such as MSCs and gene-modified MSCs [45]. MSC-secreted HGF probably attenuates EMT in type II AECs by increasing intracellular levels of Smad7 upon binding to c-Met and by upregulating the expression of matrix metalloproteinases-1, -3, and -9 in injured sites in a PI3K/Akt/p70-dependent manner, thereby promoting apoptosis of myofibroblasts [46–48]. Wang et al. further showed that HGF-modified MSC therapy can increase endogenous HGF/c-Met expression in a mouse RILI model [49]. S1P/S1PR1 may also participate in the HGF/c-Met-mediated process [49–51], suggesting that HGF-modified MSCs may exert anti-inflammatory and antifibrotic effects via paracrine secretion of HGF.

The cytokine PGE2 is secreted by MSCs to reprogram host macrophages to increase their anti-inflammatory IL-10 production [52]. Moreover, PGE2 can inhibit TGF-β1-induced activation and fibroblast proliferation, thereby reducing the production of the fibrosis marker α-SMA and collagens by elevating intracellular cAMP levels, and PGE2 also induces apoptosis in myofibroblasts by increasing the activity of the PTEN

protein, which blocks the PI3K/Akt signaling pathway [53–58], These findings suggest the potential application of MSCs in RILI therapy via activation of anti-inflammatory pathways and inhibition of profibrotic signaling in a paracrine factor-associated manner.

Protection of Injured Cells against ROS by MSC Secretion of Superoxide Dismutases

RILI starts with radiation-induced energy deposition and generation of ROS [3, 30, 31]. Inflammatory diseases are also accompanied by excessive production of ROS and depletion of endogenous antioxidants, but antioxidant enzymes such as superoxide dismutase 1 (SOD1, Cu/Zn SOD) are known to be very effective scavengers of ROS [59]. It was reported that MSC-secreted SOD1 protected lungs from radiation-induced endothelial cell damage [60]. Similarly, SOD3 or manganese superoxide dismutase-modified MSCs also have more anti-inflammatory and antifibrotic effects on RILI, compared with nongene-modified MSCs [61, 62]. This effect of superoxide dismutase is probably based on their ability to catalyze the dismutation of the superoxide radical into oxygen and hydrogen peroxide, thereby protecting injured cells against ROS generated during RILI [29, 35, 63, 64].

MSC Secretion of Soluble Factors that Inhibit Proinflammatory Signaling and Immune Cell Activation

Expression levels of the key proinflammatory cytokines IL-1 and TNF-α in the lung correlate with the development of pulmonary injury in rodents exposed to radiation [33, 65]; however, MSC-secreted interleukin 1 receptor antagonist (IL1RN) can function as a competitive inhibitor of IL-1α and IL-1β, and block the production and/or activity of TNF-α and IL-1α signaling in lung tissue [66], suggesting the possibility of using MSC-secreted IL1RN as a paracrine mediator for treating RILI. Moreover, increased NF-κB activity often triggers inflammation-related pathologies including RILI [67], but Yagi et al. reported that human MSCs can neutralize TNF-α by secreting significant quantities of soluble TNF receptor 1, which consequently blocks activation of NF-κB by TNF-α [68]. Such neutralization of TNF-α would contribute to the anti-inflammatory effect of MSC therapy on TNF-α/NF-κB signaling in RILI via a paracrine manner. A similar effect was found in TGF-β type II receptor-modified MSCs and MSC-conditioned medium [26, 69]. Notably, Xue et al. reported that only 0.1% of lung cells are derived from transplanted MSCs, a level too low to support the observed protective effects. Other studies have also reported that MSCs can repair injured lung tissues without significant engraftment or differentiation in some situations [26, 70], suggesting that other factors, including paracrine cytokines (Table 1), may be mainly involved in regulating anti-inflammatory responses and repair mechanisms.

Like infectious, thermal, or physical damage, radiation-induced damage of lung tissue can lead to the activation of the immune system [60]. Infiltration of innate and adaptive immune cells is a common response of normal tissues to ionizing radiation in the lung [71]. The pneumonitic phase is characterized by the recruitment of immune cells and a subsequent cascade of cytokines/chemokines that results in various degrees of lung inflammation after ionizing radiation [71]. MSCs not only have a reparative and regenerative ability via the secretion of paracrine cytokines, including EGF, FGF, PDGF, TGF-β, VEGF, HGF, Ang-1, KGF, SDF-1, IGF-1, and others [72], but also have immunosuppressive properties through the secretion of IL-10, TSG6, IL-6, LIF, PGE2, HO-1, and other cytokines [72]. These immunosuppressive cytokines can minimize organ damage caused by inflammation and cells activated by the immune system via inhibiting activation and proliferation of immune cells, including T cells, B cells, NK cells dendritic cells, monocyte, macrophages, and neutrophils [71–73]. Therefore, we hypothesize that MSCs have strong potential for treating RILI by secreting various paracrine cytokines that regulate inflammatory and fibrotic responses and immunomodulatory actions in injured lung tissue. Interestingly, Chen et al. reported that preactivation of MSCs with TNF-α, IL-1β, and nitric oxide can enhance paracrine effects on radiation-induced injury by a heme oxygenase-1 dependent mechanism [74]. Similarly, Block et al. reported that MSCs can be activated by UV-irradiated fibroblasts to secrete stanniocalcin-1, a peptide hormone that modulates calcium metabolism [75]. The antiapoptotic effect of stanniocalcin-1 secretion by MSCs was also observed in a coculture system with injured lung cancer epithelial cells, suggesting that the inflammatory environment is probably a key factor in regulating the paracrine responses of MSCs [75]. However, although utilizing the paracrine functions of MSCs to treat RILI shows considerable promise, further investigation of the paracrine-associated mechanisms of MSCs in RILI therapy is still needed.

Table 1. Summary of soluble paracrine factors of MSC derived secretome for RILI therapy

Soluble factors	Species	Role for RILI therapy	References
sTβR	Murine MSCs	Reduces TNF-α, IFN-γ, IL-6, TGF-β	[26]
HGF	Human MSCs	Reduces TNF-α, IFN-γ, IL-6, TGF-β, and inhibits myofibroblasts	[43, 49]
PGE2	Murine MSCs	Increases their anti-inflammatory IL-10 production	[43, 52]
SOD1	Murine MSCs	Scavenges ROS	[60]
SOD3	Human MSC	Reduces collagen deposition, inflammatory cell infiltration, and oxidative stress	[61]
MnSOD	Human MSCs	Attenuates lung inflammation, ameliorates lung damage, and protects the lung cells from apoptosis	[62]
IL1RN	Murine MSCs	Inhibits IL-1α and IL-1β,and reduces TNF-α	[65, 66]

The Potential of MSC Therapy for RILI Based on the Release of Extracellular Vesicles

Advantages of Using MSC-Derived Exosomes Compared with MSCs

In addition to secreting an array of soluble cytokines that could attenuate RILI (Fig. 1), MSCs also release large numbers of extracellular vesicles (EVs) that mediate tissue repair and anti-inflammatory effects in lung pathogenesis [66]. Indeed, exogenously administered MSCs may exert some of their complex paracrine anti-inflammatory, antifibrogenic actions and proregenerative roles through released EVs [66]. Therefore,

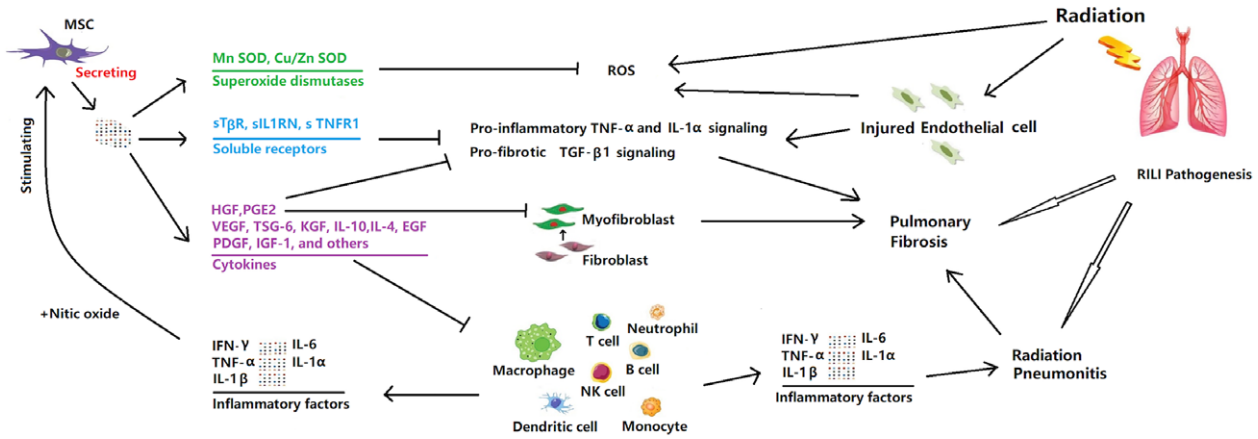

Figure 1. Mesenchymal stem cells (MSCs) regulate inflammatory signaling, fibrotic response and immune cells to attenuate radiation-induced lung injury (RILI) via secreting an array of soluble factors. Radiation causes delayed damage to resident lung cells, leading primarily to the injury; however, MSCs can protect injured lung cells against ROS via secreting superoxide dismutases including SOD1 and SOD3. Radiation can also stimulate lung fibroblasts to differentiate into myofibroblasts. Myofibroblasts can further promote the synthesis of additional collagens, leading to excessive deposition and abnormal remodeling of the extracellular matrix, which is a hallmark of RILI. MSCs may inhibit lung myofibroblasts via secreting HGF and PGE2. Moreover, radiation can also activate proinflammatory signaling pathways and trigger the recruitment of various immune cells into the lung, such as monocytic cells, neutrophils, and lymphocytes. MSCs can inhibit proinflammatory signaling and immune cell activation via secreting soluble receptors and various cytokines including sTβR, sIL1RN, TNFR1, VEGF, KGF, EGF, IL-10, TSG6, IL-6, HGF, PGE2, and so forth.

there is growing interest in the possibility of using EVs derived from cultured MSCs as a safe and, effective cell-free therapy [76], especially because of the potential carcinogenic effect of administering MSCs [77]. EVs are typically categorized based on their biogenesis. The 3 main classes of EVs are exosomes, microvesicles and apoptotic bodies [66], all of which are enclosed by a lipid bilayer and which range from 30 to 2,000 nm in diameter depending on the biogenesis pathway [66]. The term "exosome" refers to an endosome-derived subclass of membrane microvesicles with a diameter of 50–100 nm, that are components of the secretome of multiple cell types, including MSCs [66, 78]. Exosomes are important facilitators in cell-to-cell interactions by impacting multiple signaling pathways [66, 78], and their contents include proteins, miRNA and lipids [79]. Compared with MSCs, MSC-derived exosomes present exciting advantages [25]. Firstly, exosomes are vesicles with a lipid bilayer membrane that protects a complex cargo of enzymes, cytokines and genetic material [80]. They can transfer their cargo to target cells due to various proteins present on the vesicle surfaces that have binding affinity for cellular surfaces. Secondly, exosomes can travel freely through blood due to their small size and can easily fuse with cells due to their surface structures [80]. In contrast, after systemic delivery, only a small number of MSCs arrive at the target site, and only a small percentage of those cells can integrate into the tissue and exert their functions for a short time. Thirdly, exosomes do not express MHC I or II antigens whereas MSCs can be induced to express higher levels of MHC II with inflammation [25]. Fourthly, exosomes can be loaded with chemotherapeutics, specific proteins, metabolites, or RNAs including miRNAs and siRNAs [81].

Modulation of Expression Levels of RILI-Related Inflammatory Cytokines by MSC-Derived Extracellular Vesicles

RP differs from other pulmonary pneumonitis arising from other causes such as allergic pneumonitis, chemical pneumonitis, or pneumonia with viral, bacterial, fungal, or parasitic origins [9]. Accumulating studies show that RP is a type of inflammatory reaction involving high expression levels of proinflammatory cytokines such as IL-1α,IL-1β, IL-6, and others, which may play a main role in RILI progression and can be regulated by MSC application [23, 26, 33, 35, 49, 82]. However, the immunomodulatory ability of MSCs cannot be sufficiently explained by the effects of only 1 secreted factor [83–85], and therefore it is likely that this ability results from the synergism of multiple factors. MSC-exosome shuttling of multiple immunomodulatory proteins is an ideal pathway for this synergism [86–88], and consistent with this, MSC exosomes can attenuate levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α and induce high levels of the anti-inflammatory IL-10 in vitro [89]. These released cytokines are also closely involved in RILI pathogenesis [33]. Similar anti-inflammatory effects were also reported in other studies of MSC-exosome applications [90–92]. Moreover, Wen et al. showed that both murine and human MSC-derived extracellular vesicles can reverse radiation damage [93]. Blazquez et al. further reported that exosomes derived from human adipose MSCs can inhibit the differentiation and activation of T cells and reduce IFN-γ production by stimulated T cells in vitro [94]. Based on these findings, we hypothesize that MSC exosomes could have therapeutic potential for RILI.

Modulation of Expression Levels of RILI-Related Inflammatory Cytokines by MSC Release of Exosomal miRNAs

Changes in miRNAs after radiation have been reported in lung cancer patients undergoing radiotherapy in the clinic and animal studies [95, 96], suggesting that miRNAs may function during the pathologic process of RILI. Information approximately miRNA changes in the lung after radiation will facilitate a better understanding of the mechanism(s) of injury. Indeed, a growing number of studies show that using MSC exosome-shuttled miRNAs can treat multiple inflammatory diseases by regulating levels of the proinflammatory

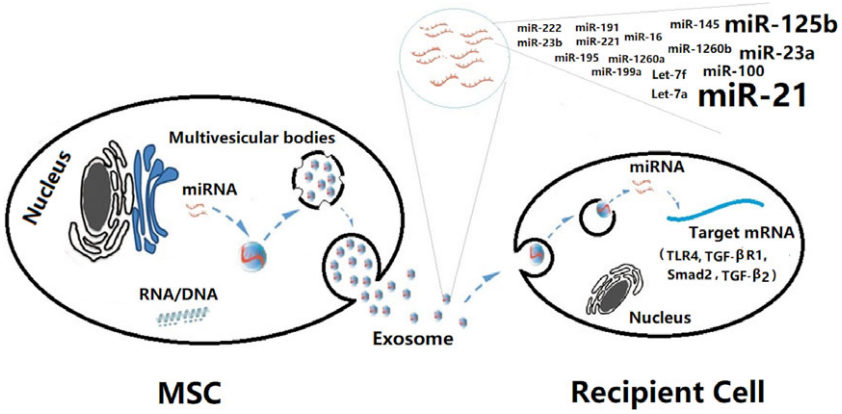

Figure 2. The anti-inflammatory and antifibrogenic potential of mesenchymal stem cells (MSCs) released exosomal miRNAs. In the RILI microenvironment, MSCs actively release miRNAs by exosomal transportation, which are taken up by recipient cells including injured endothelial cells, immune cells, myofibroblast, fibroblast, and so forth. These activities can downregulate IL-β, IL-6, and TNF-α by targeting the mRNA of proinflammatory and profibrogenic genes including TLR4, TGF-β, Smad2, and so forth, and then attenuate radiation-induced lung injury.

cytokines IL-1β, IL-6, TNF-α, and others [97]. Li et al. further reported that MSC exosome-shuttled miRNA-181c can attenuate levels of IL-1β and, TNF-α and induce high levels of IL-10 via targeting the TLR4/p65 signaling pathway [98], and therefore 1 possible strategy would be to use MSC-released exosomal miRNAs to treat RILI. Indeed, increasing evidence has suggested that MSCs release exosomal miRNAs as vital extracellular communicators to mediate the regenerative and immunomodulatory effects that prevent inflammatory and fibrogenic activity in injured tissue, including: miR-let-7b targeting TLR4 [99]; miR-21, miR-23a, and miR-145 targeting TGF-β2 [100]; miR-125b targeting Smad2 [100]; and miR-let-7c targeting TGF-βR1 [101]. These targets of MSC-released miRNAs are closely involved in production of various cytokines or in relevant inflammatory pathways in RILI, again strongly suggesting the potential of MSC-released miRNAs for RILI therapy. Moreover, evidence indicates that highly abundant miRNAs shuttled by hucMSCs play a major role in preventing inflammatory and fibrogenic activity [100, 102] (Fig. 2), further supporting the potential of MSC-released miRNAs for RILI therapy (Table 2).

Problems and Translational Challenges of the Therapies Based on MSC-Derived Secretome and Exosomes

The evidence above indicates the therapeutic potential of the MSC paracrine secretome and exosomes for RILI; however, the problems and translational challenges of the MSC secretome and exosomes have to be addressed before the therapies can be routinely applied in RILI patients.

The Tumor-Promoting Effect of MSC-Derived Soluble Factors

Although results from clinical studies using MSCs for the treatment of various lung diseases show that MSC treatment in some patients is safe [103], the carcinogenic potential of MSCs has been the subject of strong controversy for some time [77, 104]. Indeed, in addition to giving rise to cancer themselves, MSCs can also secrete a plethora of paracrine cytokines to promote tumor progression [104, 105]. For example, cell proliferation is a key process of tumor growth in tumor progression, and MSCs can promote this process by releasing IL-6 [106], IL-8 [107], MCP-1 [108], CXCL16 [109], and glutamine [110], by activating the JAK2-STAT3 pathway [106] and AMPK/mTOR-mediated NF-κB signaling [107], and through a β1-integrin-dependent mechanism [111]. Moreover, angiogenesis, as an essential component of tumor growth and survival, can also be promoted by key vasculogenic factors secreted by MSCs, including CXCL1 [112], CXCL8 [112], GDNF [113], VEGF [114], and TGF-β [114]. In addition, tumor cell invasion and migration are further malignant behaviors in tumor progression, and MSCs can promote these behaviors by producing and releasing IL-6 [115], IL-8 [115], CCL5 [116], IL-17B [117], soluble NRG1 [118], β2-microglobulin [119], FGF10 [120], VEGFC [120], MMPs [120], and nitric oxide [121], by activation of NF-κB, STAT3, PI3K/AKT signaling [117, 122], and in part through the Wnt pathway [120]. In addition to promoting tumor progression, MSCs can also elicit drug resistance in tumors by secreting CXCL1 and by activation of the STAT3 pathway [123, 124], and further promote tumor stemness by producing Gremlin-1, BMP2, BMP4, CXCL7, CXCL12, and others [114].

The Tumor-Promoting Effect of MSC-Derived Exosomes

Despite the fact that exosomes do not elicit acute immune rejection and lack the potential to directly form tumors, MSC-derived exosomes are also capable of inducing physiological processes in tumor development, for example, proliferation, angiogenesis, metastasis, and drug resistance. For example, MSC-derived exosomes can promote these processes in gastric cancer cells via activation of ERK, the PKB pathway [125], and Hedgehog signaling [126]. Moreover, MSC-derived exosomes can promote EMT effects via the FGF19-FGFR4 axis in nasopharyngeal carcinoma [127] and by TGF-β1 signaling in lung cancer [128]. In addition, such exosomes can also deliver mir-222/223 or mir-9 to induce drug resistance in tumors [125]. Similarly, Dong et al. showed that MSC-derived EVs can promote lung cancer growth by transferring mir-410, which is probably involved in PTEN downregulation [129]. On the other hand, MSC-derived exosomes are reported to exert proapoptotic functions in hepatoma [130], Kaposi's sarcoma [130] and ovarian tumors [130],

Table 2. Anti-inflammatory or antifibrotic effects of top 18 abundant miRNAs in umbilical cord MSCs-derived exosomes

MicroRNA	Targets	Effects on inflammation or fibrosis	References
miR-21-5p	PTEN, PDCD4	Dampens NF-κB/TNFα signaling, induces IL-10 expression	[143]
miR-125b-5p	Smad2	Inhibits TGF-β2/SMAD2 pathway	[100]
miR-23a-3p	TGFβ2	Inhibits TGF-β2/SMAD2 pathway	[100]
miR-100-5p	mTOR	Modulates the expression of IL-6	[144, 145]
Let-7f-5p	IL-6	Targets IL6 to inhibit inflammation	[146]
Let-7a-5p	LIN28B, TGFBR1	Targets Lin28B to regulate IL6 and NF-κB pathway	[147–149]
miR-145-5p	Smad3	Negatively regulates proinflammatory cytokine release from in COPD by targeting SMAD3	[150]
miR-1260b	Smad4	Regulates TGF-β pathway via targeting Smad4	[151, 152]
miR-1260a	COL1A1	Targets the fibrosis marker COL1A1	[153, 154]
miR-199a-3p	COX2	Targets COX2 to block TNF-α pathway	[149, 155]
miR-16-5p	Smad3	Decreases IL-1β, TNF-α, and NF-κB	[156, 157]
miR-195-5p	DLL1	Inhibits notch-induced IL-22 secretion	[158, 159]
miR-191-5p	STX3	Inhibits secretion of IL-1α, IL-1β, IL-12b, and CCL4 via targeting STX3	[160, 161]
miR-221-3p	SDF1	Prevents IL-1β-induced ECM degradation	[162]
miR-222-3p	IRF-2, ICAM-1	Inhibits inflammation via targeting IRF-2, ICAM-1	[163, 164]
miR-23b-3p	PTEN	Inhibits PTEN to promote the phosphorylation of Akt which leads to a decrease in proinflammatory cytokine production	[165, 166]
miR-3,120-5p	Hsc70	Inhibits HSC70-triggered activation of TLR signaling and inflammatory cytokine production via target HSC 70	[167, 168]
miR-214-3p	EZH1, EZH2	Prevents fibrosis-associated genes in myofibroblasts via targeting EZH1 and EZH2	[169]

suggesting a complex role for MSC-derived exosomes in tumor progression and one that warrants further investigation.

Hurdles in Clinical Translation

Compared with the risk of tumor-promoting effect of MSCs, the following major translational challenges should be considered seriously before clinical application of the MSC paracrine secretome and exosomes in the future.

First, according to the European and United States regulatory bodies, acquisition and processing of MSCs need to be in accordance with Good Manufacturing Practices (GMPs), which demand a high level of standardization, regarding the isolation of MSCs, the culture medium and serum for MSCs, and the use of closed-system bioreactors. GMP standards also demand stringent and standardized quality control measures for MSC production with reference to microbiological safety and the absence of any transformation due to genetic instability [77]. In addition, lack of a comparative characterization of murine and human MSCs may also limit the direct translation of preclinical animal model findings to clinical trials [24]. The etiology and progression of human inflammatory diseases are multifactorial, and animal models of inflammatory diseases do not fully represent human inflammatory diseases [24]. Translation of cellular or biological therapy from an animal model of inflammatory disease to human inflammatory disease remains a challenge. Additionally, MSCs derived from mice and humans are not identical in their capacity to suppress inflammation or in their mechanisms of action due to specific species differences [24]. For these reasons, preclinical animal studies with murine MSCs cannot precisely predict the outcome of human MSC-based clinical trials. Moreover, differences in MSC source, preparation, and handling methods may affect the quality and therapeutic efficacy of the cellular product and subsequently affect the clinical outcome [24]. For example, cryopreserved MSCs exhibited attenuated biodistribution and immunosuppressive properties compared with actively growing MSCs in cultures in vitro. The effects of MSC transplantation may also be limited not only by the transplantation site of MSC injection but also by the number of transplanted MSCs [131]. Indeed, it has been reported that <1% of MSCs survive for more than 1 week after systemic administration [132, 133]. Thus, many issues should be considered when translating the therapeutic promise of MSCs in preclinical studies to the clinical setting. Another big concern regarding MSC therapy for RILI is perhaps the potential for fibrosis. Yan et al. reported that Flk-1 + MSCs injected into the lung immediately after irradiation could differentiate into functional lung cells, but those injected at a later stage after irradiation may be involved in fibrosis development [134], possibly because the TGF-β1 level is markedly increased in the middle and later stages after irradiation. The dramatic change in the microenvironment of the injured lung might also inhibit differentiation of transplanted MSCs into lung epithelial cells and induce them to differentiate into myofibrocytes, which then participate in lung fibrosis.

Second, to understand the mechanisms of how cytokines are expressed during RILI and how they modulate the therapeutic effects of stem cells are a significant challenge due to the myriad complex interactions of paracrine factors in the secretome of MSCs. In addition, despite the fact that the secretome of MSCs is normally present in conditioned medium, the components of conditioned medium do not only reflect the secretome because the medium also contains proteins that are released during cell death. Avoiding leakage of intracellular proteins from dead cells is thus a challenge in the careful optimization of the secretome from MSCs. Furthermore, the production and concentration of secreted molecules in quantities sufficient for clinical

administration are also challenging. Other limitations of secretome therapy include tissue transport, pharmacokinetics, and protein stability.

Third, as with the use of MSCs, the defined and standardized methods used to isolate and identify exosomes will also be required, according to the International Society of Extracellular Vesicles [135]. Thus, a universally accepted protocol for exosome isolation, large-scale GMP production guidance, as well as validated methods for quantifying and evaluating the potency of exosomes are lacking. According to preclinical studies, the amount of MSC EVs required to produce the equivalent effect of MSCs in lung injury is generally 10 times higher [25]. Therefore, if the average dose of MSCs is 10×10^6 cells per kilogram per body weight, the number of MSCs required to generate enough exosomes may be greater than 100×10^6 of MSC per kilogram, which may make the production costs prohibitive. In addition, expanding big batches of MSCs for exosome production will impact the costs of derivation, testing, and validation, because the biological properties of MSCs may become altered with repeated passages [25]. Although a potential approach to significantly increasing extracellular vesicle production could be the use of bioreactor systems to culture MSCs, different bioreactor culture conditions may impact the content and therapeutic efficacy of EVs, including the build-up of metabolic byproducts, pH balance, hydrodynamic shear stress, and oxygen supply [25]. The very significant barriers above underline the need for caution on rapid clinical translation. Although clinical effects of using MSC exosomes have been explored in phase I and II trials in lung diseases [136], the latent side effects, based on the recipients' long-term follow-up, was unclear. In addition, the uptake mechanisms for exosomal miRNAs and the mechanisms that regulate incorporation of a particular miRNA into exosomes are still unclear, so the best methods for confirming their safety and proving their efficacy in vitro and especially in vivo still need to be resolved. Finally, other issues related to clinical application of these therapies, including ethical, legal, technical and regulatory concerns, are also challenging.

Summary and Future Perspectives

RILI is a major limiting factor in the application of thoracic radiation and a major obstacle to the use of advanced dose escalation modalities and ablative hypofractionation radiotherapy regimens. Therefore, developing alternative strategies to protect the lungs from RILI is essential. However, there are still existing drawbacks in the application of various therapeutic drugs for RILI therapy. MSCs, as an immunomodulatory and regenerative tool, exhibit considerable promise for targeting RILI pathogenesis by secreting paracrine cytokines, as demonstrated in a growing number of studies. However, tumor-promoting features of the MSC paracrine secretome may limit the translational application of MSCs for clinical RILI patients. Indeed, it has been reported that MSCs may possess a distinct tropism to tumors after systemic administration [137, 138]. And some studies have demonstrated that MSCs that are cultured for a long-term may exhibit some neoplastic transformation [139, 140]. The progression of existing tumors may be problematic due to direct effect on the tumor or surrounding stroma as a result of reducing inflammation and promoting tumor evasion from the immune system. Thus, the limitations of tumorigenic potential of MSCs in clinical trials should be also considered. Furthermore, studies should focus on more clearly identifying factors responsible for the therapeutic effects of MSCs. These efforts will help to develop more effective protein-based conditioning approaches. Modification of the secretion profile to augment the therapeutic effects of the secretome may be achieved via physical, physiological, and pharmaceutical preconditioning of stem cells including hypoxia induction, treatment with disease-specific drugs, small molecules, specific growth factors/cytokines, and cellular reprogramming/genetic manipulation strategies.

Compared with MSCs, although MSC-derived exosomes exhibit many advantages for treating lung diseases [25], the promoting and proapoptotic role of MSC-derived exosomes on tumor progression raises controversy with reference to their suitability for clinical translation. Fortunately, it was recently reported that miRNAs are transported by exosomes in lung disease [136], suggesting that, instead of native exosomes, exosomes modified by the expression of different miRNAs will be a promising alternative for RILI therapy. In particular, taking advantage of miRNAs that can inhibit both cancer and inflammation will be a better alternative for RILI therapy based on MSC-derived exosome cargo.

In addition, MSC-derived exosomes contain not only miRNA but also lipids [79] and long noncoding RNAs, which regulate multiple signaling pathways related to inflammation [141]. Moreover, immune cells can interact with various classes of lipids to regulate the plasticity of macrophages and T lymphocytes [142]. Whether MSC-derived exosomes can attenuate RILI via shuttled long noncoding RNAs, lipids, or both is unknown. Thus, the progress made so far on the potential of MSCs in RILI therapy suggests that the MSC-derived secretome and exosomes function in RILI through a variety of mechanisms but that further research is needed to understand their potential.

Author Contributions

S.X.: manuscript preparation, financial support; C.L.: literature search; S.X. and H.L.J.: conception and design, manuscript revision, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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