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Concise Review: MSC-Derived Exosomes for Cell-Free Therapy

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Key Words. Mesenchymal stem cells • Mesenchymal stromal cells • Microvesicles • Exosomes • Cellular therapy

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ABSTRACT

Mesenchymal stem cell transplantation is undergoing extensive evaluation as a cellular therapy in human clinical trials. Because MSCs are easily isolated and amenable to culture expansion in vitro there is a natural desire to test MSCs in many diverse clinical indications. This is exemplified by the rapidly expanding literature base that includes many in vivo animal models. More recently, MSC-derived extracellular vesicles (EVs), which include exosomes and microvesicles (MV), are being examined for their role in MSC-based cellular therapy. These vesicles are involved in cell-to-cell communication, cell signaling, and altering cell or tissue metabolism at short or long distances in the body. The exosomes and MVs can influence tissue responses to injury, infection, and disease. MSC-derived exosomes have a content that includes cytokines and growth factors, signaling lipids, mRNAs, and regulatory miRNAs. To the extent that MSC exosomes can be used for cell-free regenerative medicine, much will depend on the quality, reproducibility, and potency of their production, in the same manner that these parameters dictate the development of cell-based MSC therapies. However, the MSC exosome's contents are not static, but rather a product of the MSC tissue origin, its activities and the immediate intercellular neighbors of the MSCs. As such, the exosome content produced by MSCs appears to be altered when MSCs are cultured with tumor cells or in the in vivo tumor microenvironment. Therefore, careful attention to detail in producing MSC exosomes may provide a new therapeutic paradigm for cell-free MSC-based therapies with decreased risk. STEM CELLS *2017;35:851–858*

SIGNIFICANCE STATEMENT

Mesenchymal stem/stromal cells (MSCs) are being exploited as an experimental therapy for a variety of human diseases. Current dogma indicates that MSCs ameliorate disease via secretion of paracrine acting factors that limit inflammation, reprogram immune cells, and activate endogenous repair pathways. Recent studies indicate that MSCs also produce extra-cellular vesicles of varying sizes including exosomes that carry as cargo mRNAs, microRNAs, and proteins, and that horizontal transfer of this cargo induces nonautonomous changes that are therapeutic. This manuscript reviews evidence that MSC-derived microvesicles/exosomes function as paracrine mediators in tissue repair and recapitulate to a large extent the therapeutic effects of parental MSCs. It also discusses their role in reprogramming endogenous MSCs to generate a self-reinforcing malignant niche.

INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) are one of the most commonly employed cell types under investigation as an experimental cell-based therapy for treating human diseases. There are over 600 clinical trials now listed at www.clinicaltrials.gov utilizing MSCs. Their widespread use stems from their demonstrated potency in a broad range of experimental animal models of disease and their excellent safety profile in humans. Nevertheless, the precise mechanism(s) of action of MSCs administered to human patients for a particular disease or condition remains an area of intensive investigation. Results indicate (Fig. 1) that MSCs play several simultaneous roles: limiting inflammation through releasing cytokines; aiding healing by expressing growth factors; altering host immune responses by secreting immuno-modulatory proteins; enhancing responses from endogenous repair cells; and serving as mature functional cells in some tissues such as bone. These mechanism are not mutually exclusive, and as such it is anticipated that MSCs yield therapeutic effects by an orchestrated response that is dictated by the unique pathophysiology of a given disease.

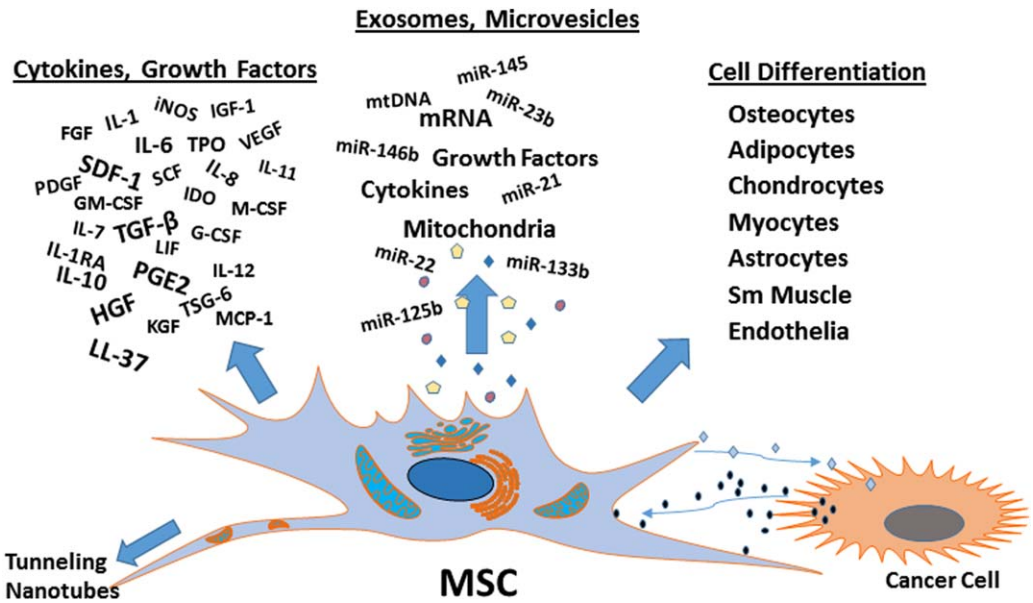

Figure 1. MSCs play multiple roles. They can differentiate to multiple lineages and can participate in organized cell replacement therapy but engraftment after delivery in vivo remains low. However, the MSCs produce many cytokines and growth factors that influence other cells producing decreased inflammation, enhanced progenitor cell proliferation, improved tissue repair and decreased infection. MSCs have also been shown to donate mitochondria via tunneling nanotubes to damaged cells. More recently, the MSC production and release of membrane bound packets—microvesicles (>200 μm) and exosomes (~50–200 μm)—that encapsulate cytokines/growth factors/RNAs/miRNAs in diverse combinations. These vesicles are being tested in experimental systems previously tested with the cells themselves. Remarkably, the vesicle preparations have shown results very similar to MSC transplantation in many cases, while avoiding many risks associated with cell transplantation. However, many important tasks remain before MSC-derived vesicle therapy can be used clinically including standardized production, vesicle characterization, improving isolation and yield optimization, reproducibility, an assay for potency, determining dosage for particular clinical indication and standardized production—all similar to parameters needed for MSC cell therapy. Abbreviation: MSCs, Mesenchymal stem/stromal cells.

Shifting Paradigms

Over the years, our understanding of the nature and function of MSCs has undergone a number of paradigm shifts. Initially characterized as osteogenic stem/progenitors [1, 2], MSC-based therapies were anticipated to augment the structure and function of damaged or diseased tissues via direct cell replacement. Indeed, in animal studies the MSCs were effective in healing bone nonunions [3, 4] and in one of their first clinical applications, MSCs were shown to produce a measurable benefit in bone strength and ambulation when administered to children with osteogenesis imperfecta [5]. When MSCs are labeled and delivered in vivo, they will migrate to sites of tissue injury such as a brain lesion or cardiac infarct [6–8]. However, it soon became apparent that relatively few MSCs engrafted at these sites of injury and studies in rodents and dogs confirmed that intravenously administered MSCs are caught in the capillaries of the lung and most MSCs are largely cleared, but some do get through to the injured target tissue [9–12]. Despite these limitations, MSCs continued to yield short-term therapeutic benefits in a large number of disease models [13–17]. Although it had been long-known that MSCs produced abundant growth factors and cytokines [18–21], many of which modulate the immune system (summarized in [22, 23]), to reconcile these disparate findings, the field adopted the revisionist viewpoint that MSCs affect tissue repair largely via their paracrine factors and stimulation of host cells, and not by cell replacement [24, 25]. This paradigm shift was spurred by studies demonstrating that culture medium conditioned by MSCs produced therapeutic effects similar to delivery of the cells in rodent models of acute myocardial infarction [26] and lung injury [27, 28], and was further bolstered by genomics data showing MSCs secrete a plethora of biologically active proteins [29–31]. In 2007, a study by Timmers et al. [32] confirmed earlier reports that medium conditioned by human embryonic stem cell (ESC)-derived MSCs (hESC-MSCs) significantly reduced infarct size in both pig and mouse models of myocardial ischemia/reperfusion (MI/R) injury. An important advance made by this work was inclusion of size fractionation studies that identified the active component in media within the 50–200 nm range. Subsequent bio-physical studies characterized the biologically active component as exosomes. Camussi and colleagues demonstrated that MSC derived microvesicles prevented kidney injury [33, 34], and Lai et al. [35] reported that a homogeneous preparation of exosomes with a hydrodynamic radius of 55-65 nm reduced the infarct size in an ex vivo mouse Langendorff heart model of myocardial ischemia/reperfusion injury at a protein dosage equivalent to ~10% of the conditioned medium dosage. Herein, the nature of the in vitro infarct model also ruled out any contribution from circulating immune cells or platelets. These studies have fostered an intense research effort to better understand the nature and function of MSC-derived exosomes. While MSCs are known to express growth factors and cytokines, many of these proteins do not have signal peptides and their packaging along with mRNAs and miRNAs in membrane-bound vesicles explained, at least partially, how MSCs exert multiple effects throughout the body.

Table 1. Translational studies employing MSC-derived microvesicles and exosomes

Target tissue/Model	Species-exosome (Origin into Target)	MSC-derived agent	Method	Dose	[a]References
Heart/infarct	Human into Pig	Cond. Med.	25 × conc	10 mg in 5 ml	[32]Timmers et al. (2007)
Heart/IR	Human into Mouse	Exosomes 55-65 nm	HPLC	0.4 μg	[35]Lai et al. (2010)
Heart/infarct	Rat into Rat	Exosomes w/GATA4	ExoQuick	(4 × 10^6 MSC)	[38]Yu et al. (2015)
Heart/IR	Human into Mouse	Exosomes, ATP	HPLC	0.1-0.4 μg	[39]Arslan et al. (2013)
Heart/infarct	Human into Rat	Extracellular Vesicles	100K × g	80 μg	[40]Bian et al. (2014)
Heart/infarct	Rat into Rat	Exosomes	ExoQuick	80 μg	[41]Teng et al. (2015)
Kidney/injury	Human into Mouse	Microvesicles	100K × g	100 μg	[34]Bruno et al. (2012)
Kidney/chronic	Human into Rat	Cond. Medium	25x	0.5mg/ml	[42]Van Koppen et al. (2012)
Kidney/gentamycin	Rat into Rat	Exosomes	100K × g	100 μg	[43]Reis et al. (2012)
Kidney/cisplatin	Human into Rat	Exosomes	100K × g	250 μg	[44]Zhou et al. (2013)
Brain/TBI	Human into Mouse	Exosomes	An Chrom	30 μg	[45]Kim et al. (2016)
Brain/stroke	Rat into Rat	Exosomes	100K × g	100 μg	[46]Xin et al. (2013)
Brain/ischemia	Human into Ovine	Extracellular Vesicles	PEG	(2 × 2 × 10^7 MSC)	[47]Ophelders et al. (2016)
Brain/TBI	Rat into Rat	Exosomes	ExoQuick	100 μg	[48]Zhang Y et al. (2015)
Brain/stroke	Human into Mouse	Exosomes	110K × g	(2 × 10^6 MSCs)	[49]Doeppner et al. (2015)
Liver/fibrosis	Human into Rat	Exosomes	100K × g	250 μg	[50]Li et al. (2013)
Liver/drug injury	Human into Mouse	Exosomes	100K × g	0.4 μg	[51]Tan et al. (2014)
Lung/hypoxia	Mouse into Mouse	Cond Med, Exsomes	PEG-S200	0.1–10 μg	[52]Lee et al. (2012)
Lung/drug	Mouse into Mouse	Exosomes	100K × g	25 μg	[53]Aliotta et al. (2016)
Lung/silicosis	Human into Mouse	Microvesicles	ExoQuick	10 μg	[54]Choi et al. (2014)
Hypertension	Human into Mouse	Microvesicles	100K × g	(3x10^6 MSCs)	[55]Zhu et al. (2014)
Lung/fluid filled	Human into Human	Microvesicles	100K × g	160 μg	[56]Gennai et al. (2015)
Lung/E.coli endotoxin	Human into Mouse	Microvesicles	100K × g	(9 × 10^6 MSCs)	[57]Monsel et al. (2015)
Intestine/enterocolitis	Human into Rat	Exosomes	PureExo	50μl IP	[58]Rager et al. (2016)
Intestine/enterocolitis	Rat into Rat	Microvesicles	100K × g	50-200 μg	[59]Yang et al. (2015)
Skin/wound	Human into Rat	Exosomes, Wnt4	100K × g	200 μg	[60]Zhang B et al. (2015)
Skin/wound	Human into Rat	Exosomes	100K × g	160 μg	[61]Zhang J et al. (2015)
Skin/wound	Human into Mouse	Exosomes, miRNA	120K × g	100 μg	[59]Fang et al. (2016)
Limb ischemia	Human into Mouse	Exosomes	100K × g	200 μg	[62]Hu et al. (2015)
Sk. Musc/cardiotoxin	Human into Mouse	Exosomes, miR-494	110K × g	50 ul	[63]Nakamura et al. (2015)
Sk. Muscle/ALS	Mouse into Mouse	Exosomes, SOD1	PureExo	0.2 μg/ml	[64]Bonafede et al. (2016)
Cancer/glioma	Rat into Rat	Exosomes, miR-146b	ExoQuick	50 μg	[65]Katakowski et al. (2013)
Cancer/breast	Human into mouse	Exosome miRNA	100K × g	1 μg/4d	[66]Ono et al. (2014)
Cancer/Myeloma	Human into mouse	Exosomes	ExoQuick		[67]Roccaro et al. (2013)
Sepsis/poly-fecal	Mouse into Mouse	Exosomes, miR-223	36K × g	2 μg/gBW	[68]Wang et al. (2015)

[a]Superscript numbers refer to citation numbers within the text. ExoQuick is from SystemBio Inc Palo Alto CA, PureExo is from 101Bio Inc., PaloAlto CA. Eighty micrograms is about the amount of exosomes released from 2 million MSCs in 48 hours.

Exosomes and Microvesicles as Paracrine Mediators in Tissue Repair

Most cells produce extracellular vesicles as a consequence of intracellular vesicle sorting including both microvesicles of >200 nm and exosomes of 50-200 nm diameter. The microvesicles are shed from the plasma membrane whereas exosomes originate from early endosomes and as they mature into late endosomes/multivesicular bodies, they acquire increasing numbers of intraluminal vesicles, which are released as exosomes upon fusion of the endosome with the cell surface [36, 37]. With respect to MSCs, most laboratories isolate exosomes/microvesicles from conditioned media via ultracentrifugation (See Table 1) although a method based on chromatography has also been described [45], and characterize these fractions based on their membrane protein content and/or cargo. For example, the tetraspanins, CD63, and CD81 are common markers enriched in exosomes [69]. While the physiological significance and evolutionary consequence for producing extracellular vesicles and a detailed description of their physical nature is beyond the scope of this review, these topics have been covered elsewhere [70–72].

The majority of the published MSC exosome literature recapitulates in large part the nature and scope of that previously devoted to the study of MSC action in animal models of disease. For example, various groups have confirmed that MSC-derived exosomes exhibit cardio and renal-protective activity [32, 42–44], are efficacious in animal models of myocardial infarction [39–41], stroke [46], peri-natal hypoxic-ischemic brain injury [47], and hind-limb ischemia [62]. The MSC-derived exosomes also ameliorated carbon tetrachloride-induced liver fibrosis [50, 51], and conferred cyto-protective effects in models of necrotizing enterocolitis [58]. In lung studies, the mouse MSC exosomes were effective in improving pulmonary hypertension [52, 53], silicosis [54], and human MSC-exosomes improved endotoxin-induced pulmonary edema [55, 57], and cleared alveolar fluid from human lungs ex vivo [56]. Other studies have shown that MSC-derived exosomes also promoted re-epithelialization of cutaneous wounds by inducing epithelial cell proliferation [60] and angiogenesis [73, 74], activated collagen and elastin secretion by fibroblasts [61], and prevented myo-fibroblast formation thereby reducing scaring [59]. The MSC-derived exosomes also promoted muscle regeneration [63], protected against experimental colitis [75], and exhibited potent neuro-protective

activities in neurons [64, 76] and in models of traumatic brain injury [45, 48]. MSC-derived exosomes are also immunologically active based on evidence that they suppressed proliferation and IFN-γ secretion by T cells stimulated with anti-CD3 and anti-CD28 antibodies [77], and also enhanced the survival of allogenic skin grafts in mice by enhancing T cell polarization to a regulatory phenotype [78]. A growing number of studies suggest that MSC-derived exosomes mimic the ability of MSCs to influence the activity of immune effector cells including B, T, NK, dendritic cells, and macrophages although not all studies show positive effects [reviewed in [79]]. Collectively, these studies readily demonstrate that MSC-derived exosomes recapitulate to a large extent the immensely broad therapeutic effects previously attributed to MSCs.

However, while much effort has been devoted to demonstrating that MSCs and MSC-derived exosomes yield similar therapeutic benefits in various disease models, most studies fall short of rigorously validating this hypothesis. For example, various groups have compared the potency of MSCs versus MSC-derived exosomes, and in some cases MSC conditioned media, in animal models of myocardial infarction [40], focal cerebral ischemia [49], gentamicin-induced kidney injury [43], and silicosis [54]. While most studies report that MSC-derived exosomes are equally effective as MSCs in sparing tissue and/or promoting functional recovery from injury, this desired outcome is compromised by lack of appropriate controls, comparable dosing, evaluation of the different disease endpoints, variations in frequency and timing of dosage, and absence of dose-dependent effects, thereby making it difficult to draw conclusions about comparable efficacy and potency. There is also the issue of lability and whether freezing/thawing effects exosome potency.

Mode of Action of MSC-Derived Exosomes

MSC-derived exosomes function largely via horizontal transfer of mRNAs, miRNAs and proteins, which then function by a variety of mechanisms to alter the activity of target cells. For example, Tomasoni et al. [80] reported that transfer of *IGF-1R* mRNA from MSC-derived exosomes to cisplatin-damaged proximal tubular epithelial cells sensitized the epithelial cells to the renal-protective effects of locally produced IGF-1. With respect to miRNAs, those contained within MSC-derived exosomes have been shown to inhibit tumor growth [65, 66], reduce cardiac fibrosis following myocardial infarction [81], stimulate axonal growth from cortical neurons [76], promote neurite remodeling and functional recovery after stroke [82], and stimulate endothelial cell angiogenesis [83]. Furthermore, several studies have validated a direct role for exosome-derived miRNAs in modulating target cell function via use of loss-of-function approaches [68, 82]. Other studies have shown that exosomes secreted by bone marrow-derived MSCs contain cystinosin (CTNS), a cystine efflux channel in the lysosomal membrane, and that coculture of fibroblasts and proximal tubular cells from cystinosis patients with MSC-derived exosomes resulted in a dose dependent decrease in cellular cystine levels [84]. Additionally, Katsuda et al. [85] demonstrated that exosomes produced from adipose-derived MSCs (ADSCs) contain neprilysin, an enzyme that degrades the amyloid beta peptide, and that coculture of N2a cells engineered to overexpress human Aβ with ADSCs significantly reduced the levels of secreted Aβ40 and Aβ42 by exosome-mediated transfer of neprilysin. A separate study by Amarnath et al. [86] reported that MSC-derived exosomes suppress human-into-mouse GvHD by inhibiting Th1 cell effector function via the release of CD73 containing exosomes, which when taken up by CD39 expressing CD4+ Th1 cells resulted in enhanced adenosine production and increased Th1 cell apoptosis. Together, these studies indicate that dissecting the therapeutic effects of MSC-derived exosomes and their mechanism of action in vivo may be equally as challenging as determining that for the parent MSCs.

Exosomes and the MSC Niche Function

It is well-established that marrow resident MSCs play a critical role in retention of HSCs within the bone marrow niche [87], and alterations in MSC function may contribute to the pathophysiology of hematological diseases [88]. Consistent with these findings, an increasing number of studies have shown that exosomes secreted from leukemic cells reprogram MSCs to promote the development of a self-reinforcing malignant niche. For example, Muntión et al. [89] found that that the miRNA cargo of exosomes was significantly altered in marrow-derived MSCs harvested from myelodysplastic syndrome (MDS) patients when compared to disease-free patients, and uptake of these exosomes by normal CD34+ progenitors enhanced cell viability and increased CFU-GM production. The cargo of MSC-derived exosomes from acute myeloid leukemia (AML) patients was also shown to differ from normal patients in that it contained elevated levels of miR155 and miR375, which independently identify AML patients at high risk for recurrence, and conferred chemo-resistance to AML cells against cytarabine and the FLT3 inhibitor AC220 [90]. Exosomes recovered from the blood of CML patients carried as part of their cargo the EGFR ligand amphiregulin (AREG), and coculture with HS5 stromal cells induced expression of MMP9 and IL-8 by increasing EGFR signaling, resulting in increased adhesion of leukemic cells to stromal cells [91]. Similarly, exosomes released by primary chronic lymphocytic leukemia (CLL) cells reprogramed MSCs to adopt a cancer-associated fibroblast (CAF) phenotype characterized predominantly by increased NF-kB signaling and elevated secretion of cytokines and chemokines [92], which enhanced tumor cell survival in vitro and tumor growth in vivo. Studies have also shown that exosome-mediated transfer of tumor associated miRNAs from multiple myeloma cells to MSCs stimulated the latter to secrete higher levels of the myeloma survival factors CXCL1, CCL5, and IL6 [93]. Moreover, MSC-derived exosomes from multiple myeloma patients were shown to express higher levels of oncogenic cytokines as compared to those from normal patients and promote growth of tumor cell lines in vivo [67].

While the role of exosomes in creating a leukemic niche is under intensive study, their role within the bone marrow niche under healthy physiological conditions has only recently garnered attention. For example, Wen et al. [94] reported that extra-cellular vesicles (EVs) derived from bone marrow MSCs were capable of protecting Lin^- hematopoietic progenitors from radiation-induced damage both in vitro and in vivo.

Herein, exposure of Lin^- cells to EVs after irradiation led to a statistically significant ($p < .05$) increase in their overall engraftment at 24 and 36 weeks post-transplant, and also enhanced engraftment when transplanted to secondary recipients. Other studies have shown that MSC-derived exosomes stimulate bone regeneration in critical-sized calvarial defects in ovariectomized rats [95], hyaline cartilage formation and repair of osteochondral defects in rat femurs after repeated intra-articular injections [96], and reversed defects in bone healing due to impaired callus formation in $CD9^{-/-}$ mice [97]. Last, Phinney et al. [98] recently demonstrated that human MSCs manage intracellular oxidative stress by targeting depolarized mitochondria to the plasma membrane via arrestin domain-containing protein 1-mediated microvesicles, that these vesicles are engulfed and reutilized by macrophages, and that MSCs simultaneously shed miRNA-containing exosomes that inhibit macrophage activation by suppressing Toll-like receptor signaling thereby de-sensitizing macrophages to the ingested mitochondria.

Not All MSC-Derived Exosomes are Created Equal

As is the case with MSC-based therapies, studies indicate that not all MSC-derived exosomes are equivalent. For example, Katsuda et al. [85] reported that exosomes isolated form adipose-derived MSCs contain up to fourfold higher levels of enzymatically active neprilysin, an enzyme important in degradation of beta-amyloid, as compared to bone marrow-derived MSCs. Del Fattore et al. [99] further showed that exosomes from marrow and umbilical cord-derived MSCs inhibited the growth and induced apoptosis of U87MG glioblastoma cells in vitro whereas those from adipose-derived MSCs promoted cell growth but had no effect on U87MG survival. Lastly, Lopez-Verrilli et al. [100] showed that exosomes prepared from different tissue-specific MSCs have measurably different effects on neurite outgrowth in primary cortical neurons and dorsal root ganglia explant cultures.

This diversity of experimental results is fascinating and complex. While the opportunities for cell-free treatment of many diseases seems at hand, have we merely replaced one variable cell therapy product with an equally variable cell extract from those cells? Moreover, does this mean each laboratory will have its own preferred method, or can we arrive at a standardized protocol to be able to assay the identity, predict the exosome contents, potency, and dosing to be assured of the in vivo effects? The exosome or microvesicle approach does avoid the transfer of cells and their DNA. However, the small payload of such vesicles suggests a production issue, and one that must be standardized by acceptable methods.

Conclusions and Future Directions

Once thought to function in cell replacement for damaged tissue-resident cells, it is now widely established that the more immediate principle mechanism of action of MSCs in vivo is paracrine in nature, and that the generation of exosomes and microvesicles by MSCs is a critical parameter in their ability to modify the function of host cells and tissues (Fig. 1). Various studies indicate that MSC-derived exosomes exert their effect via horizontal transfer of proteins, mRNAs and regulatory microRNAs. The ability of diseases and transformed cells to also affect the function of tissue resident MSCs is of importance, as usurping the MSCs ability to modify the cancer niche likely plays a critical role in survival and expansion of cancerous cells both in dispersed and solid tumors. Despite the rapid progress made in exosome research to date, a number of important questions remain with respect to their role in MSC biology. For example, few studies have explored whether endogenous niche resident MSCs that play a role in hematopoiesis and skeletal homeostasis secrete exosomes or microvesicles, and the role they play in niche maintenance under normal physiological conditions. Whether the essence of the MSC can be captured by its secreted products and used therapeutically is another critical question to be addressed. This is of particular importance owing to the fact that the broad therapeutic efficacy of MSCs is predicated on their ability to rapidly respond to the injury microenvironment, whereas isolated exosomes would not be anticipated to do so. We can also expect that the very low number of endogenous MSCs, and the constantly diminishing number of isolatable MSCs found in the aging individual make the assessment of the role of endogenous MSC exosomes a challenging question. But these problems of understanding cell to cell communication via exosomes and microvesicles are some of the most interesting problems in biology and not only confined to MSCs or stem cells, and helpful answers may come from the broader biology community.

Use of MSC-derived exosomes/microvesicles in human patients has several potential advantages. First, their use avoids the transfer of cells which may have mutated or damaged DNA. Second, the vesicles are small and circulate readily whereas MSCs are too large to circulate easily through capillaries and many MSCs do not get beyond the first pass capillary bed, usually the lungs (although some clearly get through). Third, the dose of infused MSCs quickly diminishes post-transplant, and it may be that the delivery of MSC-derived vesicles can achieve a higher "dose" that circulates to a greater extent than the larger cells. The disadvantage of using MSC-derived vesicles is that they are static and more cannot be produced in vivo as may be possible when transplanting the cell itself. The question then arises as to the potency of the vesicles and the therapeutic dose. This means a potency assay must be developed for the vesicles, a task that still challenges many labs developing MSC cellular therapeutics. While it is almost assured MSC-derived exosomes will advance toward clinical testing, their utility and efficacy will depend on a number of critical parameters including reducing to practice reproducible methods to manufacture exosomes/microvesicles of defined content, developing methods of storage and recovery of these products that maintain vesicle potency, and evaluating their therapeutic efficacy in well controlled, appropriately powered clinical trials that are rationally designed based on supporting scientific and translational data. One may anticipate that by building on knowledge gained from MSC-based clinical trials the development of exosome/microvesicle-based therapies may experience more rapid advancement.

Author Contributions

D.G.P.: Manuscript writing, final approval of manuscript; M.F.P.: Manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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