Exosomes: Vehicles of Intercellular Signaling, Biomarkers, and Vectors of Cell Therapy

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Keywords

Abstract

Mesenchymal stem cells (MSCs), whose mechanism of action is predominantly paracrine, are being widely tested for the treatment of a variety of human diseases. No one factor has been proven sufficient to mediate the therapeutic effects of MSCs. However, exosomes—membrane vesicles secreted by many cells, including MSCs—are appealing candidates as vectors of their efficacy. Exosomes can transport and deliver a large cargo of proteins, lipids, and nucleic acids and can modify cell and organ function. In addition to their key role as vehicles of intercellular communication, exosomes are increasingly recognized as biomarkers and prognosticators of disease. Moreover, they have the potential to be used as vehicles of gene and drug delivery for clinical application. This article reviews the biogenesis of exosomes, their molecular composition, and their role as messengers of intercellular communication, focusing on their potential as therapeutic vectors for stem cell therapy.

INTRODUCTION

Mesenchymal stem or stromal cells (MSCs) are multipotent adult stem cells that are capable of self-renewal and differentiation into mesenchymal lineages such as muscle, bone, fat, and cartilage, as well as into cells of nonmesenchymal lineage such as neurons and keratinocytes. However, with the possible exception of bone and cartilage formation, the differentiation of MSCs into other cell types is a rare event in vivo. Several studies have confirmed that in vivo administration of MSCs ameliorates disease in preclinical models but that these cells are rapidly cleared within 48 h. Cell differentiation and direct tissue repair contribute minimally to the beneficial effects attributed to MSCs, and paracrine, immunomodulatory pathways are the predominant mechanisms of their in vivo effect. Stem cell–based therapies represent a novel and promising approach to the treatment of many complex diseases with inflammatory underpinnings due to their potent anti-inflammatory effects as well as their ability to target several cellular pathways required for optimal organ function. With the recognition that MSCs are not retained in organs beyond a few hours to days, many investigators, including our group, have administered culture medium conditioned by MSCs and have demonstrated cytoprotective effects in models of heart, lung, renal, or neuronal injury (1–5). Paracrine factors released by MSCs have long been recognized and include the secretion of growth factors and cytokines—such as vascular endothelial growth factor, stromal cell–derived factor-1, fibroblast growth factor, transforming growth factor β, and interleukin 1 receptor antagonist—that promote angiogenesis and protect against tissue inflammation from ischemia, hypoxia, and other injuries (6–12). Not surprisingly, none of these growth factors administered individually has been shown, or should be expected, to effectively treat tissue injury and reverse human diseases that often result from the dysfunction of complex and multifactorial pathways. The delivery of these factors to the right cell type and compartment while maintaining stability and biological potency is a key limiting factor. However, the transport and delivery of a large cargo load within a membrane vesicle would confer protection from degradation as well as facilitate targeted-cell delivery through membrane receptors.

Recently, increasing attention has been paid to the exosome—a membrane-bound vesicle produced by almost all cell types, including MSCs—which is released into the culture medium and may be a critical messenger for cell-cell communication (reviewed in Reference 13). Exosomes are one of several groups of secreted vesicles, which include ectosomes, which are large, membranous vesicles that are shed directly from the plasma membrane, and apoptotic blebs that are released by dying cells. Exosomes are surrounded by a phospholipid bilayer and can be distinguished by their size and composition. Exosomes range in size from 30 to 100 nM in diameter, whereas ectosomes range between 50 and 1,000 nM and apoptotic bodies between 50 and 5,000 nM. Exosomes are of endosomal origin and are stored within multivesicular bodies (MVBs) to be released into the environment by fusion with the cell membrane (14, 15). They contain numerous proteins and lipids, as well as nucleic acid material in the form of DNA, mRNA, microRNA (miRNA), and noncoding RNA. As such, exosomes can be used by cells as a major route of excretion to dispose unused or harmful RNA and proteins or, more importantly, as messengers and carriers of important signals to other cells, modifying their function in normal physiology as well as in states of disease. Moreover, exosomes can be isolated from cultured cells and delivered in vivo to target disease and hence have the potential for therapeutic application once their characterization and biology are better delineated.

BIOGENESIS OF EXOSOMES

Exosomes were described in the early 1980s as small vesicles that formed during reticulocyte maturation and that mediated the selective externalization and removal of the transferrin receptor
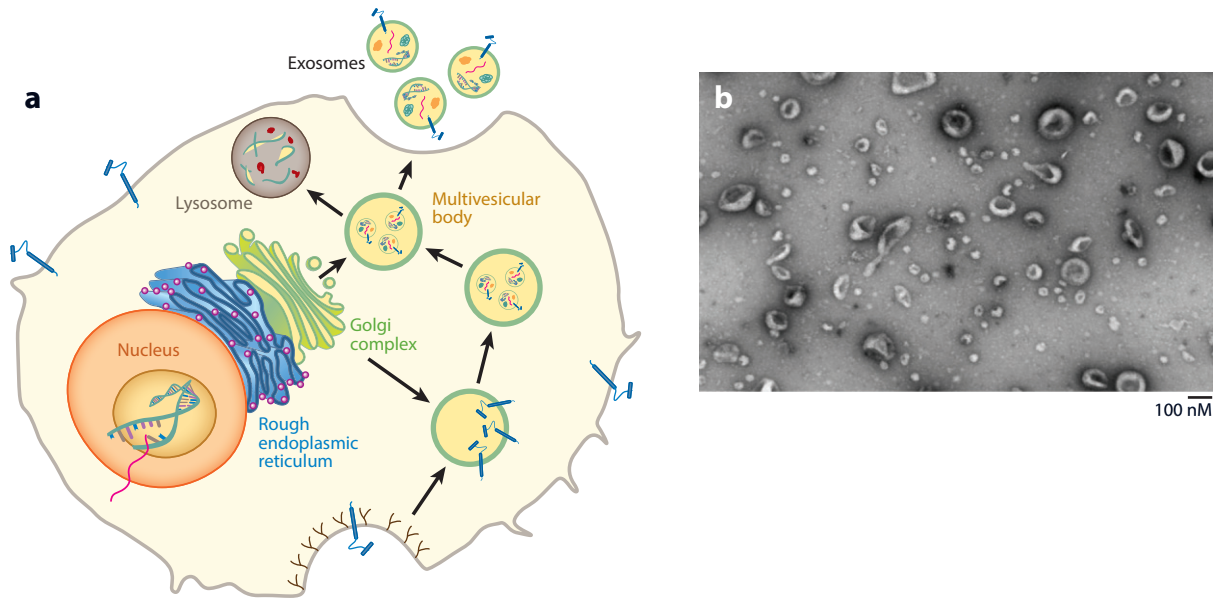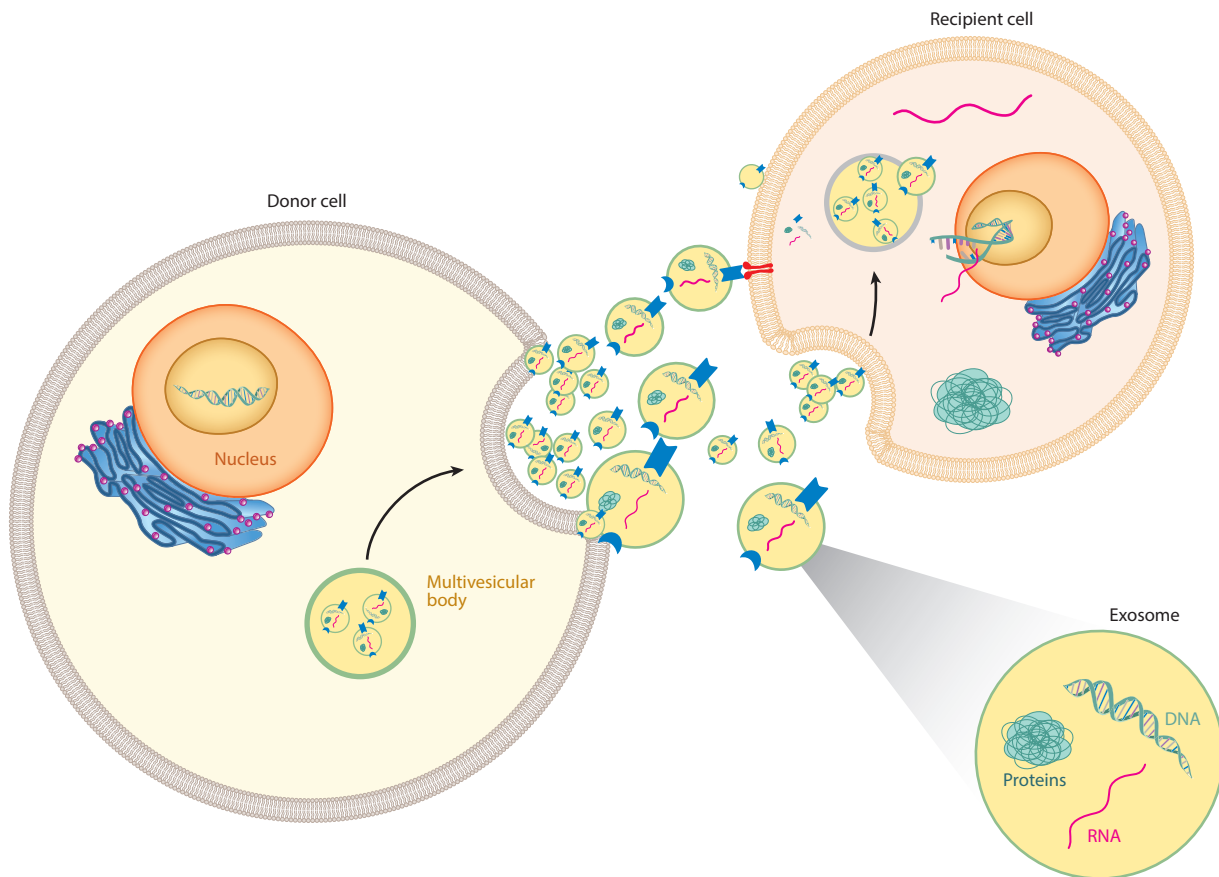
Figure 1

(*a*) Exosomal content and biogenesis in the late-endosomal compartment. Exosomes are incorporated into multivesicular bodies and can be targeted for lysosomal degradation or can fuse with the plasma membrane for externalization. (*b*) Negative staining electron microscopy of mesenchymal stem cell exosomes. Their size ranges from 30 to 100 nM in diameter, and they have a typical cup-shaped appearance.

from the erythrocyte (16–18). Subsequently, it was realized that many cell types—including B and T lymphocytes, dendritic cells, mast cells, intestinal epithelial cells, neurons, tumor cells, and MSCs—release exosomes (19–25). Exosomes are also found in physiological fluids such as urine, plasma, cerebrospinal fluid, and human milk and in exudates (26–32). A better understanding of the biogenesis of exosomes has led to a better understanding of their function. Exosomes originate from internal budding of the plasma membrane during endocytic internalization (**Figure 1*a***). The early endosome matures through a process that includes an interaction with the Golgi complex to form late endosomes. The bilayer membrane of late endosomes gives rise to intraluminal vesicles, i.e., exosomes, that are contained within MVBs and that have incorporated recycled proteins from coated pits in the cellular membrane; proteins directly from the Golgi complex; and mRNA, miRNA, and DNA. The MVBs can fuse with the plasma membrane to release exosomes through exocytosis or can be sent to lysosomes for degradation (33). Therefore, exosomes differ from ectosomes or apoptotic bodies that are released from the cell as a result of a direct budding process of the plasma membrane. The structure of exosomes by transmission electron microscopy appears cup shaped and is likely a result of the processing and fixation that result in the collapse of these circular molecules (**Figure 1*b***). Quickly frozen exosomes analyzed by cryo-electron microscopy have a perfectly round shape (34). Typically, exosomes have a diameter of 30–100 nm and a density of 1.13–1.19 g/mL and are isolated through sucrose cushion or density gradient by ultracentrifugation at 100,000g (35).

Secretion of exosomes occurs by fusion of the MVB and the cell membrane; such fusion depends on several Rab GTPase proteins (36). Exosomes released from cells can act in a paracrine or even an endocrine manner to modify the behavior of adjacent cells or distant cells. The transfer of signals to cells occurs through direct contact between the exosome and the cell membrane, either by cell
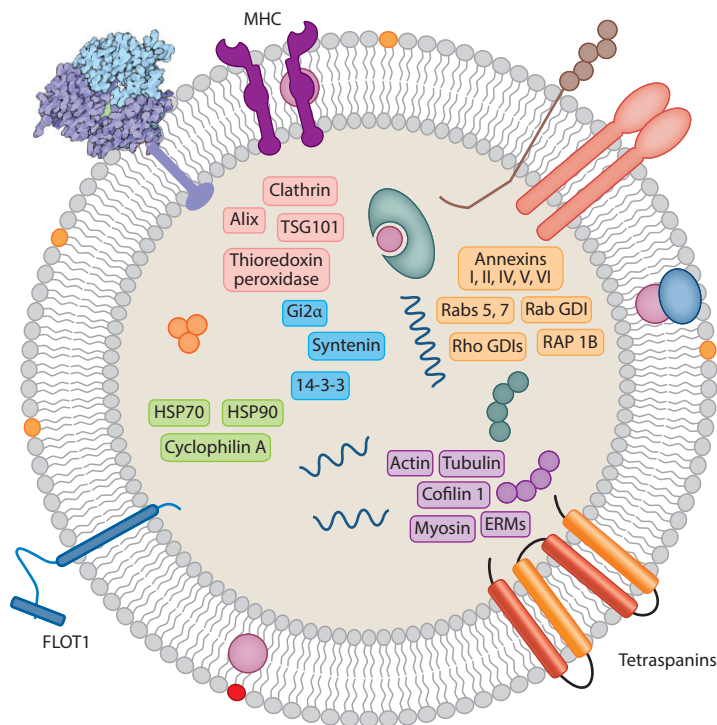
Figure 2

Exosomes are secreted through fusion of multivesicular bodies with the plasma membrane and carry a cargo that includes proteins, DNA, and RNA. Intracellular uptake of exosomes occurs via endocytosis, membrane fusion, or receptor-mediated internalization.

surface receptors, by fusion of the two membranes, or by endocytosis (**Figure 2**). A combination of specific cell surface molecules on exosomes is critical for cell targeting and cell adhesion. Many exosomes contain major histocompatibility complex (MHC) class I and class II molecules (37, 38) that are involved in antigen binding and presentation. In addition, proteins like integrins and annexins play an important role in cell adhesion, as do tetraspanins, which can direct targeting to specific cells such as endothelial cells to promote angiogenesis and vasculogenesis (39). The long-range targeting and tissue uptake of exosomes and their stability in the circulation or in other biological fluids make exosomes attractive as a biomarker and as a therapeutic vehicle in the treatment of disease.

MOLECULAR COMPOSITION OF EXOSOMES

MHC: major histocompatibility complex

Exosomes carry a unique cargo of proteins, lipids, and RNAs that can be distinct from those of the cell of origin. Because exosomes originate from endosomes, they contain proteins, such as annexins and flotillin, that are important for transport and fusion; tetraspanins involved in cell targeting; and other proteins, such as Alix and TSG101, that are involved in their biogenesis from MVBs.

Figure 3

Typical structure and content of exosomes. Exosomes are surrounded by a phospholipid bilayer and contain proteins, such as annexins, that are important for transport; tetraspanins for cell targeting; and other proteins, such as Alix and TSG101, that are involved in exosomal biogenesis from endosomes. Abbreviations: ERMs, ezrin/radixin/moesin proteins; FLOT1, flotillin 1; HSP, heat shock protein; MHC, major histocompatibility complex; Rab GDI, Rab GDP-dissociation inhibitor; RAP 1B, Ras-related protein 1B; TSG101, tumor susceptibility gene 101.

In addition, endosomes contain proteins involved in lipid metabolism (40) (**Figure 3**). High-throughput proteomic analyses of exosomes have revealed the presence of extracellular matrix and cell surface proteins such as collagens, integrins, and galectin; cell surface receptors such as platelet-derived growth factor receptor B and epidermal growth factor receptor (EGFR); and signaling molecules and intracellular cytoskeletal components plus metabolic enzymes and G proteins (41, 42). The lipid composition of exosomes is not fully characterized, but it is evident that, in contrast to their cells of origin, exosomes are enriched in cholesterol, ceramide, phosphoglycerides, and long and saturated fatty-acyl chains, all of which may provide structural stability (reviewed in Reference 43). Exosomes do not carry the soluble form of prostaglandins but rather carry prostaglandins bound to the exosomal membrane for delivery to target cells with potentially enhanced biological activity (44). An active area of discovery is focused on the RNA content of exosomes because they have been reported to carry functional mRNAs and miRNAs that can be transferred between cells (45). As is the case for other moieties, the RNA content of exosomes is a subset of the cellular RNA or, in certain cases, may be completely distinct or tissue specific, whereas other RNAs are ubiquitous among all exosomes regardless of their cell of origin due to their specific targeting into MVBs during their biogenesis (46). In addition to containing RNA, microvesicles released by tumors contain single-stranded DNA, genomic DNA, cDNA, and transposable elements (47).

EXOSOMES AS MESSENGERS OF INTERCELLULAR COMMUNICATION

Once secreted from the cell, exosomes can deliver their cargo to adjacent or distant cells and can modify the target cell's gene expression, signaling, and overall function. They are engulfed by different cells, including macrophages, endothelial cells, and tumor cells (48–50). Exosomes play a signaling role in the immune system whereby immature dendritic cells transfer MHC peptide molecules through exosomes to other dendritic cells to activate the immune response (51). Selective loading of specific mRNA and miRNA into exosomes can be a vector of genetic exchange and communication between cells (45). Specifically, the horizontal transfer of miRNA from activated T cells to antigen-presenting cells points to an important role for exosomal transport in the immune synapse (52). The RNA species transferred via exosomes can be transcribed into cDNA or translated within the recipient cell. Exosomes from cultured glioblastoma tumor cells contain several angiogenic peptides and RNA that can be transferred and translated, respectively, into recipient brain microvascular endothelial cells, conferring proangiogenic properties and thus disseminating malignancy (50). Moreover, a tumor-specific mRNA, EGFRvIII, is carried in serum exosomes of glioblastoma patients (50). Further studies showed that exosomes from mast cells contain both mRNA and miRNA and transfer mRNA to recipient cells for translation into proteins (45). These important findings point to the multifaceted role of exosomes as biomarkers of disease (e.g., malignancy), as mediators of signals between cells that alter their function, and as potential vehicles for drug delivery given exosomes' ease of isolation, of in vitro modulation to express a protein/RNA of interest, and of in vivo administration (53). An advantage of exosomes as vectors and mediators of intercellular communication is that the message can be targeted to multiple cells and multiple locations. The transfer of miRNA allows for rapid alterations in gene expression and control of critical processes such as growth, differentiation, cell survival, angiogenesis, and immunomodulation.

The therapeutic potential of exosomes in cardiopulmonary diseases is just beginning to be explored, with promising results in various preclinical models. Human cardiomyocytes were reported to produce exosome-like vesicles, and in a mouse model of ischemic injury, exosomes having a cup-shaped appearance and an appropriate diameter of 30–100 nM were detected in the intracellular space of border zone cardiomyocytes (54). In addition to modulating inflammation and oxidative stress to protect cells from hypoxia and ischemia/reperfusion (I/R), additional processes such as microvascular angiogenesis are also required to counter tissue ischemia. Therefore, in the context of ischemic heart disease, angiogenesis and collateral vessel formation are critical pathways to target for therapy. Angiogenesis requires communication between endothelial cells, stromal cells, and potentially progenitor/stem cells. Exosomes, among other signaling moieties, may play a key role in this interaction because transfer of exosomes between cells can regulate biological signals required for angiogenesis. Indeed, endothelial cell–derived exosomes stimulated endothelial cell migration and angiogenesis in target endothelial cells via *miR-214*-dependent pathways (55). Furthermore, exosomes isolated from conditioned media (CM) of CD34^{+} stem cells have proangiogenic activity in vitro and in vivo (56). In contrast to the case for unmodified control cells, Mackie et al. (57) reported that CD34^{+} cells modified to overexpress sonic hedgehog (Shh), a factor with angiogenic properties, protected against ventricular dilation and cardiac dysfunction post–myocardial infarction in a mouse model. These investigators demonstrated that CD34^{+} cells selectively shuttled 25% of their secreted Shh protein into exosomes and that exosomes transport and deliver Shh to other cells. Interestingly, intramyocardial injection of recombinant Shh protein had no protective effect, pointing to the delivery system imparted by the exosome as being critical in conferring therapeutic efficacy. One could speculate that selective packaging

of the Shh protein into exosomes protects it from extracellular proteases, thereby prolonging its half-life and enhancing its biological activity. Another example of cell-to-cell signaling orchestrated by exosomes involves the transfer of miRNA. In this case, extracellular vesicles secreted by endothelial cells exposed to shear stress are enriched in *miR-143/145* and regulate target gene expression in cocultured smooth muscle cells to prevent their dedifferentiation (58). Shear stress induces the level of expression of *miR-143/145* through the enhanced activity of the transcription factor Krüpel-like factor 2 (KLF2), and KLF2-transduced endothelial cells release vesicles with a selective enrichment in *miR-143/145*. When exosomes derived from endothelial cells overexpressing KLF2, but not those from mock-transfected controls, were delivered into ApoE$^{-/-}$ mice, the mice were protected against high-fat diet–induced atherosclerosis, demonstrating that exosome-mediated transfer of miRNAs such as *miR-143/145* is not just an in vitro phenomenon but also occurs in vivo to reduce atherosclerotic lesion formation (58).

Depending on the cellular source and disease context, exosomes can be vectors of anti- or proinflammatory signals and can promote resolution or exacerbation of disease. For instance, exosomes isolated from the bronchoalveolar lavage fluid of patients with sarcoidosis stimulated IL-8 production in epithelial cells (59). When exposed to compressive stress simulating the stress generated during bronchoconstriction in asthma, bronchial epithelial cells produce exosomes bearing tissue factor (60). Tissue factor carried in exosomes may thus be released from epithelial cells to promote the subepithelial angiogenesis that has been associated with the reduced lung function underlying the asthma phenotype. Also, the Notch ligand delta-like 4, which is critical for angiogenesis, accumulates within endothelial cell exosomes or tumor exosomes and regulates Notch signaling at a distance to enhance angiogenesis (61). The truncated oncogenic form of EGFR highly expressed in aggressive glioblastoma multiforme tumor cells can be transferred via microvesicles into other cells, leading to enhanced expression of its target genes and propagating the oncogenic transformed phenotype (62). In the central nervous system, proteins such as amyloid-β peptide may be transferred via exosomes and thus exacerbate and extend neuronal injury (63, 64). These examples demonstrate the diverse biological functions of exosomes as vectors of signaling molecules that alter pathways and processes affecting cell function in normal physiology and disease.

EXOSOMES AS VECTORS OF STEM CELL THERAPY

Stem cell therapies exhibit great potential for the treatment of various diseases. The therapeutic effects of adult stem cells remain to be fully elucidated. However, exosomes carrying a cargo of packaged signals in the form of RNA, miRNA, or protein, among others, may be a key mechanism and the vehicle of their action to reduce inflammation, alter cellular signaling, and result in tissue repair. MSC therapy, for example, is being tested in animal models and in multiple clinical trials for the treatment of disorders including acute lung injury, myocardial infarction, diabetes, sepsis, graft-versus-host disease, and hepatic and acute renal failure (65–72). The therapeutic effect has been recapitulated in several preclinical models with administration of cell-free media from MSC cultures that, among other moieties, contain exosomes. In 2005, using a myocardial infarction model, Gnecchi et al. (73) first proposed that the effect of genetically engineered MSCs takes place in less than 72 h, before any regeneration can take place, and showed that CM from MSCs (MSC-CM) cultured in vitro have efficacy comparable to that of whole-cell transplantation in preventing ventricular remodeling. In the hyperoxia-induced lung injury mouse model, bone marrow MSC-CM were more effective than cells in preventing vascular changes associated with pulmonary hypertension (PH) (2). A single dose of bone marrow MSC-CM shortly after hyperoxic exposure abrogated inflammatory lung infiltration and suppressed right ventricular hypertrophy (RVH) and lung vascular remodeling in hyperoxia-treated neonatal mice.

PH: pulmonary hypertension

RVH: right ventricular hypertrophy

In the same model of established bronchopulmonary dysplasia (BPD), by delivering MSC-CM, Hansmann et al. (74) reversed the hyperoxia-induced parenchymal fibrosis and peripheral pulmonary artery (PA) devascularization, partially reversed alveolar injury, and normalized lung function. In addition, MSC-CM fully reversed the RVH and attenuated the peripheral PA muscularization associated with hyperoxia-induced BPD. Further support for the anti-inflammatory mediators of MSC secretome came from Lee et al. (25), who showed that factors secreted by MSCs prevent hypoxia-induced pulmonary inflammation and pulmonary vascular remodeling. Pretreatment of hypoxia-exposed mice with bone marrow MSC-CM prevented early lung inflammation with suppression of inflammatory cytokines such as monocyte chemoattractant protein-1, with inhibition of macrophage accumulation, and with prevention of later hypoxic PH. Using an in vitro system, Liang et al. (75) showed that bone marrow MSC-CM can inhibit the proliferation of PA smooth muscle cells, an effect not observed with fibroblast-CM. This observation demonstrated that, in addition to the immunomodulatory effect of MSCs on lung inflammation, MSCs release factors with antiproliferative activity on smooth muscle cells, directly inhibiting vascular remodeling. Treatment with allogeneic human MSCs or their CM administered 1 h following endotoxin-induced lung injury reduced extravascular lung water, improved lung endothelial barrier permeability, and restored alveolar fluid clearance (76). Thus, the recurrent theme in preclinical studies is that the therapeutic action of MSC transplantation is mediated by factors that are released in the MSC medium and that have a paracrine effect on the lung. The reported findings are consistent with the notion that the therapeutic effects observed with these treatments may be mediated by exosomes present in the CM, although the above studies did not specifically investigate this possibility.

In proteomic analysis of the MSC secretome, many immunomodulators and matrix components, as well as proteins commonly associated with extracellular vesicles derived from different cell types, were detected. Many of these proteins, including CD63, CD81, moesin, Alix, TSG101, and heat shock protein 70 (HSP70), are enriched in exosomes (**Figure 3**). Research on the effect of exosomes in vitro has focused mostly on their interaction with the immune system, including dendritic cell maturation and Treg and B cell responses (13, 77, 78). Exosomes isolated from cardiac progenitor cells in culture protect from I/R injury when injected into the myocardium (79). Exosomes derived from dendritic cells modulate immune responses and inhibit rejection from heart transplantation (80). Lee et al. (25) demonstrated that exosomes mediate the cytoprotective effect of bone marrow MSCs in hypoxia-induced PH. In this model, administration of MSC-derived exosomes, identified through widely accepted exosomal markers and visualized by electron microscopy, protected against the elevation of right ventricular systolic pressure and against the development of RVH after 3 weeks of hypoxic exposure, whereas microvesicle-depleted CM had no effect. Exosomal treatment also abrogated early hypoxic macrophage influx and downregulated hypoxia-activated inflammatory pathways, thus mediating the anti-inflammatory properties of MSCs. Researchers have isolated and characterized exosomes from MSCs originating in almost all sources, including from human embryonic stem cells, and the MSC exosome has been proposed as the alternative therapeutic vehicle for MSCs in many disease models (81–84). Such models of disease include a myocardial I/R model in which MSC exosomes decreased infarct size and ameliorated reperfusion injury (24); a cisplatin-induced acute kidney injury model in the rat in which adipose tissue MSC exosomes ameliorated oxidative stress and cell apoptosis, promoting cell proliferation in vivo and in vitro (14, 85); and an acute kidney injury model in which MSCs activated a proliferative program in tubule cells (86).

Exosomes released by MSCs may activate kinase pathways that are critical for ischemic preconditioning by increasing extracellular ATP levels and decreasing oxidative stress and inflammation (87–89). In a mouse model of hind limb ischemia, microvesicles from endothelial progenitor cells

improved neovascularization (90) by the transfer of miRNA or mRNA (91). The protection was lost with RNase treatment or depletion of *miR-126* and *miR-296*. The same mechanism is thought to protect the kidney from I/R through the miRNA-dependent reprogramming of renal resident cells imparted by the delivery of microvesicles released by endothelial progenitor cells or MSCs (92, 93).

EXOSOMES AS BIOMARKERS AND VEHICLES OF DRUG DELIVERY

Exosomes are also being recognized as critical mediators of health and disease and may be biomarkers of specific conditions. Exosomes isolated from bronchoalveolar lavage fluid of patients with asthma contain unique miRNA species that differ from those of control patients (94). Cancer cell–derived exosomes contain several components, including miRNAs, that are distinct from those of nonmalignant cells, and such exosomes can be sentinels of disease. Sera from patients with globlastoma multiforme and sera from controls differ in exosomal RNA content (95). Exosome-derived miRNA and proteomic profiling may be used as diagnostic indicators of cancer, such as prostate, ovarian, and lung cancer (96–100). Proteins in urinary exosomes reflect kidney injury (101), and plasma prostasome microvesicles secreted by prostate acinar cells can be biomarkers of prostate cancer (102).

The recognized function of exosomes as vehicles of intercellular communication has been further explored in the delivery of therapeutic signals such as small interfering RNAs (siRNAs) for drug delivery, as was shown for the brain (53). The development of an exosome-based drug delivery system may improve targeting of specific cargos to treat disease. Types of therapeutic cargo include interfering RNAs, such as siRNAs and miRNAs (103). Exosome-mediated transfer of *miR-133b* from MSCs to neural cells stimulated neurite growth (104). Also, MSC-derived exosomes that express *miR-146b* inhibited glioma growth (105). Because MSCs are efficient and high producers of exosomes, they can be engineered to overexpress specific miRNAs that are incorporated into the exosomal cargo and delivered in vivo for the specific targeting of disease (84). In addition to the therapeutic transfer of interfering RNAs, chemotherapeutics such as doxorubicin have been loaded onto exosomes and used to inhibit growth of breast and colon cancers (106, 107). The use of exosomes as drug delivery systems is just beginning to be explored.

CONCLUSIONS AND FUTURE PROSPECTS

The nascent era of the clinical application of exosomes is rapidly exploding. Mounting evidence suggests that exosomes are released under physiological and pathophysiological conditions from various tissues and carry signaling molecules for intercellular communication and regulation of organ function. Exosomes in peripheral blood likely reflect the state of the organ of exosomal origin and are thus biomarkers of disease and prognostic indicators of the response to treatment or progression of disease. Exosomes from stem cells such as MSCs have promising potential as therapeutic vehicles of epigenetic immunomodulatory signals to treat cardiovascular, pulmonary, renal, and neurological injuries. There is still a great deal to be learned about the control of exosome generation and modification by the parental cells and about the quality and function of the released exosomes; we also need a detailed characterization of the different types of extracellular vesicles released and their role in transferring signals that may propagate or limit disease. Exosomes have the potential to replace cellular therapy and to alleviate the theoretical concerns of neoplastic transformation from blood-, tissue-, or bone marrow–derived stem cell transplantation. Loaded with therapeutic contents, exosomes may ultimately be designed and engineered for highly selective and effective targeted treatment of disease.

DISCLOSURE STATEMENT

ACKNOWLEDGMENTS

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