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REVIEW ARTICLE

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Biology and therapeutic potential of mesenchymal stem cell-derived exosomes

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Abstract

Mesenchymal stem cells (MSC) are multipotent stromal cells with the potential to differentiate into several cell types. MSC-based therapy has emerged as a promising strategy for various diseases. Accumulating evidence suggests that the paracrine effects of MSC are partially exerted by the secretion of soluble factors, in particular exosomes. MSC-derived exosomes are involved in intercellular communication through transfer of proteins, RNA, DNA and bioactive lipids, which might constitute a novel intercellular communication mode. This review illustrates the current knowledge on the composition and biological functions as well as the therapeutic potential of MSC-derived exosomes in cancer, with a focus on clinical translation opportunities.

KEYWORDS

cancer, exosomes, immunomodulation, mesenchymal stem cells, MSC-derived exosomes

1 | INTRODUCTION

Mesenchymal stem cells (MSC) are a heterogeneous subset of multipotent precursors that exist in many tissues and can differentiate into several types of cells.[1] They not only possess self–renewal capacity to undergo numerous cell divisions but also exert anti–inflammatory and immunosuppressive properties via the direct interaction with a series of immune cells.[2] There has been growing interest in MSC secretome, including cytokines, chemokines, growth factors and extracellular vesicles (EV). Generally, ectosomes and exosomes are two distinct subtypes of EV.[3] Ectosomes are vesicles typically 50 nm to 1 μm in diameter generated by direct budding with plasma membrane; in contrast, exosomes, ranging in size from 40 to 160 nm, originate from the endosomal compartments, and are ubiquitous in body fluid.[4] Exosomes are composed of a lipid bilayer membrane and contain all molecular constituents, including DNA, RNA, lipids and proteins.[5] Notably, exosomes have distinct abilities to influence many activities through exchanging bioactive components with neighboring cells and transporting genetic contents towards distal cell subpopulations, which likely reflect the molecular and genetic profiles of the parent cells.[6] MSC-derived exosomes function as potent therapeutic vehicles; they also appear to recapitulate the broad therapeutic effects that have been described in MSC themselves.[7] A comparison of MSC and MSC-derived exosomes is presented in Table 1.

1.1 | Biogenesis and secretion of exosomes

Exosome generation involves the inward budding of the plasma membrane and formation of intracellular multivesicular bodies (MVB), followed by further invagination of endosomal membranes, which was dependent on their physiological or pathological status.[5]

Abbreviations: mtDNA, Mitochondrial DNA; MVBs, Intracellular multivesicular bodies; Ndfip1, Nedd4 Family Interacting Protein 1; PD-L1, Programmed death-ligand 1; PEG2, Prostaglandin E2; PGE2, Prostaglandin E2; P-gp, P-glycoprotein; PLK-1, Serine/threonine-protein kinase; ROS, Reactive oxygen species; rRNA, Ribosomal RNA; siRNA, Small-interfering RNA; ssDNA, Single-stranded DNA; TASP6, Tumor suppressor activated pathway-6; TEMs, Tetraspanin-enriched microdomains; Th17, T helper 17; TME, Tumor microenvironment; TRAIL, TNF-related apoptosis-inducing ligand; tRNA, Transfer RNA; UCMSC, Human umbilical cord mesenchymal stem cell.

TABLE 1 Comparison between mesenchymal stem cells (MSC) and MSC-derived exosomes

	MSC	MSC-derived exosome	Reference
Therapeutic effects	Regeneration medicine, immunomodulation and cancer	Retain the therapeutic effects of MSC	4,115,116
Drug carriers	Only the drugs that could be internalized by MSC, such as paclitaxel and gemcitabine, but not pemetrexed. Transfection efficiency is a primary limitation for the nucleic acid delivery	Promising carriers for all the drugs; nucleic acid with increased efficiency compared to MSC	103-106,115
Target tissue	Injury site, inflammation and cancer	Injury site, inflammation and cancer	6,117
Immunogenic	Can induce allogenic immune rejection	Considered to be non–immunogenic	99,118
Clinical settings	Both in preclinical and clinical settings	Both in preclinical and clinical settings	119,120
Production	Undergo senescence after only a few passages, expensive to have a large-scale production	No senescence and easy to generate a large-scale production for clinic application	99,113

The uncovered mechanisms for MVB formation as well as exosome release will be briefly summarized.

1.1.1 | Sorting complex required for transport machinery

The endosomal sorting complex required for transport (ESCRT) machinery is critical for the regulation of exosome biogenesis. Four complexes (ESCRT-0, -I, -II and -III) and the associated accessory proteins (ALIX, VPS4 and VTA1) were identified and involved in recognizing the targeting proteins to exosomes and orchestrating a discrete step for MVB vesicle formation.[8] All the accessory proteins allow dissociation and recycling of the ESCRT machinery.[9]

1.1.2 | Endosomal sorting complex required for transport-independent mechanisms

Recent reports also suggest that ESCRT independence is important during exosome biogenesis, including ceramides and tetraspanin proteins (eg CD81, CD82 and CD9), which are responsible for the sorting and loading of various cargoes to exosomes.[8] Tetraspanins are ubiquitous transmembrane proteins enriched in exosomes, which can directly interact with various types of receptors in the plasma membrane, and participate in the formation of the microdomains that bud.[10] However, both ESCRT-dependent and ESCRT-independent pathways work synergistically, and different subpopulations of exosomes depend on different machineries.

1.1.3 | Exosome secretion regulation

It is well known that the RAB family of small GTPase proteins, such as Rab27a, Rab27b, Rab35 and Rab7, has been implicated in transferring vesicles between intracellular compartments, and has also been implicated in intracellular vesicular trafficking to the plasma membrane for exosome release.[11] Furthermore, the biogenesis and secretion of exosomes released from MSC also depend on external stimuli. For instance, MSC undergoing hypoxia or inflammation could influence the biomolecule packaging process into exosomes and affect their functional properties, such as the hypoxia-conditioned MSC-derived exosomes exerting more angiogenic activity than normoxic exosomes.[12] The Wnt and mTOR pathways are considered as "master regulators" involved in the enhancement of self-renewal in MSC by increasing β-catenin expression, and are also required for exosome secretion.[13-15] In summary, the process of exosome biogenesis and secretion is complex but varies depending on cell type and cellular homeostasis, which is also influenced by the surrounding microenvironment. Figure 1 illustrates the intracellular composition, release and uptake of MSC exosomes.

1.2 | Contents of mesenchymal stem cell-derived exosomes

Exosome associated plasma membranes are composed of several kinds of lipids, including hexosylceramides, cholesterol, phosphatidylserine, sphingomyelin and saturated fatty acids.[16] Exosomes are also enriched in various proteins with multiple functions, including the proteins associated with exosome biogenesis (eg ESCRT complex, ALIX, TSG101 and syntenin), membrane transporter and fusion proteins (eg Rab GTPases and annexins).[17] In addition, an evolutionary conserved set of proteins are also packaged into exosomes during biogenesis, including tetraspanins (eg CD9, CD63, CD81 and CD82), integrins, major histocompatibility complex (MHC) class II proteins and heat shock proteins.[4] Exosomes appear to be as diverse as the original cell types and may serve as a critical determinant in their function. Specifically, MSC-derived exosomes not only express the common surface markers like CD81 and CD9 but also express CD73, CD44 and CD90, which are characteristic of MSC.[18] Characterization of the contents based on the proteomics of bone marrow mesenchymal stem cell (BMSC)-derived exosomes identified 730 functional proteins, among which are proteins controlling cell growth, proliferation, adhesion, migration and morphogenesis capacities of MSC.[19] Comprehensive proteomic analysis from human primed MSC-secreted exosomes demonstrated that higher fractions of specific extracellular-associated proteins were packaged into

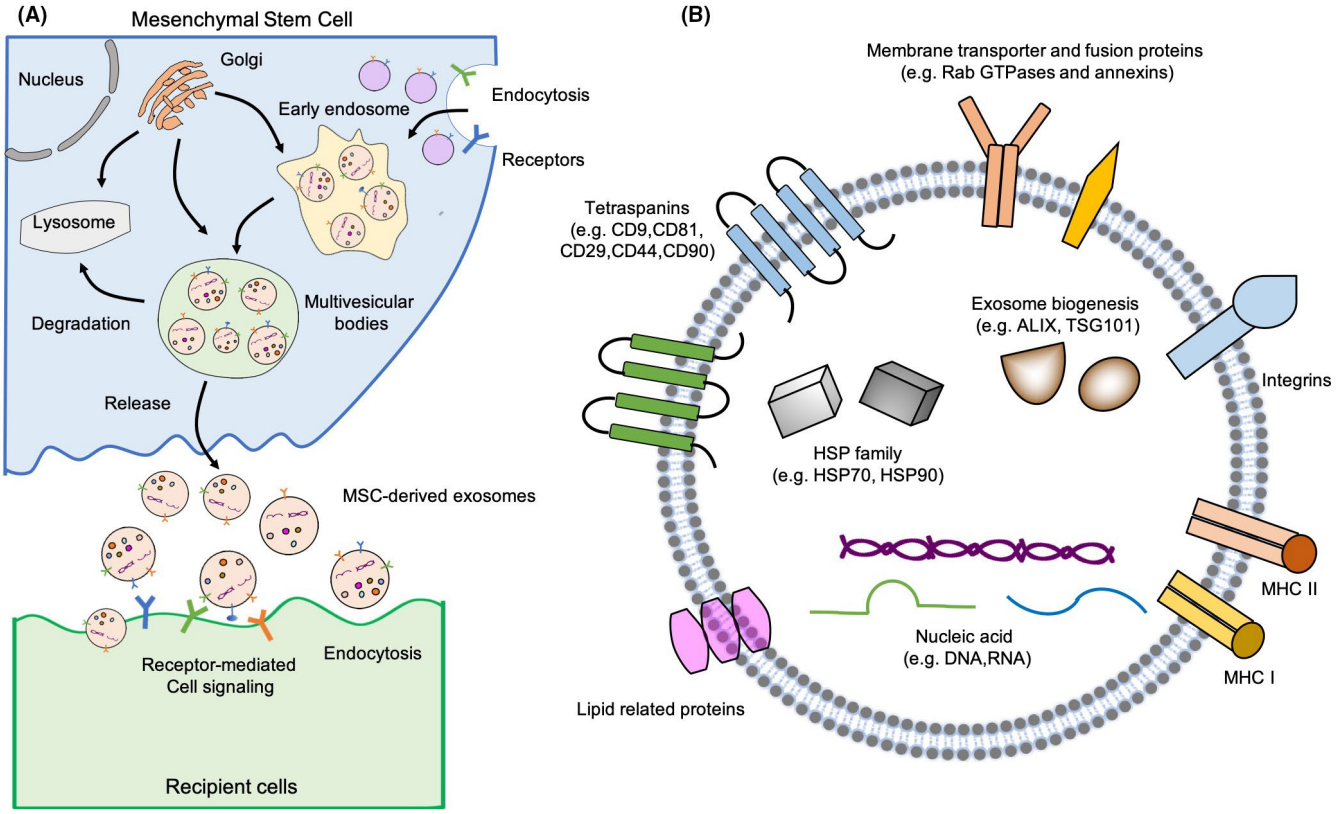

FIGURE 1 Biogenesis, secretion and molecular composition of the mesenchymal stem cell (MSC)-derived exosomes. A, Exosome biogenesis, secretion and uptake by recipient cells. First, multiple proteins were internalized from the cell surface or transported from the Golgi; nucleic acids were endocytosed and delivered into the endosomes, followed by the formation of intracellular multivesicular bodies (MVB). Further invagination of late endosomal membranes ultimately results in the secretion of exosomes. MVBs then either taken up by lysosomes for degradation or fused with plasma membrane for releasing all their cagos into extracellular spaces. The secreted exosomes could be taken up by recipient cells through either direct fusion of their membrane or endocytosis. B, MSC-derived exosomes are enriched in various proteins with multiple functions, such as the biogenesis-related proteins (eg ALIX, TSG101), common surface markers (eg CD9, CD81, CD29, CD44 and CD90), membrane transporter and fusion proteins (eg Rab GTPases and annexins), integrins, heat shock proteins (eg HSP60, HSP70 and HSP90) and MHC class I and II proteins

exosomes compared with their cells of origin.[20] All of the findings provided a molecular basis for the distinct functional properties of MSC-derived exosomes, such as the induction of mitosis and potentiation of growth factor secretions.[20]

Aside from selected proteins, exosomes are also rich in RNA cargoes and make up an important fraction of exosomal contents.[21] RNA packaging into exosomes is not random but, rather, specific, indicating preferential accumulation of certain RNA within exosomes that can finally be incorporated into recipient cells. In other words, the abundant specific mRNA in exosomes may partly reveal a special "zip-code" sequence.[22] The RNA present in MSC-derived exomes are usually involved in the regulation of cell survival and differentiation but also related to immune system modulation.[23] A system view of the miRNA of MSC-derived exosomes identified that the top 23 miRNA were capable of angiogenesis promotion, tissue remodeling and cardiomyocyte proliferation.[23] Comprehensive information on the complete RNA content between BMSC and adipose-MSC (AMSC)-derived exosomes revealed that both were similar in RNA composition but strikingly different in tRNA species that were associated with the MSC differentiation status.[24] The comparison of exosome mRNA from BMSC and the human liver stem cells (HLSC) shows that BMSC-derived exosomes contained a specific number of cellular mRNA, which were related to differentiation into the mesenchymal phenotype, cell transcription and proliferation regulation.[25] However, a specific subset of mRNA found in HLSC-derived exosomes was associated with the liver cell metabolism and proliferation.[26]

In addition to protein and RNA species, several types of DNA have been detected in exosomes.[5,27,28] Previous studies had identified large fragments of double-stranded DNA (dsDNA) (>10 kb), which indicated that mutations in *KRAS* and *p53* can be detected using genomic DNA from exosome DNA for pancreatic cancer prediction.[5] The transfer of exosome DNA into target cells was also reported to exert multiple biological activity in recipient cells transiently.[29] Tumor-derived exosomes contain immunostimulatory DNA, which could be recognized by cytoplasmic DNA receptors in activated dendritic cells (DC) through the induction of the STING-dependent pathway and drove anti–tumor immunity.[29] The horizontal DNA gene transfer by exosomes released from BMSC was identified. It carries high-molecular DNA, which was mainly associated with the outer exosome membrane for the exchange of genetic

information mediating the intercellular communication during cell evolution and development.[30] In addition, exosomes were able to package and transfer their mitochondrial DNA to breast cancer cells, leading to restoration of metabolic activity and increased self-renewal potential.[27]

1.3 | Functions of mesenchymal stem cell-derived exosomes in cancer

Recently, much interest has shifted to the field of cancer therapy as MSC-derived exosomes have demonstrated a potential role in cancer progression. Cancer cells are surrounded by a complex tumor microenvironment (TME), which is a highly heterogenous and dynamic intricate ecosystem that consists of different cell types. The crosstalk of MSC-derived exosomes in TME seems to be pivotal for cancer progression.

1.3.1 | Tumor growth

Accumulating evidence has linked the transfer of tumor-associated miRNA enriched in MSC-derived exosomes with the promotion or inhibition of cancer cell proliferation. The function of BMSC-derived exosomes has been widely investigated. It was shown that the enriched miR-222-3p in exosomes could directly target IRF2 that negatively regulated IRF2/INPP4B signaling, which contributed to the suppression of the tumor growth in acute myeloid leukemia (AML) cells.[31] Exosomes also enable the delivery of miR101-3p and lead to the inhibition of oral cancer progression via targeting COL10A1.[32] Along with BMSC-derived exosomes, several groups have also reported that exosomes isolated from human umbilical cord mesenchymal stem cells (hUCMSC) possess tumoricidal properties themselves.[33] They could inhibit the growth of human lymphoma cells by blocking the cell cycle, induction of superoxide dismutase and hydrogen peroxide activity, as well as reduction of glutathione peroxidase.[33] Similarly, AMSC-derived exosomes demonstrated a suppressive effect through the delivery of miR-145, leading to the induction of apoptosis via the activation of the caspase-3/7 pathway and reduction of Bcl-xL activity in prostate cancer.[34] It also exerted inhibitory effects on human ovarian cancer cells through cell cycle arrest, activation of mitochondria-mediated apoptosis signaling, as well as downregulation of the anti–apoptotic protein BCL-2, which partly resulted from a rich population of suppressor miRNA.[35] Fonsato et al showed that the transfer of several miRNA (eg miR451, miR223, miR24, miR125b miR31 and miR122) by exosomes into target HepG2 cells could suppress tumor cell proliferation and induce apoptosis, which also exerted potential anti–tumor activity in vivo.[36]

Conversely, the role of exosomes in the tumor promoting effect was also reported. It has been shown that BMSC-derived exosomes exert a tumor promotion effect through the activation of extracellular signal-regulated kinase 1/2 (ERK1/2) signaling in gastric cancer.[37] It has also been demonstrated that exosomes could facilitate multiple myeloma disease progression through transferring tumor suppressor miR-15a and result in the alteration of cytokines and adhesion molecules secretion.[38] In addition, the transfer of miR-410 from hUCMSC-derived exosomes promoted lung adenocarcinoma cell growth through direct inhibition of *PTEN* expression.[39] Sun et al revealed that hUCMSC-derived exosomes exerted a protective role from cell stress and decreased tumor cell apoptosis, indicating a possible protective role from chemotherapy of tumor cells.[40] Yang et al also demonstrated that the incubation of hUCMSC with human breast cells promoted the exchange of biological content through exosomes, including matrix metalloproteinase-2 (MMP-2) and ecto-5′-nucleotidase acquisition, which was associated with the increased tumor heterogeneity via the alteration of cellular functionalities and TME.[41]

1.3.2 | Angiogenesis

It is well documented that exosomes derived from various cell types have the potential to deliver complex information to endothelial cells, which are implicated in the angiogenetic signaling, exerting either a pro–angiogenic or an anti–angiogenic effect.[42,43] So far, the limited studies investigating the functions of MSC-derived exosomes on angiogenesis have yielded contradictory results. Considering their pro–angiogenic properties, it was demonstrated that BMSC-derived exosomes could enhance the expression of CXCR4 in human gastric carcinoma and colon cancer cells and promote tumor growth.[37] Gong et al revealed that exosomes isolated from conditioned medium of BMSC could transfer several miRNA to HUVEC and promote angiogenesis in vivo.[42] Activation of Wnt signaling plays a pivotal role in the pro–angiogenic activity of exosomes isolated from BMSC, which could transport Wnt3a exteriorly to stimulate fibroblast proliferation and enhance angiogenesis.[44] AMSC-derived exosomes could significantly promote angiogenesis by transferring miR-125a to endothelial cells through the inhibition of DLL4 expression.[45]

The anti–vascular remodeling ability was also observed in BMSC-derived exosomes by targeting hypoxia-inducible factor-1 alpha (HIF-1α) and Smad2, which contribute to the inhibition of angiogenesis.[43] Lee et al reported that exosomal miR-16 was involved in suppression of angiogenesis through downregulation of VEGF and CD31 expression in breast cancer cells.[46] It has also been shown that miR-100 from BMSC-derived exosomes can significantly reduce VEGF expression through the mTOR/HIF-1α signaling axis and inhibition of angiogenesis in breast cancer cells.[47]

1.3.3 | Metastasis

Metastasis represents progressive outgrowth of tumor cells leaving the primary site and moving to a distant location, which is then influenced by tumor cell signaling and interaction with a modified

microenvironment.[48] Emerging evidence has suggested the crucial role of exosomes in the TME mediating cancer metastasis.[49] For instance, the treatment of BMSC-derived exosomes in combination with radiotherapy could systematically increase tumor cell death and decrease metastatic tumor foci, suggesting its potential therapeutic benefit in anti–metastatic therapy.[50] Numerous studies have examined the role of MSC-derived exosomes, which encompass various miRNA and are widely implicated in provoking dormant cells as well as stimulating the formation of metastatic tumors.[48] It was demonstrated that BMSC-derived exosomes were able to shuttle miR-205 and miR-31 into tumor cells, resulting in the reduction of metastatic potential via the suppression of the ubiquitin-conjugating enzyme E2 N pathway in MDA-MB-231 cells.[51] The delivery of miR-143 via exosomes could alter the biological functions of osteosarcoma cells and also reduced their migration ability in vitro.[52] However, exosomes also served as a critical mediator to facilitate tumor migration. BMSC-derived exosomes could also promote cycling quiescence and early breast cancer dormancy through the transferase of the miR-222/223.[53] Exosomes from AMSC were also found to be involved in the promotion of breast cancer cell migration and metastasis through the Wnt signaling pathway via the induction of *Axin2* and *Dkk1* expressions.[54] AMSC-derived exosomes favor the invasiveness of P2X-mediated purinergic signaling, leading to more cell proliferation and invasion.[55]

1.3.4 | Drug resistance

Growing evidence suggests that exosomes play divergent roles in the acquisition of therapeutic resistance and regulation of tumor progression. Interestingly, several mechanisms appear to be involved, such as the promotion of active drug sequestration and the reduction of drug concentration, the ability to deliver specific RNA molecules or proteins into target cells, and exerting crosstalk between cancer cells and stromal cells, contributing to the dysregulation of relevant signaling pathways.[56-58]

Bone marrow mesenchymal stem cell-derived exosomes are reported to act as communicators during drug treatment. Transcriptomic profiles further demonstrated that the underlying mechanism was through the enhancement of cell migration and induction of relevant gene expression, such as *Ccl3/4*, *Ccl4* and *Cxcr4*.[59] In addition, it was found that exosomes were able to promote breast cancer cell metastasis through the transfer of miRNA-23b and to suppress target *Marcks* mRNA expression.[60] The exosomal transfer of miR-222/223 was involved in the regulation of drug resistance.[53] BMSC-derived exosomes also play a vital role in bortezomib resistance in multiple myeloma cells through the activation of the relevant signaling pathways.[61] The exosomes derived from hUCMSC were able to confer 5-fluorouracil (5-Fu) drug resistance in gastric cancer through the induction of multidrug resistance-associated proteins, and were associated with the activation of MAPK and Raf/MEK/ERK kinase signaling in gastric cancer.[62]

1.4 | Manipulation of their microenvironment

Mesenchymal stem cell-derived exosomes recapitulated the therapeutic effects of MSC via the modulation of both innate and adaptive immune responses.[7] The dichotomic role of exosomes in the regulation of the immune system was largely dependent on their original cells and the functional state of both parental and target cells.[6] However, most previous studies have demonstrated that MSC-derived exosomes exhibit an immunosuppressive effect.

1.4.1 | Regulation of the immune response

Natural antigen carriers or direct antigen presentation

Exosomes are not only natural antigen carriers but also act as presenters modulating direct and indirect antigen presentation, and stimulating several different components of both adaptive and innate immune responses.[63] It was reported that exosomes derived from DC, macrophages as well as natural killer (NK) cells, contain or could transfer specific peptides/antigens to DC, leading to the activation of $CD4^+$ T cells.[64] The shared tumor antigens served as a novel source of immunorelevant antigens for naïve T priming/B-cell activation, allowing specific human cytotoxic T cell (CTL) and NK cell activation.[65]

Indirect antigen presentation through transfer to antigenic peptides/bioactive molecules

Exosomes also served as vehicles for transfer of antigenic peptides or delivery of bioactive molecules, which, in turn, led to the modulation of other immune cell subpopulations.[66] Mature DC-released exosomes bear certain adhesion molecules, such as MHC Class II, CD86 and intercellular adhesion molecule 1 (ICAM1), all of which were involved the enhancement of T-cell stimulation and initiation of innate anti–cancer immunity.[67] It was shown that the delivery of cytosolic dsDNA derived from cancer cells was involved in the exosome elicited adaptive and innate immune reactions through the activation of cGAS-STING signaling.[29,68] In contrast, exosomes have also been reported to mediate immunosuppressive properties by carrying molecules and facilitating tumor progression. For instance, transforming growth factor (TGF)-β1 from tumor-released exosomes mediated the suppressive activity of T-cells through the promotion of myeloid cell differentiation.[69] Exosomes derived from a subset of cancer cell lines carrying PD-L1 were involved in the suppression of T-cell activity, leading to the promotion of tumor growth.[70,71]

Immunosuppression by mesenchymal stem cell-derived exosomes

Mesenchymal stem cell-derived exosomes possess a broad spectrum of immunomodulatory capabilities similar to MSC. The immunosuppression activities are summarized in Table 2. BMSC-derived exosomes have multiple roles in the modulation of T cells, NK cells, macrophages, DC and B cells. Recently, Krampera's group examined the inhibitory effects of BMSC-derived exosomes on unfractionated peripheral blood mononuclear cells (PBMC).[72] It was demonstrated

TABLE 2 Immunosuppressive activities by mesenchymal stem cell-derived exosomes

Cell sources	Target cells	Effect	Reference
BMSC	PBMC	Inhibited NK and B-cell proliferation	72
BMSC	PBMC	Suppressed T helper cell activation	73
BMSC	T-lymphocytes	Increased Treg cell generation and decrease activated T cells	74
BMSC	T-lymphocytes	Inhibited the proliferation and induced the apoptosis of $CD4^+$ cells; also increased the Treg/Teff ratio	75
BMSC	T-lymphocytes	Inhibited Th1 cell function via the adenosine A2A receptor	76
BMSC	NK cells	Inhibited NK cell proliferation and function through the upregulation of TGF-β mediating downstream TGF/Smad2/3 signaling	77
BMSC	DC and T cell	Increased the anti–inflammatory cytokines levels	78
BMSC	DC and T cell	Decreased Th17 cells and TFN-γ and IL-17 levels, increased Treg cell and TGF-β, IL-10 and IL-6 levels	79
BMSC	DCs and T cell	Suppress DC differentiation and maturation, inhibit T cell immune response	80
BMSC	DCs	Impair antigen uptake by immature DCs and halt DC activation	81
BMSC	B cells	Induce naïve to $CD24^{hi}CD38^{hi}$, IL-10 producing regulatory B cells	82
BMSC	B cells	Inhibit B cells proliferation and differentiation	83
BMSC	B cells	Inhibit B cells proliferation	84
BMSC	Macrophages	Inhibit LPS-induced inflammatory M1 toward M2 polarization	85
BMSC	Macrophages	Promote the polarization from pro-inflammatory M1 toward M2 via the activation of CCL2/CCR2 axis	86
BMSC	Macrophages	Induced the conversion of Th17 to macrophages via the down-regulation of IL-23 and IL-22 production	87
ESC	T-lymphocytes	Induce M2-like phenotype in monocyte, promote Treg cells	89
AMSC	T-lymphocytes	Inhibit differentiation, activation and proliferation of T cells	90
AMSC	PBMCs	Suppress the proliferation of PBMC	91
BMSC	Mast cells	Decrease TNFα secretion and exert immunosuppression	93

BMSC, bone marrow mesenchymal stem cell; DC, dendritic cells; NK, natural killer; PBMC, peripheral blood mononuclear cells; TGF, transforming growth factor.

that most of the exosomes were internalized by monocytes rather than by B or T cells, which could also suppress the proliferation of NK and B cells but without any direct impact towards T-cell division.[72] They could suppress the expansion of activated T cells in PBMC and inhibit the functional differentiation of T cells but preserved regulatory T (Treg) cell populations in vivo.[73] It also inhibited T lymphocyte proliferation in splenic mononuclear cells and promoted the secretion of more anti–inflammatory cytokines.[74] Del Fattore et al report that exosomes could inhibit the proliferation and induce apoptosis of $CD3^+CD4^+$ T cells, and increase the Treg proportion as well as the immunosuppressive cytokine interleukin (IL)-10 levels.[75] They were also able to harness purinergic signaling and remarkably inhibited T helper 1 (Th1) cell function via the adenosine A2A receptor.[76] Fan et al showed that MSC-derived exosomes could inhibit NK cell proliferation and function, which was through the upregulation of TGF-β mediating downstream TGF/Smad2/3 signaling.[77] He et al found that the coculture of exosomes from indoleamine 2,3-dioxygenase-1 (IDO-1) overexpressed in BMSC with DC and T cells could decrease the expression of interferon gamma (IFNγ).[78] In addition, an increased number of Treg and decreased CD8 + T cells were also observed, which may have been due to the alteration of miR-540-3p and miR-338-5p levels.[78] BMSC-derived exosomes were able to recapitulate MSC-mediated DC maturation and function.[79] It was reported that they could induce immature IL-10 secreting DC activation, increase Foxp3 + Treg cell numbers and inhibit inflammatory T helper 17 (Th17) cell responses, which appear to be enriched in higher levels of TGF-β, IL-10 and IL-6, but decrease IFN-γ and IL-17 secretion.[79] Cristina et al found that exosomes suppressed monocyte-derived DC differentiation, maturation and T-cell immune response, which relied on expression of HLA-G.[80] Exosomes were shown to impair antigen uptake by immature DC and to halt DC activation, which contributed to increased anti–inflammatory cytokine TGF-β production and decreased pro–inflammatory cytokine secretion.[81] In addition, transfection of DC with exosomes carrying miR-21-5p mimic showed diminished C-C motif chemokine receptor 7 (CCR7) expression and significant inhibition of migratory ability toward chemokine CCL21.[81] BMSC-derived exosomes are also involved in the transition from naïve B cells to $CD24^{hi}CD38^{hi}$, IL-10-producing Breg cells, but has no influence on their proliferation.[82] It was also shown that exosomes exerted an immunomodulatory effect on CpG-stimulated PBMC proliferation via the downregulation of B-cell proliferation and differentiation, as well as inhibition of

IgM, IgG and IgA production.[83] The inhibitory effects of exosomes on B-lymphocyte proliferation as well as B-cell function were also observed, which was through the alteration of relevant mRNA levels, such as *CXCL8* and *MZB1*.[84] It was demonstrated that MSC-derived exosomes could suppress M1 macrophage function, while directly increasing M2 polarization through the activation of the miR-let7/HMGA2/NF-κB pathway.[85] CCR2 on MSC-derived exosomes was also involved in polarization from M1 to M2 through the activation of the CCL2/CCR2 axis.[86] Hyvärinen et al revealed that it could induce the conversion of anti–inflammatory activated regulatory macrophages from T helper type 17 (Th17) cells through the down-regulation of IL-23 and IL-22 production as well as the upregulation of prostaglandin E2 ($PGE2_2$) levels.[87]

Besides BMSC-derived exosomes, other types of MSC-derived exosomes were also involved in the modulation of the immune system. Toll-like receptor (TLR) signaling can serve as a regulator of MSC immunomodulation, and TLR activation can modulate MSC to switch from a predominately immunosuppressive to a pro–inflammatory phenotype but are more likely depending on experimental settings.[88] Human embryonic stem cell (ESC)-derived exosomes induced the activation of TLR-dependent signaling and, in turn, activated more Treg cells, which attenuated an activated immune response.[89] AMSC-derived exosomes were reported to suppress stimulated T-cell proliferation and activation.[90] hUCMSC-derived exosomes preferably bound to the monocytes in PBMC, and gave rise to M2 macrophages with more production of immune suppression-associated cytokines.[91] Exosomes derived from hUCMSC stimulated with TGF-β and IFN-γ could enhance Treg differentiation.[92] In addition, PGE2 was the key mediator responsible for BM-derived mast cell immunosuppression by significantly decreasing TNFα secretion.[93]

1.5 | Exosomes as drug delivery vehicles

Among the abundant drug platforms, exosomes by themselves or as drug carriers are being actively explored as therapeutic agents. They have of a bilayer lipid membrane, which is similar to cell membrane and confers lower toxicity when injected in mice repeatedly.[6] The MSC-derived exosomes exhibited an innate targeting tendency to prefer the inflamed tissues and tumor tissues upon in vivo delivery, which appears to hold possible benefits for genetic modification to express therapeutic proteins or targeting surface proteins to provide treatment potential.[94]

1.5.1 | Exosomes engineering and functional cargo loading

There is growing extensive literature documenting the application of genetic and nongenetic approaches for the generation of modified exosomes from in vitro modification of MSC.[6] To date, the package of nucleic acid, proteins and other active molecules into exosomes has been widely applied.

1.5.2 | miRNA and siRNA

It was shown that BMSC-derived exosomes equipped with synthetic miR-143 could lead to the suppression of migration in osteosarcoma cells.[52] Mark Katakowski et al engineered MSC to shed exosomes that contain miR-146b, and found that intra-tumor injection of miR-146b through MSC-derived exosomes could significantly inhibit glioma growth, reduce its invasion, migration via targeting on epidermal growth factor receptor (EGFR) signaling.[95] It was reported that BMSC that secrete exosomes encapsulated with miR-379 could significantly reduce the rate of tumor proliferation and survival in breast cancer cells, as well as decrease lymph node metastasis.[96] It was shown that the systemic administration of exosomes equipped with anti–miR-222/223 could significantly increase host survival and be more sensitized to carboplatin-based therapy in breast cancer.[53] Ohno et al showed that the active loading of let-7a into exosomes enabled the delivery of let-7a into EGFR-expressing breast cancer cells and exerted significant anti–tumor effects in various breast cancer models.[97] In addition, clinical-grade MSC-derived exosomes with $Kras^{G12D}$ siRNA payload have been applied for the treatment of pancreatic cancer in vivo, which yielded a robust increase in overall survival and enabled specific target engagement without any obvious toxicity.[98,99] Similarly, serine/threonine-protein kinase (PLK-1) silencing by siRNA via BMSC-exosomes delivery resulted in the depletion of PLK-1 and contributed to cell cycle arrest and more cell apoptotic cell death in bladder cancer cells.[100]

1.5.3 | Functional proteins

Engineered exosomes also served as an attractive vehicle for the delivery of biologically active proteins. Sterzenbach et al labeled an intracellular target protein Cre recombinase with a WW tag, which was recognized by the L-domain motifs containing protein Nedd4 family interacting protein 1 (Ndfip1); the Ndfip1 expression acts as a molecular switch for exosomal packaging of WW-Cre and a test for intracellular protein delivery in vivo.[101] Recently, it was found that TRAIL delivery via MSC-derived exosomes served as an effective anti–cancer therapy that induced pronounced apoptosis in a series of cell lines.[102] Furthermore, the combination therapy of exosomes derived from TRAIL-overexpressed MSC with CDK9 inhibitor could significantly increase apoptotic cancer cell death.[102]

1.5.4 | Small molecule therapeutics

Exosomal delivery of small therapeutic molecules, including doxorubicin, paclitaxel, porphyrin, curcumin, taxol, methotrexate, cisplatin and withaferin, has been widely investigated, and some of them have been registered in clinical trials.[103-106] Those drugs are slowly released from the exosomes and result in higher drug accumulation in target cells and longer blood circulation time and enhanced

therapeutic efficacy.[107] MSC-derived exosomes have been engineered as an efficient delivery system reported to exert higher anti–tumor efficacy and reduced toxicity.[108,109] For instance, BMSC-derived exosomes encapsulated with doxorubicin are preferentially taken up by human epidermal growth factor receptor 2+ (HER2+) cells in vitro, leading to an inhibitory effect in a breast tumor model.[110] It was observed that paclitaxel-treated BMSC mediated obvious anti–tumor activity because of their capacity to take up the drug inside exosomes, which results in an obvious inhibition of cell growth and proliferation in pancreatic cancer cells.[105] Melzer et al reported that exosomes served as cellular carriers for taxol, which were extensively distributed around the breast tumor after their direct intratumoral injection and significantly inhibited tumor growth and reduced metastasis.[111] In addition, ESC-derived exosomes were highly versatile and could deliver therapeutic porphyrin to target breast cancer cells, thereby leading to the improvement of cellular uptake of porphyrins associated with a strong induction of reactive oxygen species.[106]

1.6 | Mesenchymal stem cell-derived exosomes in clinic trials

Currently, there are nine studies undergoing clinical trials, and they are listed in www.clinicaltrials.gov.[109] A combination of ascites-derived exosomes with granulocyte-macrophage colony-stimulating factor (GM-CSF) underwent a phase I clinical trial for the treatment of advanced colorectal cancer, which was shown to be feasible and safe and also capable of triggering more CTL infiltration in tumor regions.[112] Another phase I clinical trial using exosome delivery of curcumin to colon tumors and normal colon tissue was started in 2011 and is estimated to end in 2020 (ClinicalTrials.gov identifier: NCT01294072). As mentioned previously, siRNA-sxosome-based therapy is being used in the treatment of pancreatic cancer patients with $Kras^{G12D}$ mutation via $Kras^{G12D}$ in a phase I clinical trial (ClinicalTrials.gov identifier: NCT03608631). Despite these encouraging results for the application of MSC-derived exosomes as a drug vehicle for cancer treatment in clinic, there are still have many challenges ahead. Although a process for production of MSC-derived exosomes with good manufacturing practice grade has been reported, effective large-scale exosome production for clinical application still needs to be developed.[99,113] In addition, there is still a lack of standard protocols for the characterization of MSC exosomes due to their heterogeneous nature, and this heterogeneity may have diverse effects on the target cells. To ensure therapeutic biosafety, investigating the relationships among delivery route, dosage and pharmacokinetics is also needed. In addition, the horizontal genetic composition transfer via exosomes also raised the potential for discrimination risks because of other genetic information exchange between cells.[114] Furthermore, it also seems necessary to closely monitor patients who have received MSC-derived exosomes to determine the optimal dosage with the best therapeutic efficiency but minimal undesired toxicity individually.

2 | CONCLUSION AND FUTURE DIRECTION

Collectively, we discussed the therapeutic potential of MSC-derived exosomes and their exciting new prospects in cancer treatment. With the substantial research being dedicated to MSC-derived exosomes, there will also be more opportunities to manipulate their composition and modulate cell-cell interactions in TME. Despite recent promising advances using MSC-derived exosomes as drug/nucleic acid delivery platforms, it is still necessary to develop appropriate strategies and techniques to tailor exosomes with high drug-carrying capacity, increased target specificity and non–cytotoxic effects. In conclusion, basic research and emerging technologies need to be integrated to fully exploit the potential of MSC-derived exosomes and to accelerate their therapeutic application in the clinic.

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CONFLICT OF INTEREST

The MD Anderson Cancer Center and RK hold patents in the area of exosome biology and are licensed to Codiak Biosciences. The MD Anderson Cancer Center and RK are stock equity holders in Codiak Biosciences. RK is a consultant and scientific adviser for Codiak Biosciences.

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