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Review Article

Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools

Song-pei LI, Zhong-xiao LIN, Xue-yan JIANG, Xi-yong YU*

Key Laboratory of Molecular Target & Clinical Pharmacology, School of Pharmaceutic Sciences & Fifth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511436, China

Abstract

Exosomes are nano-sized vesicles that serve as mediators for intercellular communication through the delivery of cargo, including protein, lipids, nucleic acids or other cellular components, to neighboring or distant cells. Exosomal cargo may vary in response to different physiological or pathological conditions. The endosomal sorting complex required for transport (ESCRT) family has been widely accepted as a key mechanism in biogenesis and cargo sorting. On the other hand, accumulating evidence show that ESCRT-independent pathways exist. Due to the critical role of exosomes in intercellular communications in delivering cargo to recipient cells, exosomes have been investigated as a vector for the delivery of endogenous or exogenous cargo for therapeutic purposes. But the number of exosomes produced by cells is limited, which hampers their application. Synthetic exosome-mimics have been fabricated and investigated as a therapeutic tool for drug delivery. This review focuses on ESCRT-independent regulation of cargo loading into exosomes, including lipid raft and ceramide-mediated mechanisms, and reported exosomes or exosome-mimics with therapeutic effects.

Keywords: exosome; extracellular vesicle; lipid raft; exosome-mimic; drug delivery

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Introduction

Exosomes are membrane-bound extracellular vesicles (EVs) released from cells into the extracellular space. It is widely accepted that exosomes are generated from inward budding of the membrane in endosomes, forming intraluminal vesicles into multivesicular bodies (MVBs) that eventually fuse with the plasma membrane and release exosomes into the extracellular space[1]. In comparison, microvesicles, which are another subpopulation of EVs, are formed by directly budding off from the plasma membrane. Typically, exosomes are 30–100 nm in diameter, which is similar to the size of intraluminal vesicles, whereas microvesicles are larger than 100 nm[2, 3]. Exosomes were considered to be a mechanism for discarding cell garbage until a number of studies suggested that they were nano-sized vesicles that serve as mediators for intercellular communication through the delivery of proteins, lipids, nucleic acids, or other components in or within their lipid bilayer membrane to neighboring or distant cells. Although accumulating studies have indicated the crucial role of exosomes in various physiological and pathological processes, including tumor metastasis, neurodegeneration, and tissue repair[4-8], the regulation of biogenesis and cargo loading is not fully understood.

The endosomal sorting complex required for transport (ESCRT) family has been believed to play a crucial role in biogenesis and cargo sorting, and thus, ESCRT-dependent exosome formation has been reviewed previously[1, 9, 10]. It has been shown that ESCRT family members, including Tsg101, Hrs[11], CHMP4[12, 13], STAM1[13], VPS4[13], and VTA1[13], or ESCRT-associated protein, ALIX[12], are involved in the regulation of exosomal biogenesis and cargo sorting in metazoan systems. Tsg101[14], Vps4[15], and ALIX[16, 17] might also mediate protein or RNA loading into exosomes. However, depletion of the components of ESCRT reduced the secretion of exosomes rather than creating a complete blockade[13, 18], thereby indicating the presence of an ESCRT-independent pathway in exosome biogenesis and cargo sorting. Due to the critical role of exosomes in intercellular communications with respect to cargo delivery to recipient cells, exosomes or synthetic exosome-mimics have been investigated as vectors for drug delivery. The present review focuses on ESCRT-independent cargo sorting in exosome biogenesis and exosomes or exosome-mimics loaded with endogenous and/or exogenous cargo,

*To whom correspondence should be addressed.
E-mail yuxycn@gzhmu.edu.cn
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which might serve as a potential therapeutic tool.

ESCRT-independent cargo loading into exosomes

Lipid raft and cargo sorting into exosomes

Association of proteins and molecules with lipid rafts may facilitate their secretion via exosomes

The lateral heterogeneity of the plasma membrane led to the hypothesis of lipid rafts, also known as detergent-resistant membranes (DRMs), which are membrane domains enriched in cholesterol, sphingolipids, and GPI-anchored proteins. The bilayer membrane of exosomes has also been shown to possess DRM domains, which often contain lipid raft-associated proteins[19-22]. The involvement of lipid rafts in protein sorting into exosomes was initially proposed in maturing reticulocytes as a putative mechanism for the selective clearance of clustered membrane receptors and lipids[23]. The presence of raft-associated proteins and molecules [ganglioside GM1, major histocompatibility complex (MHC) class II molecules and flotillin-1] in the DRM of exosomes in two different cell populations was demonstrated by the same research group. This phenomenon suggested that the incorporation of proteins and molecules into raft domains may facilitate their assembly into exosomes[19]. Therefore, an association with raft domains was proposed as a mechanism underlying the export of a transmembrane glycoprotein *via* exosomes in a breast carcinoma cell line[21]. Furthermore, in retinal pigment epithelial cells, αB-crystallin, a small heat-shock protein located in DRM, together with caveolin-1, Hsp70, and flotillin-1, has been shown to be released via exosomes. The secretion of αB-crystallin can be blocked by disruption of the lipid raft, indicating that the process is associated with the lipid raft[24].

Shift of proteins and molecules to raft domains may elicit their secretion via exosomes

Lipid raft-mediated invagination of the plasma membrane has been well recognized as an endocytosis mechanism; therefore, the shift of proteins or molecules to lipid raft domains may facilitate their assembly into early endosomes[25, 26]. Thus, it could be speculated that the proneness of proteins or molecules towards raft domains may be involved in protein sorting into exosomes. This raft-mediated protein sorting mechanism was observed in the loss of stemness of mammary epithelial stem cells, wherein prostaglandin E receptor 4 antagonist promoted the trafficking of membrane receptors and other signaling proteins to raft domains. Subsequently, these proteins were shuttled together with stem cell surface markers out of the cells through exosomes[27]. Notably, disruption of the lipid raft by cholesterol or caveolin depletion decreased the level of raft-associated proteins and stem cell surface markers in exosomes, suggesting a regulatory role of lipid rafts in protein sorting in exosomes. Similarly, heat-shock stress might induce the secretion of chemokine-containing exosomes from tumor cells via the incorporation of chemokines into lipid raft domains that can be blocked by cholesterol scavengers[28]. Treatment with Ca^{2+} ionophores triggered the recruitment of a Ca^{2+}-dependent phospholipid-binding protein to lipid rafts and therefore increased the secretion of exosomes containing this protein[29]. The release of TNFα converting enzyme through EVs (80–120 nm, including exosomes and microvesicles) was also mediated by the incorporation of this enzyme into lipid rafts in 293T cells[30]. Thus, the association or incorporation of proteins or molecules into lipid rafts might facilitate their secretion through exosomes (Figure 1).

Ceramide and cargo sorting into exosomes

In a mouse oligodendroglial cell line, the knockdown of ESCRT components Tsg101 and Alix did not affect the number of secreted exosomes or the release of proteolipid proteins through exosomes, suggesting an ESCRT-independent pathway in exosome biogenesis and cargo loading[31]. The inhibition or deletion of neutral sphingomyelinase, an enzyme that catalyzes the formation of ceramide, reduced the release of proteolipid proteins through exosomes. Introducing sphingomyelinase promoted the budding of small vesicles from lipid-ordered domains in the artificial unilamellar vesicles that contain both lipid-ordered and lipid-disordered domains. Sphingomyelinase-induced ceramide production was required for the secretion of miRNA via exosomes[32, 33]. C6 ceramide has been shown to stimulate exosome secretion and increase the exosomal content of tumor-suppressive miRNA in a dose-dependent manner in a multiple myeloma cell line[34]. Notably, ceramide-induced CD63 secretion through exosomes can be blocked by the inhibitor of sphingosine kinase, an enzyme that catalyzes the formation of S1P[35], indicating that the effect of ceramide on cargo loading into exosomes may rely on its metabolite sphingosine 1 phosphate (S1P).

RNA sorting into exosomes

RNA sorting into exosomes is unlikely to be random

The RNAs enriched in exosomes include coding mRNAs and non-coding RNAs such as ribosomal RNA (rRNA), micro RNAs (miRNAs), long noncoding RNAs (lncRNAs), and recently identified circular RNAs (circRNAs)[36-39]. Accumulating evidence has shown that miRNA can be shuttled via exosomes to neighboring cells, where they regulate gene expression and biological functions[32, 40-44]. Therefore, the mechanisms underlying miRNA sorting into exosomes has gained increasing attention and are the focus of this review.

Early miRNA array analysis indicated that a sorting mechanism of miRNAs into exosomes might exist since the level of miRNAs with smaller numbers was lower than that of miRNA with larger numbers in prostate cancer cell-derived exosomes[45]. Further studies have shown that the miRNA profiles of secreted exosomes are distinct from those of cells[15, 44, 46-49], indicating that miRNAs are sorted into exosomes under specific mechanisms rather than being sorted randomly.

Cellular abundance and miRNA sorting into exosomes

It has been shown that sorting of miRNAs into exosomes can be regulated by the levels of miRNAs and endogenous target sequences[47]. Deletion of Dicer in bone marrow-derived cells induced an obvious reduction of miRNAs in secreted

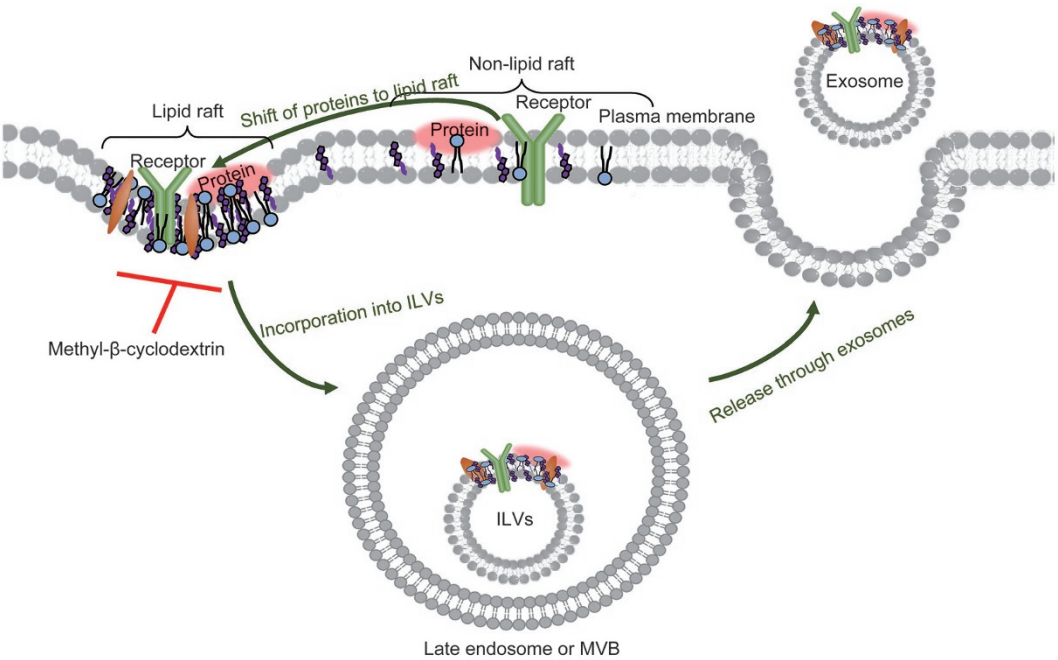

Figure 1. Lipid raft-mediated cargo loading into exosomes. Lipid raft is a membrane domain enriched in cholesterol, sphingolipids, and GPI-anchored proteins. Proteins associated with lipid rafts can be sorted into intralumenal vesicles (ILVs) in multivesicular bodies (MVBs). Shift of membrane proteins in non-lipid raft domain to lipid raft might facilitate their incorporation into ILVs of MVBs. MVBs fuse with the plasma membrane and release exosomes into extracellular space. Disruption of lipid raft by methyl-β-cyclodextrin inhibits the secretion of these proteins through exosomes.

exosomes compared to their levels in the cells. Overexpression of miRNA-511-3P (miR-511-3p) in marrow-derived macrophages increased the level of this miRNA in exosomes to a greater extent than its level in cells. Conversely, knockout of an miRNA target gene or an increase in an miRNA target sequence has been shown to increase or decrease the miRNA in exosomes. *In silico* analysis also indicated that exosomal miRNAs that shared the same seed sequence exhibited similar fold changes compared to random miRNA pairs. These results indicated that sorting of miRNAs into exosomes might depend on their sequence and could be affected by their cellular abundance and competition with miRNAs targets.

Exo-motifs and miRNA sorting into exosomes

RNA-binding proteins that play a major role in intracellular RNA trafficking have been suggested to be involved in RNA sorting into exosomes. It has been suggested that annexin A2, a non-canonical RNA-binding protein[50, 51], may be required for the viral RNA sorting into exosomes that are released from infected cells to trigger the immune response in dendritic cells[40]. Mutation of RNA-binding protein Y-box protein 1 was found to impair the sorting of two exosome-enriched miRNAs (miR-144 and miR-233) into exosomes in HEK293T cells, leading to intracellular accumulation of these two miRNAs[49].

Analysis of the miRNAs sequences presented in exosomes identified common seed sequences, termed EXO-motifs, that facilitated binding to RNA-binding proteins, such as hnRNPA2B1 and SYNCRIP[52, 53]. SUMOylated hnRNPA2B1 has been shown to control the loading of miR-198 into exosomes, which was blocked by mutation of the EXO-motif of miR-198[52]. In hepatocytes, suppression of SYNCRIP by short hairpin RNAs induced the retention of exosomal miRNAs within cells, and GGCU was identified as an EXO-motif for sorting of miRNAs into exosomes by SYNCRIP[53]. Interestingly, knockdown of hnRNPA2B1 did not affect the loading of GGCU-containing miRNAs into exosomes in hepatocytes. Analyses of binding activities of SYNCRIP and hnRNPA2B1 revealed different binding capacities to EXO-motifs, suggesting that RNA-binding proteins may cooperate with each other during miRNA sorting into exosomes via specific binding to EXO-motifs[53].

However, the mechanisms underlying the sorting of RNA-binding protein-miRNA complexes into exosomes have not yet been clarified. Most likely, RNA-binding protein-miRNA complexes may be loaded into exosomes via mechanisms of protein sorting, such as the ESCRT-dependent pathway and lipid raft-dependent mechanisms. Lipid rafts have been shown to facilitate the trafficking of annexin A2 to intralumenal vesicles in MVBs[29]. Ubiquitinated Y-box protein 1 can be secreted into the extracellular space through interactions with TSG101, a component of the ESCRT-1 complex[14]. hnRNPA2B1 has been shown to colocalize with ceramide, which might facilitate its secretion into exosomes[52].

Exosomes or synthetic exosome-mimics serve as a potential therapeutic tool

Naturally occurring exosomes

Exosomes with therapeutic effects include naturally occurring

exosomes, exosomes secreted by cells with modifications, and exosomes loaded directly with exogenous cargo (Figure 2). Naturally occurring exosomes derived from stem cells have been suggested to exert protective effects on neighboring cell populations[54, 55] (Table 1). Intramyocardial injection of exosomes derived from embryonic stem cells (ESCs) promoted the proliferation and survival of cardiomyocytes and reduced fibrosis in a mouse model of myocardial infarction[54]. These exosomes also increased the number of resident cardiac progenitor cells, and cardiac progenitor cells pretreated with exosomes from ESCs showed enhanced capacity for survival and proliferation. These protective effects might result from miR-294, which was enriched in ESC-derived exosomes. Amniotic fluid stem cells secreted exosomes that prevented ovarian granulosa cells from damage during chemotherapy through the delivery of anti-apoptotic miRNAs[55].

Naïve exosomes derived from cells with modifications

Exosomal cargo can vary in response to different physiological and pathological conditions[47, 56-58]. Exosomal miRNAs and proteins from cells with modifications (pathological factors, transfection and drug loading) can be delivered to recipient cells as a therapeutic approach (Table 1). Our previous studies have investigated the role of stress-treated stem cell-derived exosomes in cardiac protection. Ischemic preconditioning triggered the secretion of miR-22-enriched exosomes from mesenchymal stem cells (MSCs), and these exosomes protected cardiomyocytes from ischemic injury[56]. Exosomes derived from H_2O_2-preincubated cardiac progenitor cells exhibited an increased level of miR-21 and attenuated H_2O_2-induced apoptosis in cardiomyocytes[57]. Moreover, heat-shock pretreated cardiac stem cells produced heat shock factor 1 enriched exosomes that could be taken up by cardiomyocytes and reduced apoptosis[58].

As reviewed above, sorting of miRNAs into exosomes may increase in response to elevations in their cellular levels[47]. Therefore, exosomes may serve as vesicles to export overloaded miRNAs from donor cells to recipient cells. THP-1 macrophages transfected with modified miR-143 increased the secretion of miR-143-containing microvesicles[59]. After uptake by recipient cells, these exogenous miRNAs were demonstrated to be functionally active. Exosomes derived from human embryonic kidney cell line 293 (HEK293) cells transfected with siRNA or miRNA together with plasmids containing GE11 peptide DNA (targets tumor cells by binding to a surface receptor) can target tumors and suppress tumor growth[60]. Exosomes secreted from marrow stromal cells that were transfected with miR-146b delivered this miRNA to tumor cells *in vitro*, and intra-tumor injection of these exosomes suppressed the xenograft growth of rat primary brain tumors[61]. Neural progenitor cells transfected with miR-210 produced exosomes that protected endothelial cells from

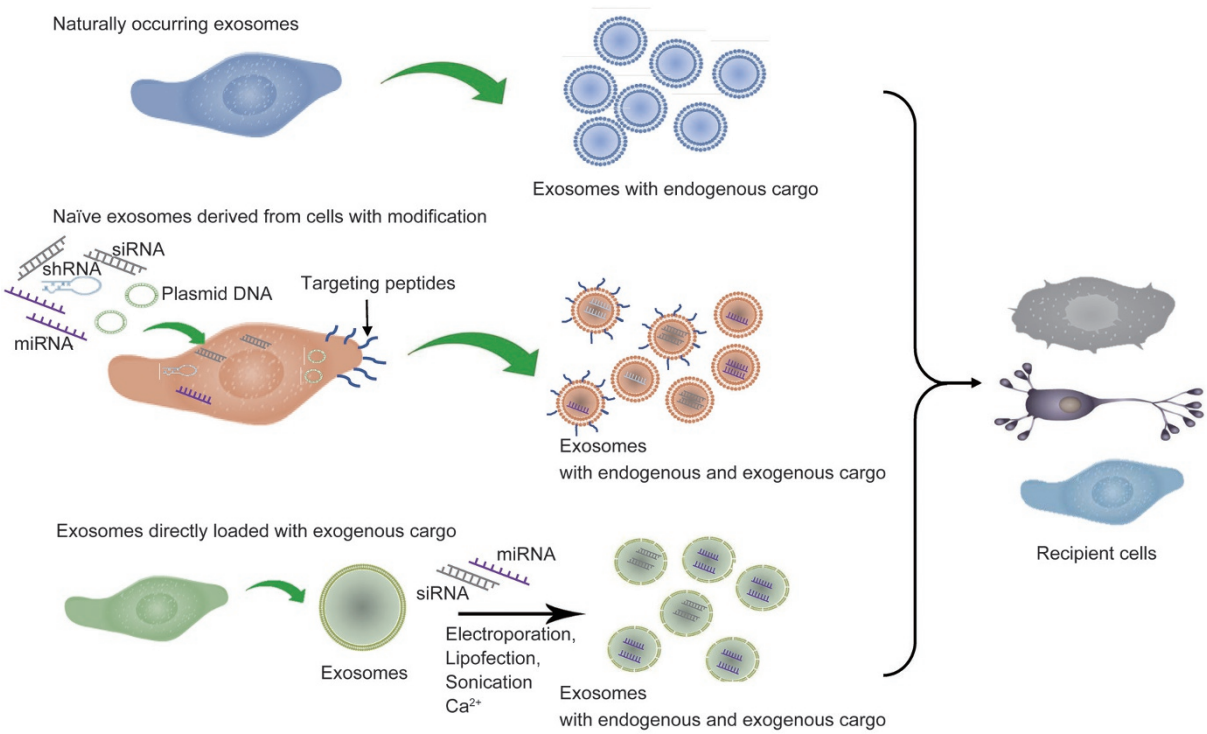

Figure 2. Naturally occurring and engineered exosomes for therapeutic purposes. Naturally occurring exosomes from stem cells have shown the protective effects on the survival and proliferation of cells such as cardiomyocytes. Cells loaded with exogenous cargo including siRNAs, miRNAs, and shRNAs secrete exosomes containing these siRNAs, miRNAs, or shRNA-derived miRNAs. Cells expressing target peptides by plasmid transfection produce exosomes that can target specific cell populations. Exogenous cargo can be directly loaded into exosomes by electroporation, lipofection, sonication or Ca^{2+}. Exosomes loaded with endogenous and/or exogenous cargo can be taken up by recipient cells for the regulation of gene expression.

Table 1. Exosomes or exosome-mimics as a therapeutic tool.

Group	Sub-group	Delivered cargo	Modification for targeted delivery	*In vivo* administration route
Naturally occurring exosomes		miR290-295[54] miR-10a[55]	–	*in situ* injection[54, 55]
Naïve exosomes from cells with modification	Stress-treated cells	miR-22[56], miR-10a[57] Heat shock factor 1[58]	–	*in situ* injection[56]
	Genetic modified cells	miR-143[59, 66] miR-let7a[60] miR-146b[61] miR-210[62] anti-miR-9[63] miR-122[64] miR-let7c[65] miR-155 and miR-125b2[91]	Tumor-targeting peptide[60]	*in situ* injection[64] iv injection[59, 60]
Exosomes loaded with exogenous cargo	Electroporation	BACE1 siRNA[67] Doxorubicin (drug)[74]	Neuron-targeting peptide[67] Tumor-targeting peptide[74]	iv injection[67, 74]
	Lipofection	RAD51 siRNA[68]	–	–
	Sonication	HER2 siRNA[69]	–	–
	$CaCl_2$	miR-15a and anti-miR-15a[70]	–	inhalation[70] (function not tested)
	Coincubation	Withaferin A[75] (drug)	Tumor-targeting ligand[75]	ip injection[75] oral delivery[75]
Exosome-mimics	Cell extrusion	Doxorubicin (drug)[76] c-Myc shRNA [77]	–	iv injection[76]
	Cell membrane-cloaked nanoparticles	sTRAIL[81] (drug) Emtansine[83] (drug) Monophosphoryl lipid A[84] (drug)	–	iv injection[81, 83]

angiotensin II-induced apoptosis[62]. Anti-miR-9 oligonucleotides can be delivered by exosomes from MSCs to glioblastoma multiforme cells, where they sensitize the cells to anti-glioblastoma multiforme drug treatment[63]. Adipose tissue-derived MSCs transfected with miR-122-expressing plasmids produced miR-122-enriched exosomes[64]. Exosomes with miR-122 could be delivered to hepatocellular carcinoma cells and sensitized cancer cells to chemotherapeutic therapy. Exosomes derived from MSCs overexpressing miR-let7c delivered miR-let7c to kidney cells and regulated the expression of genes related to fibrosis[65].

A comparison of the efficiency of exosomes from miR-143-loaded MSCs with direct lipofection of miRNAs into recipient cells revealed that exosomes exhibited similar inhibition of cell migration with lipofection; however, the loading efficiency of exosomes was much lower than that of direct lipofection[66]. The authors proposed that miRNAs in naïve exosomes were incorporated with RNA-induced silencing complexes that might facilitate the appropriate location of miRNAs to target mRNAs.

Exosomes directly loaded with exogenous nucleic acids or drugs

Exogenous RNAs can be directly loaded into exosomes by electroporation, lipofection, sonication, and calcium chloride (Table 1). Purified exosomes from dendritic cells that were transfected with a neuron-targeting peptide-encoding plasmid were loaded with siRNA by electroporation[67]. SiRNA-containing exosomes could be delivered to neurons in the brain and knocked down specific genes without inducing an obvious immune response. Naked siRNA could not be delivered to the brain, and unmodified exosomes could not induce gene silencing. Exosomes originating from HeLa cells were transfected with siRNA by lipofection, and these exosomes could silence specific genes in recipient cells[68]. siRNA loaded into EVs by sonication could be delivered to breast cancer cells, which induced a 50% knockdown of an oncogene, although only a limited number of siRNAs were incorporated into the recipient cells[69]. It has been shown that calcium chloride can mediate the transfection of miRNAs or their inhibitors into exosomes in the case of heat shock, and these RNAs were functionally active after delivery to recipient cells[70].

However, several studies showed that nucleic acids directly loaded into EVs might not be functionally active when taken up by recipient cells. It has been suggested that transfection of siRNA into EVs by electroporation might induce the formation of insoluble siRNA aggregates[71]. EVs loaded with plasmid DNAs by electroporation delivered DNAs to recipient cells; however, these DNAs were not functionally active[72]. It has been shown that mRNAs, siRNAs and plasmid DNAs transfected into HEK293FT cell-derived exosomes by lipofection could not induce or downregulate protein expression in recipient cells[73]. It has been shown that siRNA loaded into

EVs by sonication induced less siRNA aggregation than electroporation; however, the amount of siRNA incorporated into recipient cells by exosomes was still limited[69].

Tumor-targeting exosomes were generated by the expression iRGD peptide together with a membrane protein in dendritic cells and were loaded with doxorubicin by electroporation; these exosomes showed anti-tumor effects *in vivo* with targeting capability and no apparent tissue damage [74]. Bovine milk-derived exosomes loaded with a chemopreventive drug (withaferin A) by direct coincubation showed enhanced anti-tumor effects *via* intraperitoneal (ip) injection in lung cancer-bearing mouse compared to the naked drug[75]. The additional loading of folic acid, a tumor-targeting ligand, into exosomes augmented the inhibition of tumor growth in mice when it was administered orally compared to exosomes loaded with the drug alone.

Exosome-mimics

Cells secrete a limited number of exosomes. A large number of nanovesicles can be produced by cell extrusion or polymer-nanoparticles coated with cell membranes (Figure 3). These nanovesicles may serve as exosome-mimics for drug delivery (Table 1).

Nanovesicles generated by cell extrusion

Two Korean groups fabricated exosome-mimetic nanovesicles by extruding cells. The forceful and sequential passage of monocytes or macrophages through 10-, 5-, and 1-μm filters led to the generation of a large amount of nanovesicles with a peak diameter of approximately 120–130 nm. Unlike liposomes, these nanovesicles loaded with doxorubicin were targeted to tumors and showed anti-tumor effects similar to exosomes *in vivo*[76]. Furthermore, GFP-silencing siRNA loaded into monocyte-derived nanovesicles by electroporation could be taken up by endothelial cells and eventually knocked down GFP[77]. Nanovesicles generated by extruding fibroblasts transfected with shRNA could also be harnessed as a vector to deliver functionally active miRNAs to recipient cells. The other group used multiple microchannels to break down murine ESCs into membrane-bound nanovesicles with a size of 60–120 nm in diameter[78]. These nanovesicles could be incorporated into fibroblasts to deliver endogenous RNAs and proteins of stem cells to fibroblasts. The same group developed a device that can generate large-scale nanovesicles using centrifugal force to extrude cells through 10-μm and 5-μm filters[79].

Cell membrane-cloaked nanoparticles

Recently, cell membrane-cloaked nanoparticles have emerged as a potential tool for drug delivery with the advantages of immunocompatibility, stability and targeting capability[80-84]. Erythrocyte membrane-encapsulated polymeric nanoparticles have been shown to have long-circulating properties[80]. The membrane of erythrocytes was ruptured in hypotonic conditions, and erythrocyte membrane-enclosed nanovesicles were generated by extrusion through a 100-nm porous membrane. Compared to conventional PEG-coated nanoparticles, these membrane-bounded vesicles were fused with PLGA particles, which were 70-nm in diameter, to produce erythrocyte membrane-coated nanoparticles with an increased half-life in the blood and prolonged circulation retention. Cell membrane coating has been used for targeted drug delivery. Platelet

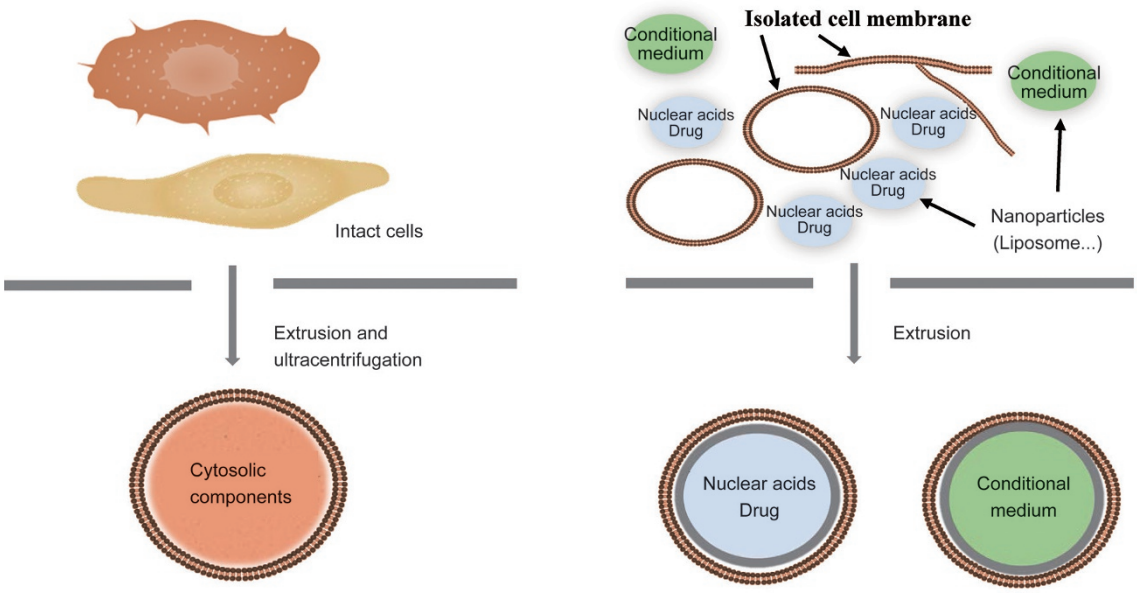

Figure 3. Fabrication of exosome-mimics. The passage of intact cells through micro-sized filters or tubes generates cell membrane-enclosed nanovesicles (left). These nanovesicles may be used as exosome-mimics for the delivery of nucleic acids and drugs to recipient cells. Cell membrane-cloaked nanoparticles can be fabricated by extruding polymer-coated nanoparticles together with the cell membrane (right). These nanoparticles may be used for the targeted delivery of nucleic acids, drugs and conditioned medium from cells.

membrane-cloaked nanoparticles showed reduced uptake by macrophages and could selectively adhere to damaged vasculature[82]. MSC membrane-generated nanoparticles delivered the anti-tumor drug sTRAIL to tumor tissue and suppressed tumor growth *in vivo*, whereas liposome-encapsulating sTRAIL did not show any anti-tumor effect[81]. Cancer cell membrane-coated nanoparticles showed homotypic targeting to tumors with immunocompatibility[83, 84] and therefore may serve as a potential vector for anti-tumor drug delivery or immunotherapy.

Recently, a Chinese group fabricated stem cell-mimics using stem cell membrane-cloaked PLGA microparticles comprised of conditional medium from stem cells[85, 86]. Intramyocardial injection of these stem cell-mimics reduced infarction size and increased left ventricle ejection fraction in a mouse model of myocardial infarction. These protective effects were equivalent to those of stem cells. Therefore, cell membrane-coated nano-sized PLGA particles containing conditional medium may serve as exosome-mimics for drug delivery.

Conclusion and perspectives

Cargo loading into exosomes is regulated by multiple mechanisms, such as the ESCRT-dependent pathway, lipid raft-mediated pathway, and ceramide-dependent pathway. Exosomes serve as mediators that modify the condition of recipient cells by delivering protein, lipid, and RNA cargo. Stem cells (with or without the treatment of pathological factors) secrete exosomes that promote cell survival and proliferation[54-58]. Modifications of exosome cargo can confer additional benefits, such as enhanced effects and targeting capability, to exosomes. As the function of exosomal cargo has become more widely recognized, exosomes have been proposed as a potential alternative to cell-based therapies[87]. A phase I trial (NCT02565264) has been registered to study autologous plasma-derived exosomes in cutaneous wound healing. Exosomes have also emerged as a nanoplatform for drug delivery[88]. An ongoing phase I trial (NCT01294072) uses exosomes as a vehicle to deliver an anti-tumor drug, curcumin, for the treatment of colon cancer. Exosomes containing therapeutic cargo could be generated by loading exogenous cargo into cells or by directly loading cargo into exosomes. However, the direct loading of nucleic acids into exosomes may not deliver functionally active cargo into recipient cells efficiently. This phenomenon may result from the low efficiency of transfection by exosomes[66, 69, 88] or the aggregation and degradation of nucleic acids during loading[69, 71]. Thus, RNAs or proteins passively loaded into exosomes by lipofection or electroporation without cellular cargo sorting regulation might be less favorable than RNAs or proteins loaded into naturally occurring or preconditioned cell-derived naïve exosomes.

Cells secrete a limited number of exosomes, which significantly hampers the development of basic research and clinical trials using exosomes. Synthetic exosome-mimics by cell extrusion or cell membrane-cloaked nanoparticles, which can be fabricated on a large-scale, provide novel platforms for drug delivery. MiRNAs have been widely studied as a functional constituent of exosomes. However, anionic miRNAs with a short half-life could not pass through cell membranes easily and were prone to accumulation in the liver and kidney[89, 90]. Exosome-mimics generated by cell extrusion or cell membrane-cloaked nanoparticles could serve as a vector for functional miRNA and drug delivery, although they are different from exosomes since cargo loading is not selected by specific mechanisms, such as the ESCRT-dependent and lipid raft-dependent pathways. Nevertheless, these exosome-mimics showed similarities to exosomes in their zeta-potential, size distribution, and morphology and possessed the immunocompatibility and stability of exosomes due to coating of the plasma membrane[76, 78, 80]. Furthermore, the incorporation of specific peptides into cell membranes[60, 67] could effectuate these membrane-bounded exosome-mimics in the targeted drug delivery.

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