Mesenchymal Stromal Cell-Derived Exosomes: Biogenesis and Cargoes

Mesenchymal stromal cells (also known as mesenchymal stem cells or MSCs) are self-renewing progenitor cells that can be isolated from various tissues, including bone marrow[1], adipose tissue[2], dental pulp[3], and Wharton's jelly[4]. They can also be generated from human embryonic stem cells (hESCs), including induced pluripotent stem cells (iPSCs).[5] The International Society for Cellular Therapy (ISCT) guidelines suggest that human MSCs must: (i) express CD105, CD73, and CD90; (ii) lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules; (iii) adhere to plastic, and (iv) have the capacity to differentiate into adipocytes, osteoblasts, and chondrocytes in vitro.[6] MSCs are also capable of modulating inflammatory responses, and this activity is believed to be mediated at least in part through the MSC secretome.[7] This includes the release of extracellular vesicles—primarily microvesicles (MVs) and exosomes. MVs (50–1,000 nm in diameter)[8–10] are shed directly from cell plasma membranes (PMs), whereas exosomes (50–150 nm in diameter)[9,10] are intraluminal vesicles (ILVs) formed by inward budding of early endosomal membranes and released through fusion with PM. The exosomal biogenesis pathway in MSCs involves packaging of signaling molecules—including nucleic acids (mRNA/microRNA), cytokines, metabolites, and enzymes—for transfer to recipient cells.[6] MSC-derived exosomes have received substantial interest for their potential use as cell-free therapeutics. Here, we will focus on their biogenesis, cargo, and impact on physiological processes.

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Biogenesis, Release, and Uptake of Extracellular Vesicles

The biogenesis of MVs and exosomes utilizes different cellular pathways and release mechanisms*. MVs are formed via the outward budding of the PM and the sequestering of export-tagged molecules to microdomain sites. Additional clustering of membrane proteins, cytoplasmic cargo, and cellular machinery triggers MV release via PM shedding.

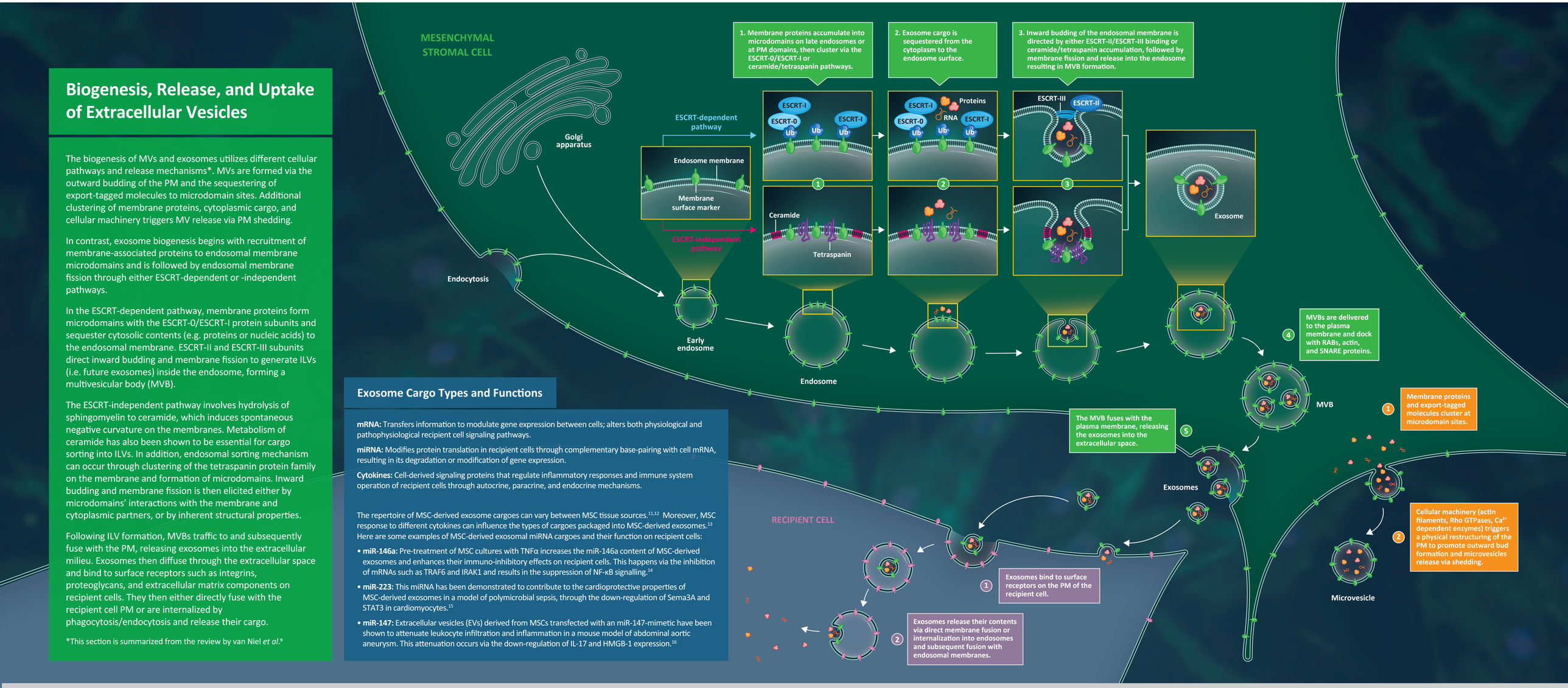
In contrast, exosome biogenesis begins with recruitment of membrane-associated proteins to endosomal membrane microdomains and is followed by endosomal membrane fission through either ESCRT-dependent or -independent pathways.

In the ESCRT-dependent pathway, membrane proteins form microdomains with the ESCRT-0/ESCRT-I protein subunits and sequester cytosolic contents (e.g. proteins or nucleic acids) to the endosomal membrane. ESCRT-II and ESCRT-III subunits direct inward budding and membrane fission to generate ILVs (i.e. future exosomes) inside the endosome, forming a multivesicular body (MVB).

The ESCRT-independent pathway involves hydrolysis of sphingomyelin to ceramide, which induces spontaneous negative curvature on the membranes. Metabolism of ceramide has also been shown to be essential for cargo sorting into ILVs. In addition, endosomal sorting mechanism can occur through clustering of the tetraspanin protein family on the membrane and formation of microdomains. Inward budding and membrane fission is then elicited either by microdomains' interactions with the membrane and cytoplasmic partners, or by inherent structural properties.

Following ILV formation, MVBs traffic to and subsequently fuse with the PM, releasing exosomes into the extracellular milieu. Exosomes then diffuse through the extracellular space and bind to surface receptors such as integrins, proteoglycans, and extracellular matrix components on recipient cells. They then either directly fuse with the recipient cell PM or are internalized by phagocytosis/endocytosis and release their cargo.

*This section is summarized from the review by van Niel *et al.*[9]



Exosome Cargo Types and Functions

mRNA: Transfers information to modulate gene expression between cells; alters both physiological and pathophysiological recipient cell signaling pathways.

miRNA: Modifies protein translation in recipient cells through complementary base-pairing with cell mRNA, resulting in its degradation or modification of gene expression.

Cytokines: Cell-derived signaling proteins that regulate inflammatory responses and immune system operation of recipient cells through autocrine, paracrine, and endocrine mechanisms.

The repertoire of MSC-derived exosome cargoes can vary between MSC tissue sources.[11,12] Moreover, MSC response to different cytokines can influence the types of cargoes packaged into MSC-derived exosomes.[13] Here are some examples of MSC-derived exosomal miRNA cargoes and their function on recipient cells:

- **miR-146a:** Pre-treatment of MSC cultures with TNFα increases the miR-146a content of MSC-derived exosomes and enhances their immuno-inhibitory effects on recipient cells. This happens via the inhibition of mRNAs such as TRAF6 and IRAK1 and results in the suppression of NF-κB signalling.[14]
- **miR-223:** This miRNA has been demonstrated to contribute to the cardioprotective properties of MSC-derived exosomes in a model of polymicrobial sepsis, through the down-regulation of Sema3A and STAT3 in cardiomyocytes.[15]
- **miR-147:** Extracellular vesicles (EVs) derived from MSCs transfected with an miR-147-mimetic have been shown to attenuate leukocyte infiltration and inflammation in a mouse model of abdominal aortic aneurysm. This attenuation occurs via the down-regulation of IL-17 and HMGB-1 expression.[16]

STEMCELL Technologies is committed to supporting scientists along the basic to translational research continuum by providing high-quality standardized media and reagents for MSC and EV research.

MesenCult™-ACF Plus Culture Kit (Catalog #05448): Use this EV- and animal component-free kit to derive and expand human MSCs. Cells cultured in MesenCult™-ACF Plus expand faster compared to cells cultured in serum-based media and demonstrate robust trilineage differentiation potential. MesenCult™-ACF Plus supports the generation of functional MSC-derived EVs. The EV content of MesenCult™-ACF Plus, as determined by the presence of tetraspanin proteins CD9, CD63 and CD81, is below the detection limit by western blot.

EasySep™ Human Extracellular Vesicle Positive Selection Kits: EVs can be easily and quickly isolated from plasma and culture-conditioned medium using the new EasySep™ kits. Four different selection kits are available, with immunomagnetic capture of EVs based on the expression of tetraspanin proteins CD9, CD63 or CD81, or pan-tetraspanin expression.

Visit www.stemcell.com/MesenCult and www.stemcell.com/EasySepEV for additional information on all products and resources available to help your MSC and EV research, including cell enrichment and selection kits, antibodies, and a range of primary cell products. You can also contact our technical support team for detailed protocol information at techsupport@stemcell.com.

Abbreviations

ESCRT: Endosomal sorting complexes required for transport; EV: Extracellular vesicle; hESCs: Human embryonic stem cells; HLA-DR: Human leukocyte antigen-DR isotype; HMGB1: High mobility group box 1; IL-17: Interleukin 17A; ILV: Intraluminal vesicles; iPSCs: Induced pluripotent stem cells; IRAK1: Interleukin 1 receptor associated kinase; miRNA: MicroRNA; mRNA: Messenger RNA; MSCs: Mesenchymal stromal cells; MV: Microvesicles; MVB: Multivesicular body; NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B cells; PM: Plasma membrane; Rho: Ras homologous; SEMA3A: Semaphorin-3A; STAT3: Signal transducer and activator of transcription 3; TNFα: Tumor necrosis factor alpha; TRAF6: TNF receptor associated factor 6; Ub: Ubiquitin.

Current Protocols has several in-depth protocols for the isolation and characterization of mesenchymal stromal cell and tissue-derived exosomes.

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