Journal of Experimental and Basic Medical Sciences 2024;5(1):19-32

Review

Exosomes and Microvesicles

Dildar Savcı[1], Gamze Işık[1], Saliha Hızlıok[1], Oytun Erbaş[1]

ABSTRACT

Extracellular vesicles (EVs) are released by all cells and have a variety of physiological functions. Microvesicles (MV), exosomes, oncosomes, and apoptotic bodies, are the four forms of EVs. Exosomes are spherical EV with an endocytic double-layered lipid membrane. As for their identification, it's based on isolation methods, cell type, and cell surface markers. Moreover, their functions differ according to the cell from which they are obtained. They are involved in the immune system, neural communication, drug delivery, and many other processes. Another type of EV is MVs, which are a diverse collection of vesicles formed by the outward budding of the plasma membrane. Differentiating these two types of EVs is mostly based on the process of biogenesis. MVs play a variety of pathological and physiological roles. MVs were hypothesized to have a role in the immunomodulatory function of mesenchymal stem cells (MSCs). Following that, it was discovered that MVs secreted by damaged cells send precise signals to MSCs. Given their physiological contact with the target cell, MVs are regarded to be therapeutic targets in cancer treatment. This chapter provides information about exosomes and microvesicles, which are extracellular vesicles.

Keywords: Exosomes, extracellular vesicles, stem cell, mesenchymal stem cell, microvesicle.

Extracellular vesicles (EVs) are secreted by practically all cell types as a continuation of membrane structures and are responsible for many vital physiological functions. Exosomes, a form of EVs, were first observed in the 1980s. These nano-sized spherical vesicles secreted by cells contain a wide variety of lipids and proteins as well as nucleic acids in their composition and can fulfill a broad range of functions depending on the source from which they are obtained.[1-9]

Waste molecule removal, intercellular contact, cargo package transportation, immune system, reproduction and development, neural communication, and cell proliferation are some of the processes these organelles can participate in.[2,4]

Microvesicles (MVs), another type of EVs, are formed directly by the budding of the cell membrane. Although MVs resemble exosomes in many ways, these vesicles have different properties and functions that distinguish them, including size distribution and biogenesis mechanism.[10-13] Moreover, having the ability to transport various bioactive molecules, MVs are involved in many pathological and physiological events as well as intercellular interactions.[14-17] Different extracellular factors, in addition to cytoskeletal components, molecular motors, and numerous signaling molecules, influence the formation of microvesicles.[13,18]

Mesenchymal stem cells (MSCs), which can differentiate into several lineages and play a key role in the physiological system in addition to their ability to self-renew, can also release various chemicals that influence the immune system. It is considered that EV secretion may influence MSCs' numerous physiological effects.[19-25] The involvement of EVs in crucial events and processes including inflammation, fibrosis, and cancer, implies that they may play a role in disease development. Furthermore, stem cell-derived EVs have been shown to be beneficial in numerous treatment processes.[26,27]

[1]ERBAS Institute of Experimental Medicine, Illinois, USA & Gebze, Türkiye

Correspondence: Dildar Savcı. Institute of Experimental Medicine, 41470 Gebze-Kocaeli, Türkiye

E-mail: ddildarsavci@gmail.com

Cite this article as: Savcı D, Işık G, Hızlıok S, Erbaş O. Exosomes and Microvesicles. JEB Med Sci 2024;5(1):19-32.

doi: 10.5606/jebms.2024.1069

Received : October 19, 2023
Accepted : October 24, 2023
Published online : February 26, 2024

EXOSOMES

Exosomes are nano-sized endocytic EV with a diameter of 30-100 nm, float at a density of 1.13-1.19 gmL-1 in a sucrose gradient solution, and are attached to a double-layered lipid membrane.[2-6] They are classified based on the cell type from whence they were derived, isolation techniques, size, and cell surface indicators.[2,5]

Exosomes are composed of a variety of molecules including nucleic acids like DNA and RNAs such as microRNA (miRNA), long non-coding RNA (lncRNA), non-coding RNA (ncRNA), circular RNA (circRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), messenger RNA (mRNA), piwi-interacting RNA (piRNA) and more. various lipids such as cholesterol, ceramide, sphingomyelin, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylethanolamine, and glycosphingolipid, along with a range of proteins like transmembrane protein, heat shock proteins (HSP), cytoskeletal proteins such as actin, cophilin, and tubulin, glycoproteins, there are also several molecules and complexes necessary for certain intra- and extracellular activities, including endosomal sorting complex required for transport (ESCRT) components, include ALG-2-interacting protein X (Alix) and tumor susceptibility gene TSG-101 as well as proteins involved in membrane trafficking and fusion such as GTPases, annexin, Rab, dynamin, and syntaxin, other molecules essential for the immune system like, cytokines, Tumor necrosis factor-alpha, Transforming growth factor (TGF)-beta, and TNF-associated apoptosis-inducing ligand (TRAIL), Major histocompatibility complex (MHC) class I and II, cluster of differentiation (CD) molecules including CD9, CD37, CD53, CD63, CD81, and CD82, signaling receptors Fas ligand (FasL), TNF receptor and Transferrin receptor.[28-30] Together with different types of biomolecules involved in transcription and protein syntheses such as Histone1,2,3 and Ubiquitin.[7-8] In exosomes, tetraspanins, antigen-presenting molecules, glycoproteins, and adhesion molecules are located among the transmembrane proteins, while HSPs, cytoskeletal proteins, components of the ESCRT mechanism, proteins responsible for membrane transport and fusion, cytokines, and developmental factors are located in the lumen.[8]

Those biomolecules cover a vast variety of activities, for instance, integrins, lactadherin, and intracellular adhesion molecule-1 are involved in cell-cell communication, Tsg101, Aliks, Rab proteins, involved in multivesicular body biogenesis, lysosomal-associated membrane proteins 1/2, CD13 and PG regulator-like protein, on the other hand, are essential for membrane transport.[31] Moreover, there are molecules involved in transcription and protein synthesis, proteins responsible for signaling, aspartate aminotransferase, aldehyde reductase, ATPase, and similar metabolic enzymes, antiapoptotic proteins like Aliks, Thioredoxin, peroxidase; death receptors FasL, TNF-associated apoptosis-inducing ligand also, transferrin receptor which is involved in iron transport.[6,31]

In the same context, Leukotrienes (LTs) like LTA4, LTB4, LTC4, and related LTA4 Hydrolase and LTC4 synthase enzymes are associated with triggering polymorphonuclear leukocyte migration in exosome functioning, cyclooxygenase isoenzymes (COX-1 and -2) are engaged in immunosuppression, and prostaglandin 2 (PGE2), as well as PGE synthase enzyme, are involved in inflammation.[32-35] Additionally, phosphatidic acid, phospholipase (PLD) 2, and diglyceride kinase increase exosome production; arachidonic acid, lysophosphatidylcholine, calcium-dependent PLA2, and calcium-independent PLA2 participate in the formation of membrane curvature and PLA- II, and V in PGE biosynthesis; and while ceramides are involved in the sorting of cargo into multivesicular bodies (MVBs), Bis (monoacylglycerol) phosphate is involved in the formation of them, phosphatidylserine (PS) determines exosome fate and sphingomyelins are known to be associated with the triggering of calcium influx.[6]

Exosome Biogenesis

Structurally, exosomes consist of early endosomes, which are intracellular, small bodies formed by the inward budding of the plasma membrane due to the drawing of intracellular fluid. Early endosomes enter the maturation process, where late endosomes are formed.[36] Intraluminal vesicles (ILVs) form as late endosomal membranes invaginate, and as ILVs accumulate, MVBs rich in ILVs form.[8,37]

The newly formed MVBs either go through hydrolyzation and fragmentation by fusion with lysosomes; or get transported to the plasma membrane via the cytoskeleton and microtubular network, where they undergo ion-dependent fusion and are released as exosomes into the extracellular space.[2,8,38]

The ESCRT is a biogenesis and secretion process. This mechanism is made up of four complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, as well

as vacuolar protein Sorting-associated protein 4A (VPS4A), Tsg101, and ALIX.[7] The MVB pathway is initiated by ESCRT-0 conveying ubiquitinated cargo proteins via their lipid domains. Following that, ESCRT-I and II drive the inward budding and induce stabilization, and ESCRT-III detaches from the MVB membrane via VPS4A, completing its role in membrane remodeling.[6,7,38] Tsg101 ensures that ESCRT-1 is active in the process by connecting with ESCRT-0, whereas ALIX is a protein implicated in ILV production by binding ESCRT-III subunits.[39]

Independent of the ESCRT mechanisms involved in cargo loading, endosomal sorting pathways, exosome release, and exosome biogenesis, there is a different pathway called tetraspanin, in which exosome-rich transmembrane proteins and lipids are involved.[37,38]

Tetraspanin-rich microdomains (TEMs) are involved in the classification of membrane-bound receptors and signaling proteins and tetraspanin CD81 participates in the sorting of intracellular components of TEMs and target receptors towards exosomes and in exosome biogenesis; meanwhile, CD63 is found to help in the loading of exosomal proteins, with Epstein-Barr virus being an example, additionally, EVs are reduced as a result of CD63 knockout, indicating that CD63's involvement in exosome biogenesis; Furthermore, tetraspanins are valuable exosome biomarkers.[40,41]

Another mechanism independent of ESCRTs that plays an important role in secretion is the sphingolipid (ceramide Cer) method where ceramides are produced by sphingomyelinase or neutral sphingomyelinase which are involved in the budding of intracellular vesicles into MVBs, and inhibition of neutral sphingomyelinases results in a decrease in exosome secretion.[38,42-45]

Aside from that, miRNAs are considered another way of sorting cargo, given exosomes are enriched with them. Thanks to a specific motif contained in miRNA, A2B1 ribonucleoprotein interacts with miRNAs and plays a role in their loading. The KRAS gene is also known to be involved in this process. Likewise, it is thought that mRNAs can be separated into vesicles due to the enrichment of fragments at their 3' ends.[37]

More importantly, it has been proposed that the Trans-Golgi network, Rab-GTPase, Rab family proteins, p53 activation, microtubules, microfilaments, SNARE complex, Na+/Ca2+ channels, H+ pumps, pH level, calcium ionophores, statins, heparanase, molecular motors, and tethering factors are all involved in the release of exosomes.[2,7,37,38]

As for the exosomes' entry to the target cells, there are three different ways. The first is direct interaction, where the ligands in exosomes bind to the receptor of the target cell.[46] The second is fusion with the plasma membrane, where the exosomes fuse with the membrane of the target cell via adhesion molecules.[47] Another method is internalization, which is divided into five subdivisions. In this method, exosomes are introduced into target cells by clathrin-mediated endocytosis, lipid rafts-mediated endocytosis, caveolin-mediated endocytosis, phagocytosis of the exosome in the target cell, and finally, macropinocytosis, in which macropinosomes are formed by inducing invagination of the plasma membrane using actin-driven lamellipodia.[48]

Exosome Functions and Relationship with Stem Cells

Exosomes are linked to various functions depending on the source of the cell from which they are obtained.[9] Including removal of unnecessary molecules, intercellular communication and transport, immune system, reproduction and development, neural communication, cell proliferation-homeostasis maturation, and drug delivery, besides functioning as vaccines and biomarkers.[2,4]

B cells and dendritic cells-derived exosomes provide co-stimulatory proteins to activate T cells together with tumor antigens, resulting in anti-tumoral and immunostimulatory effects. These effects are observed in melanoma, prostate, lymphoma, and kidney cancer patients.[49]

Additionally, the epithelial cell adhesion molecule (EpCAM) and CD24 found in tumor-derived exosomal (TD-exosomes) miRNAs act as an essential diagnostic for the early identification of ovarian cancer and detection of TD-exosomes in general.[50] likewise, exosomal miRNAs in saliva serve as a biomarker of aging and as a marker for diagnosing various central nervous system diseases, cancer types, and cardiovascular diseases. Moreover, the ones found in amniotic fluid serve as a biomarker of fetal sex.[51,52]

As for their role in the immune system, they participate in a range of functions including acting as an immune activator, and immune suppressor, taking part in antigen-presenting, immune tolerance, immunoregulation, as well as establishing communication between mast cells, dendritic cells, natural killer cells (NKs), and antigen-presenting cells

(APCs).[5] It was demonstrated that exosomes released *in vivo* from rodent mast cells promote dendritic cell maturation and present antigens to T lymphocytes, the ones released from immunosuppressive dendritic cells suppress autoimmunity, and finally, exosomes derived from bone marrow-derived dendritic cells showed its ability to inhibit the development of inflammatory bowel disease.[53-56]

An excellent example of exosome involvement in immunosuppression is helping neoplastic lesions to form and grow *in vivo*, leading to a decreased proliferation and cytotoxicity of NKs and T cells, as well as decreased numbers of APCs.[5] These effects are accomplished through autocrine signaling depending on the type of cell line from which the exosomes originate. For instance: exosomes from pancreatic cancer cells can inhibit the growth of some neoplastic lesions by inducing apoptosis via the Notch signaling pathway, whereas exosomes from breast cancer and gastric cancer promote neoplastic lesions.[55] They are also implicated in developing a matrix for neoplastic cell adhesion, aiding their movement to the sentinel lymph node, promoting angiogenesis, and establishing an environment that encourages metastases by suppressing the immune system.[5,57]

Moreover, the significance of exosomes in the nervous system's signaling between sensory and motor neurons, interneurons, and glial cells, as well as their slowing influence on the hypothalamus's endocrine function concerning aging, added to that, the exacerbation of some diseases such as Alzheimer (AD), Huntington, and Parkinson's due to the release of amyloid beta peptide, misfolded proteins, and prions by exosomes, suggests the role the exosomes play in the pathogenesis of neurodegenerative diseases.[4,9,58]

As for the process of homeostasis and cell maturation, exosomes reduce intracellular stress by clearing toxic materials and selectively removing unwanted cargo in the maturation process of reticulocytes to erythrocytes, maintain homeostasis by secretion of exosomes, however, when secretion is suppressed, the innate immune response is exacerbated, causing reactive oxygen species (ROS)-dependent DNA damage responses and inducing apoptosis.[59]

Interestingly, exosomes contribute to reproductive and developmental processes such as sex cell maturation, cell behavior, horizontal transfer of genetic material, protection of the fetus by reducing immune cell functions during pregnancy, organ development, protection of the placenta against infections with exosomal miRNAs and resident immunity in the genital area, fertilization, and embryo implantation. Likewise, exosomes in breast milk, sometimes called tolerosomes, influence immunological tolerance modulation and development.[60-63] In addition, TD-exosomes can be employed as immunotherapeutic vaccines against cancer with modifications of various antigen-presenting proteins due to their effects on the immune system.[2,4] In that context, exosomes derived from intestinal epithelial cells induce immunotolerance in an antigen-specific manner during pregnancy, whereas exosomes derived from pancreatic carcinoma cells induce tolerance by stimulating migration in NKs, exosomes derived from breast milk are involved in immune tolerance regulation.[63,64] Moreover, breast tumor-derived exosomes have a role in the regulation of the immune system. The FasL on the exosomes' surface demonstrates immunosuppressive effects by inducing apoptosis.[2] In contrast, Some tumor-derived antigen-containing exosomes suppress tumor growth by triggering antitumoral T-cell responses. And on top of that, exosomes generated from MSCs have immunomodulatory and cytoprotective properties and can even be used as therapeutic agents.[65,66] Several studies proposed that exosomal miRNAs increase anticancer activity in mammary carcinoma and glioma and that dendritic cell-derived exosomes modified with rabies virus glycoprotein have therapeutic effects.[67-69]

Exosomes released by human retinal pigment epithelial cells (ARPE-19) in response to oxidative stress were shown to promote apoptosis and inflammatory responses.[9,70]

Furthermore, exosomes can act as intermediaries in drug delivery due to their biodistribution and biocompatibility properties, their ability to cross the blood-brain barrier, and their immunogenicity.[9] For instance, RVG exosomes are used to reduce the loss of dopaminergic neurons in the treatment of parkinsonism, while cisplatin-loaded M1 exosomes are given as chemotherapeutics in ovarian cancer.[71,72]

Exosomes originating from immature dendritic cells also play a role in targeted drug delivery due to their low immunogenicity and toxicity. superparamagnetic iron oxide nanoparticles (SPION)-equipped exosomes, significantly inhibit the growth of tumor cells by inducing the apoptotic pathway.[73] It was found that quercetin-loaded exosomes potentiate brain targeting of the drug in AD and are involved in the amelioration of cognitive

dysfunctions.[9] Further, increasing the solubility of resveratrol in primary microglia-derived exosomes in spinal cord injuries has the potential to boost targeted drug delivery and improve neuronal function.

Additionally, catalase-loaded exosomes showed neuroprotective effects in a mouse model of Parkinson's disease, and treatment with gemcitabine-loaded autologous exosomes resulted in tumor suppression in tumor-bearing mice.[74,75]

As for stem cell-derived exosomes effects, human adipose tissue-derived MSCs (hADSCs) and human bone marrow-derived MSCs (hBM-MSCs) exosomes were found to be beneficial in AD, with the former being more effective due to the noticeable reduction in amyloid beta they cause.[76] Meanwhile, human menstrual blood MSCs (MenSCs) and hBM-MSCs-exosomes help neurite outgrowth in cortical and sensory neurons unlike exosomes derived from the human chorionic plate MSCs and human umbilical cord MSCs (hUC-MSCs) in neurodegenerative diseases. In comparison with exosomes obtained from human synovial membrane-derived MSCs (SMSCs), exosomes from induced pluripotent stem cells (iPSCs)-derived MSCs have more significant therapeutic effects with chondrocyte migration and proliferation in osteoarthritis disease.[77,78]

The anti-inflammatory properties of MSC exosomes are mediated by macrophage polarization from the pro-inflammatory phenotype M1 to the anti-inflammatory M2 macrophages. Exosomes derived from human jaw BM-MSCs and BM-MSCs, accelerate wound healing and decrease bronchopulmonary dysplasia, and exosomes obtained from MenSCs reduce inflammation and repair wounds in diabetic rats. They are both examples of M2 macrophage polarization.[79] Furthermore, it was reported that hUC-MSCs-exosomes' immunosuppressive capabilities alleviate inflammation, help in diabetic cutaneous conditions, enhance wound healing and burn inflammations, and exosomes from mouse BM-MSCs (mBM-MSCs) regress osteosclerosis, and decrease atherosclerotic plaques, myocardial ischemia-reperfusion damage, and infarct size.[80-82]

Exosomes derived from hBM-MSCs are therapeutically effective by inducing M2 macrophages in intestinal bowel disease in mice and are also useful in improving motor skills in a mouse model of multiple sclerosis.[83] On top of that, exosomes derived from ADSCs depending on the species from which they are derived can demonstrate various effects including, showing a decrease in cardiac damage, fibrosis, and apoptosis when derived from rats; increasing the expression of M2 macrophages when derived from humans; increasing the polarization of M2 macrophages in obese mice when derived from mice; and are effective in the treatment of autoimmune type 1 diabetes mellitus.[84,85] Exosomes from hBM-MSCs and hADSCs not only help reduce skin photoaging ensure skin flap longevity and reduce inflammation respectively, but also they are useful in reducing the pathological symptoms of atopic dermatitis, meanwhile, exosomes from rat BM-MSCs (rBM-MSCs) help improve regeneration with a decrease in histopathological findings, blood urea nitrogen, creatine levels, oxidative stress, apoptosis, and inflammation in kidney injury.[86-89] Human placental MSCs exosomes are reported to be useful in reducing tissue fibrosis and inflammation in Duchenne muscular dystrophy.[90] In neonatal mice, models of bronchopulmonary dysplasia, exosomes from hUC-MSCs exhibit improvements in lung, heart, and brain pathologies, and in post-stroke neurodegeneration, hBM-MSCs-exosomes can demonstrate neuroprotection, induction of neurogenesis, and angiogenesis. In addition to that, in diabetic peripheral neuropathy, exosomes derived from mBM-MSCs are capable of increasing the nerves' conduction velocity, intraepidermal nerve fibers' density, myelinization, and axon diameter, as well as preventing disease progression in intervertebral disc degeneration.[91,92]

Along with that, inhibition of cardiac fibrosis, reduced inflammation, improvement in cardiac function in myocardial infarction, as well as induced survival and injury healing with decreased apoptosis and inflammation in acute lung injuries, were reported as impacts of rBM-MSCs-exosomes.[93,94] In graft versus host disease (GVHD), exosomes derived from hUC-MSCs and hBM-MSCs modulate immune cells and prevent acute GVHD. While, in status epilepticus, they reduce inflammation in the hippocampus, decrease glutamatergic and gamma-aminobutyric acid (GABA) loss, and improve learning and memory disorders.[95,96] With similar effects, exosomes derived from rBM-MSCs and hADSCs enhance spatial learning and motor behavior in traumatic brain injury, reduce neural inflammation, increase cognitive performance, increase white matter connectivity, improve autistic-like behaviors, and improve social behaviors in Autism spectrum disorder.[77,97] Besides, exosomes derived from human Wharton's jelly MSCs, also known as UC-MSCs, help reduce neuroinflammation and neuron-specific cell death in perinatal brain injury.[98]

Exosomes inhibition of the M1 phenotype in macrophages, and promotion of M2 polarization, along with the suppression of macrophages migration, lead to suppression of proliferation, activation, migration, and cytotoxicity of NKs; dendritic cells maturation and activation suppression, increasing in Treg cells; inhibition of proliferation, differentiation, and immunoglobulin secretion in B cells, in addition to suppression in T cells proliferation and activation.[99]

With that mechanism, MSC-exosomes prevent inflammation in the central nervous system and relieve neurobehavioral symptoms in multiple sclerosis. When they target the spinal cord, they reduce inflammation by the inflammatory inactivation of NALP3 autoimmune damage is prevented by inducing Treg cells with anti-inflammatory factors when T and B lymphocytes are targeted. Further, when the inflammation of islet cells is prevented, it leads to an increase in plasma insulin levels; likewise, they promote the recovery of cognitive impairment by repairing neurons and astrocytes.[100-105] Similarly, when exosomes target suppressor cells of myeloid origin in Sjögren's Syndrome, the disease's progression is slowed by raising levels of reactive oxygen species and nitric oxide, which is accomplished by activating the Janus Kinase 2/ Signal Transducer and Activator of Transcription 3 (JAK2/STAT3) signaling pathway, and when salivary gland epithelial cells are targeted, serum autoantibody levels are suppressed by preventing APC activation.[106]

Using exosome targeting abilities, RNA loading is becoming a popular application in drug delivery. Nowadays, BM-MSCs-exosomes can be applied for various tumors treatments like loading miRNA for glioma inactivation, reducing breast cancer activation via miRNA-379, playing a neuroprotective role via miRNA-133b, promoting angiogenesis via miRNA-132, and repairing spinal cord injury via miRNA-29b, while, hUC-MSC-exosomes are used for the inhibition of pancreatic ductal adenocarcinoma via miRNA-145-5p, treatment of aging-related vascular dysfunction via miRNA-675, treatment of diabetes via miRNA-21. Additionally, dental pulp MSCs suppress the proliferation of breast cancer cells via miRNA-34a, and SMSCs are involved in the treatment of osteoarthritis via miRNA-155-5p.[107-113]

In a similar mechanism, BM-MSC-exosomes are loaded into Norcantharidin to treat hepatocellular carcinoma and into Doxorubicin to treat osteosarcoma; hUC-MSCs-exosomes into Paclitaxel to target breast cancer; and MSC-exosomes are loaded into Honokiol to treat some cancer cell lines.[114]

Aside from that, they are loaded into proteins. Exosomes obtained from MB-MSC are used in the treatment of cystic fibrosis by loading zinc finger protein and for anti-tumoral purposes with TRAIL proteins; while exosomes obtained from hUC-MSCs increase angiogenesis with Angiopoietin-2 protein and play a role in the progression of cardiac regeneration through Akt protein.[115,116]

MICROVESICLES

Microvesicles are a diverse set of membrane-enclosed vesicles that are discharged into the extracellular space as a result of the plasma membrane being compressed by budding outward. Their diameters range from 100 to 1000 nm.[10,11] Surface markings distinctive to MVs are absent. They can, however, express the cell surface markers from which they are produced.[14] DNA, mRNA, miRNA, and proteins are examples of bioactive molecules that can be transported by MVs. They act as a conduit for communication between cells.[12,14,17] As a result, they can play a role in a variety of physiological and pathological processes, including cell apoptosis, autophagy, tumor development, and metastasis.[14-16]

Microvesicle Biogenesis

One of the main points in the classification and naming of EVs is their biogenesis mechanism. It has been shown that the biogenesis mechanism of MVs is different from the biogenesis mechanism of other EVs such as exosomes, in which intracellular events are effective in their formation, and apoptotic bodies, which are formed indiscriminately by bubbling of almost any surface.

The biogenesis mechanism of EVs is one of the key factors in their classification and nomenclature. Further, it has been demonstrated that the biogenesis method of MVs differs from that of other EVs such as exosomes, which are effectively generated by intracellular events, and apoptotic bodies, which are created randomly by outward budding of virtually any surface. In contrast to exosomes, which are formed by the continuation of intracellular events, MV biogenesis involves budding and compression directly outward from the plasma membrane. In this process, the vertical transport of molecular cargo across the plasma membrane and the use of contractile mechanisms for vesicle compression can be observed.[117-119]

Another point that distinguishes MVs from the better-characterized exosomes is the size difference arising from their formation mechanism.

Since MVs are formed by directly bulging from the cell membrane, they are larger than exosomes and can be found in a wider range of diameters.[11-13] However, the biogenesis mechanism is primarily taken into account in the separation of the two types of EVs due to their common size range.[18,120]

Biofluids such as urine, blood, and cerebrospinal fluid can also be a source for EVs, and the sources from which they are obtained may play a major role in their naming. However, the fact that some cells such as endothelial cells, platelets, and cancer cells can release exosomes as well as MVs into the circulation makes it difficult to distinguish between them both. Nevertheless, EVs can also be named variously in the literature as epididymosomes, argosomes, exosome-like vesicles, microvesicles, archaeosomes, promininosomes, dexosomes, exosomes, nanovesicles, apoptotic bodies, and oncosomes depending on the isolation source or isolation method.[11,120-122]

Depending on their cell of origin, MVs, which may have a plasma membrane, endosome, cytosol, or other cellular protein contents and molecular loads, are released directly into the extracellular space and shed from the surface of the cells. Microvesicles, which mostly contain proteins clustered on the surface of the plasma membrane, may also contain some marker proteins and various proteins with translational modifications and therefore are thought to be used as biomarkers.[11-13]

Regulation of microvesicle formation is a process involving various cell molecules including cytoskeletal components such as actin and microtubules and molecular motors such as myosins. Here, Ras homology family GTPases (Rho) play a critical role in actin reorganization, and thus, MV secretion.[13,18,27,123].

At the same time, studies are showing that the redistribution of heterogeneous phospholipids in the cell, and mainly the translocation of PS to the outer leaflet leads to the formation of MVs.[11,120]

Regulation of the process of microvesicle release, however, is achieved by a signaling cascade that starts with PLD activation in the cell. Here, as a result of the activity of ADP-ribosylation factor 6, one of the GTP-binding proteins, extracellular signal-regulated kinase is recruited to the plasma membrane and phosphorylation of myosin light chain and MV release occurs with its increased activity.[120,124] In addition, RhoA/Rho-associated kinase signaling is thought to support MV release via a different pathway.[11,18,27]

The number of MVs formed varies depending on the physiologic state of the released and retrieved cells as well as their microenvironment. More importantly, temperature and extracellular calcium concentration are thought to be effective in the formation of these vesicles.[13,18] Microvesicles content can also be analyzed to understand the mechanisms involved in the release of MVs loaded with various proteins and nucleic acids.[11,124]

While it was initially thought that MVs, like most EVs, were involved in the removal of waste material from the cell, it was revealed that these vesicles, like exosomes, have an important role in intercellular interaction.[11,125] With this discovery, interest in MVs has increased and the processes of packaging and delivering active cargoes containing various proteins, nucleic acids, and lipids to the target cell have begun to be investigated. Additionally, it is thought that glycan-binding proteins on the surface of MVs may be an effective factor, especially in the transportation of MVs to target cells and regulation of the function of the recipient cell.[13,126]

Relationship of Microvesicles with Stem Cells

Stromal cells, also known as MSCs, were initially identified only in the bone marrow.[127] Nevertheless, to date, they have so far been isolated from a wide variety of other tissues, including umbilical cord blood, Wharton's jelly, and placental, adipose, and lung tissue.[128,129]

The perspective on the nature and function of MSCs has undergone several paradigm shifts over time. They play multiple roles in the physiological system as well as their ability to differentiate into multiple lineages and contribute to organized cell replacement therapy.[19] They not only have the ability to self-renew by undergoing multiple cell divisions but also exhibit anti-inflammatory and immunosuppressive properties by directly interacting with several immune cells.[20,21] Moreover, MSCs have provided short-term therapeutic benefits to numerous diseases.[130,131] To reconcile these different findings, it has long been known that MSCs produce growth factors and cytokines, many of which modulate the immune system. However, despite this knowledge, most of these proteins lack signal peptides. The fact that they are packaged in membrane-bound vesicles with mRNAs and miRNAs partly explains how MSCs can exert multiple effects on the physiological system.[19,22-26]

Bioactive molecules secreted by MSCs can function as paracrine or endocrine mediators that modulate

immune responses, interact with neighboring cells, and promote self-repair.[129]

According to several recent researches, MSCs' capacity to release MVs may play a role in mediating some of their immunomodulatory function.[132] Therefore, MVs released by injured cells may provide specific signals to stem cells that enable their differentiation. They may also represent a mechanism involved in physiologic tissue repair.[133] Stem cell-derived MVs can cause changes in the phenotype of tissue cells that regulate regeneration and cell differentiation.[134] It has been shown that MVs isolated from MSC culture supernatants may perfectly replicate the inhibitory effects of MSCs on CpG-induced B cell proliferation and differentiation in the co-culture system of peripheral blood mononuclear cells in a dose-dependent way. Allogeneic and syngeneic T lymphocyte proliferation has been shown in certain investigations to be effectively inhibited by MVs derived from BM-MSCs. Additionally, it was shown that these microparticles can cause activated T-cells to undergo apoptosis. Additionally, spleen cells grown with MSC-MVs showed enhanced production of TGF-1 and IL-10. These findings imply that tolerogenic signaling may be induced by MSC-MVs.[132]

Numerous studies have demonstrated that EVs play a key role in numerous crucial cellular processes and events, including fibrosis, cancer, and inflammation. Considering different pathological situations, EVs may also effectively contribute to the onset of illness.[135,136]

Some of the EVs released by tumor cells influence their microenvironment, promote immune suppression, and promote the proliferation and spread of cancer cells.[136-141] The fact that EVs promote the spread of cancer while simultaneously offering hope for a cure for the disease is particularly intriguing.[142-144]

The potential of tumor cells to produce MVs, like many other cells, raises the possibility that MVs play a significant role in the development, progression, and dissemination of cancer.[135,137,145-147] The thought that MVs might be exploited as potential therapeutic targets in the treatment of cancer boosts interest in them given their capacity to package and securely deliver a variety of cargo, including nucleic acids, to the target cell as well as their function in intercellular communication.[148-152] In particular, MSCs can be treated with chemotherapeutics and the treatment process can be shortened with drug-carrying EVs released by them. [153,154]

Stem cells have a significant role in cancer in addition to microvesicles. It has been found that MVs are effective in protecting cancer stem cells, which are known to have a significant impact on the development of tumors in liver cancer, one of the malignant malignancies. Additionally, it is believed that cell-to-cell pathways created by EVs safeguard the stem cell lineages of ESCs.[136,137]

Even though there have been several studies on cancer, there is still a great demand for investigation into metastasis and other topics. The importance of gaining MVs is rising at this stage. Patients' serum may simply be used to isolate MVs, and urine and plasma can also be used in this way.[137,155,156] A liquid biopsy of cancerous tissue can also yield MVs, but this approach is still relatively new.[153,157,158]

In conclusion, the molecular makeup of exosomes and MVs and the kind of stem cell or tissue they originate from the impact their function. The capacity of EV to biodistribution, pass the blood-brain barrier and reflect the molecular characteristics of the cells from which they arise has drawn attention in pharmacology and translational medicine. Stem cells are prominent in drug delivery by loading drugs through EVs and thus play a role in the treatment of different types of cancer; at the same time, EVs have been noted to cause positive clinical effects in the treatment of neurodegenerative diseases, antitumoral effects, regeneration and repair processes by loading miRNA and protein types. As biomarkers, EVs are used in the early detection and diagnosis of diseases and even the gender determination of the fetus. As a result, it is thought that the therapeutic effects of EVs in cancer will also lead to vaccine studies. Although EV-stem cell studies offer many promising developments, their negative effects such as creating a microenvironment supporting metastasis, promoting lesions, inducing apoptosis, and immune suppressor behaviors should also be considered.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

REFERENCES

1. Stahl PD, Raposo G. Extracellular Vesicles: Exosomes and Microvesicles, Integrators of Homeostasis. Physiology (Bethesda). 2019 May 1;34:169-77.
2. Beach A, Zhang HG, Ratajczak MZ, Kakar SS. Exosomes:

an overview of biogenesis, composition and role in ovarian cancer. J Ovarian Res. 2014 Jan 25;7:14.
3. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. Int J Nanomedicine. 2020 Sep 22;15:6917-34.
4. He C, Zheng S, Luo Y, Wang B. Exosome Theranostics: Biology and Translational Medicine. Theranostics. 2018 Jan 1;8:237-55.
5. Zhang HG, Grizzle WE. Exosomes: a novel pathway of local and distant intercellular communication that facilitates the growth and metastasis of neoplastic lesions. Am J Pathol. 2014 Jan;184:28-41.
6. Old Protein, New Medicine - Brain-Derived Neurotrophic Factor [Working Title] [Internet]. Biochemistry. IntechOpen; 2024. Available from: http://dx.doi.org/10.5772/intechopen.111201
7. Yue B, Yang H, Wang J, Ru W, Wu J, Huang Y, et al. Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. Cell Prolif. 2020 Jul;53:e12857.
8. Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. Cell Commun Signal. 2021 Apr 23;19:47.
9. Gurunathan S, Kang MH, Kim JH. A Comprehensive Review on Factors Influences Biogenesis, Functions, Therapeutic and Clinical Implications of Exosomes. Int J Nanomedicine. 2021 Feb 17;16:1281-312.
10. Zhu S, Li S, Yi M, Li N, Wu K. Roles of Microvesicles in Tumor Progression and Clinical Applications. Int J Nanomedicine. 2021 Oct 18;16:7071-90.
11. Clancy JW, Schmidtmann M, D'Souza-Schorey C. The ins and outs of microvesicles. FASEB Bioadv. 2021 Mar 4;3:399-406.
12. Tricarico C, Clancy J, D'Souza-Schorey C. Biology and biogenesis of shed microvesicles. Small GTPases. 2017 Oct 2;8:220-32.
13. Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells. 2019 Jul 15;8:727.
14. Guo Y, Tan J, Miao Y, Sun Z, Zhang Q. Effects of Microvesicles on Cell Apoptosis under Hypoxia. Oxid Med Cell Longev. 2019 Apr 17;2019:5972152.
15. Noman MZ, Janji B, Berchem G, Chouaib S. miR-210 and hypoxic microvesicles: Two critical components of hypoxia involved in the regulation of killer cells function. Cancer Lett. 2016 Sep 28;380:257-62.
16. Ji Y, Ma Y, Chen X, Ji X, Gao J, Zhang L, et al. Microvesicles released from human embryonic stem cell derived-mesenchymal stem cells inhibit proliferation of leukemia cells. Oncol Rep. 2017 Aug;38:1013-20.
17. Cocucci E, Racchetti G, Meldolesi J. Shedding microvesicles: artefacts no more. Trends Cell Biol. 2009 Feb;19:43-51.
18. Sedgwick AE, D'Souza-Schorey C. The biology of extracellular microvesicles. Traffic. 2018 May;19:319-27.
19. Yildirim N, Yigitturk G, Sahingoz Yildirim AG, Akdemir A, İlgen O, Yeniel O, et al. Octreotide protects ovary against ischemia-reperfusion injury in rats: Evaluation of histological and biochemical parameters. J Obstet Gynaecol Res. 2015 Oct;41:1591-7.
20. Xunian Z, Kalluri R. Biology and therapeutic potential of mesenchymal stem cell-derived exosomes. Cancer Sci. 2020 Sep;111:3100-10.
21. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol. 2014 Nov;15:1009-16.
22. Fontaine MJ, Shih H, Schäfer R, Pittenger MF. Unraveling the Mesenchymal Stromal Cells' Paracrine Immunomodulatory Effects. Transfus Med Rev. 2016 Jan;30:37-43.
23. Galipeau J, Krampera M, Barrett J, Dazzi F, Deans RJ, DeBruijn J, et al. International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials. Cytotherapy. 2016 Feb;18:151-9.
24. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005 Feb 15;105:1815-22.
25. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol. 1996 Mar;166:585-92.
26. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol. 2014;30:255-89.
27. Sedgwick AE, Clancy JW, Olivia Balmert M, D'Souza-Schorey C. Extracellular microvesicles and invadopodia mediate non-overlapping modes of tumor cell invasion. Sci Rep. 2015 Oct 13;5:14748.
28. Villarroya-Beltri C, Baixauli F, Gutiérrez-Vázquez C, Sánchez-Madrid F, Mittelbrunn M. Sorting it out: regulation of exosome loading. Semin Cancer Biol. 2014 Oct;28:3-13.
29. Munich S, Sobo-Vujanovic A, Buchser WJ, Beer-Stolz D, Vujanovic NL. Dendritic cell exosomes directly kill tumor cells and activate natural killer cells via TNF superfamily ligands. Oncoimmunology. 2012 Oct 1;1:1074-83.
30. Van Niel G, Charrin S, Simoes S, Romao M, Rochin L, Saftig P, et al. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. Dev Cell. 2011 Oct 18;21:708-21.
31. Natasha G, Gundogan B, Tan A, Farhatnia Y, Wu W, Rajadas J, et al. Exosomes as immunotheranostic nanoparticles. Clin Ther. 2014 Jun 1;36:820-9.
32. Solmaz V, Tekatas A, Erdoğan MA, Erbaş O. Exenatide, a GLP-1 analog, has healing effects on LPS-induced autism model: Inflammation, oxidative stress, gliosis, cerebral GABA, and serotonin interactions. Int J Dev Neurosci. 2020 Nov;80:601-12.
33. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and

microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9:654-9.

34. Alonso R, Rodríguez MC, Pindado J, Merino E, Mérida I, Izquierdo M. Diacylglycerol kinase alpha regulates the secretion of lethal exosomes bearing Fas ligand during activation-induced cell death of T lymphocytes. J Biol Chem. 2005 Aug 5;280:28439-50.
35. Laulagnier K, Grand D, Dujardin A, Hamdi S, Vincent-Schneider H, Lankar D, et al. PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. FEBS Lett. 2004 Aug 13;572:11-4.
36. Keller S, Sanderson MP, Stoeck A, Altevogt P. Exosomes: from biogenesis and secretion to biological function. Immunol Lett. 2006 Nov 15;107:102-8.
37. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci. 2018 Jan;75:193-208.
38. Frydrychowicz M, Kolecka-Bednarczyk A, Madejczyk M, Yasar S, Dworacki G. Exosomes - structure, biogenesis and biological role in non-small-cell lung cancer. Scand J Immunol. 2015 Jan;81:2-10.
39. Henne WM, Buchkovich NJ, Emr SD. The ESCRT pathway. Dev Cell. 2011 Jul 19;21:77-91.
40. Perez-Hernandez D, Gutiérrez-Vázquez C, Jorge I, López-Martín S, Ursa A, Sánchez-Madrid F, et al. The intracellular interactome of tetraspanin-enriched microdomains reveals their function as sorting machineries toward exosomes. J Biol Chem. 2013 Apr 26;288:11649-61.
41. Verweij FJ, van Eijndhoven MA, Hopmans ES, Vendrig T, Wurdinger T, Cahir-McFarland E, et al. LMP1 association with CD63 in endosomes and secretion via exosomes limits constitutive NF-κB activation. EMBO J. 2011 Jun 1;30:2115-29.
42. Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. J Biol Chem. 2010 Jun 4;285:17442-52.
43. Kogure T, Lin WL, Yan IK, Braconi C, Patel T. Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. Hepatology. 2011 Oct;54:1237-48.
44. Mittelbrunn M, Gutiérrez-Vázquez C, Villarroya-Beltri C, González S, Sánchez-Cabo F, González MÁ, et al. Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. Nat Commun. 2011;2:282.
45. Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science. 2008 Feb 29;319:1244-7.
46. Mettlen M, Chen PH, Srinivasan S, Danuser G, Schmid SL. Regulation of Clathrin-Mediated Endocytosis. Annu Rev Biochem. 2018 Jun 20;87:871-96.
47. Jahn R, Südhof TC. Membrane fusion and exocytosis. Annu Rev Biochem. 1999;68:863-911.
48. Gonda A, Kabagwira J, Senthil GN, Wall NR. Internalization of Exosomes through Receptor-Mediated Endocytosis. Mol Cancer Res. 2019 Feb;17:337-47.
49. Andre F, Schartz NE, Movassagh M, Flament C, Pautier P, Morice P, et al. Malignant effusions and immunogenic tumour-derived exosomes. Lancet. 2002 Jul 27;360:295-305.
50. Hortu I, Ozceltik G, Sahin C, Akman L, Yildirim N, Erbas O. Granulocyte Colony-Stimulating Factor Prevents Ischemia/Reperfusion-Induced Ovarian Injury in Rats: Evaluation of Histological and Biochemical Parameters. Reprod Sci. 2019 Oct;26:1389-94.
51. Keller S, Rupp C, Stoeck A, Runz S, Fogel M, Lugert S, et al. CD24 is a marker of exosomes secreted into urine and amniotic fluid. Kidney Int. 2007 Nov;72:1095-102.
52. Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Transl Med. 2011 Jun 8;9:86.
53. Kim SH, Lechman ER, Bianco N, Menon R, Keravala A, Nash J, et al. Exosomes derived from IL-10-treated dendritic cells can suppress inflammation and collagen-induced arthritis. J Immunol. 2005 May 15;174:6440-8.
54. Cai Z, Zhang W, Yang F, Yu L, Yu Z, Pan J, et al. Immunosuppressive exosomes from TGF-β1 gene-modified dendritic cells attenuate Th17-mediated inflammatory autoimmune disease by inducing regulatory T cells. Cell Res. 2012 Mar;22:607-10.
55. Skokos D, Botros HG, Demeure C, Morin J, Peronet R, Birkenmeier G, et al. Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. J Immunol. 2003 Mar 15;170:3037-45.
56. Ristorcelli E, Beraud E, Verrando P, Villard C, Lafitte D, Sbarra V, et al. Human tumor nanoparticles induce apoptosis of pancreatic cancer cells. FASEB J. 2008 Sep;22:3358-69.
57. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res. 2011 Jun 1;71:3792-801.
58. Rajendran L, Honsho M, Zahn TR, Keller P, Geiger KD, Verkade P, et al. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. Proc Natl Acad Sci U S A. 2006 Jul 25;103:11172-7.
59. Baixauli F, López-Otín C, Mittelbrunn M. Exosomes and autophagy: coordinated mechanisms for the maintenance of cellular fitness. Front Immunol. 2014 Aug 20;5:403.
60. Chiu YJ, Cai W, Shih YR, Lian I, Lo YH. A Single-Cell Assay for Time Lapse Studies of Exosome Secretion and Cell Behaviors. Small. 2016 Jul;12:3658-66.
61. Williams AM, Dennahy IS, Bhatti UF, Halaweish I, Xiong Y, Chang P, et al. Mesenchymal Stem Cell-Derived Exosomes Provide Neuroprotection and Improve Long-Term Neurologic Outcomes in a Swine Model of Traumatic Brain Injury and Hemorrhagic Shock. J Neurotrauma. 2019 Jan 1;36:54-60.
62. Admyre C, Johansson SM, Qazi KR, Filén JJ, Lahesmaa R, Norman M, et al. Exosomes with immune modulatory features are present in human breast milk. J Immunol. 2007 Aug 1;179:1969-78.

63. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "Tolerosomes" are produced by intestinal epithelial cells. Eur J Immunol. 2001 Oct;31:2892-900.
64. Costa-Silva B, Aiello NM, Ocean AJ, Singh S, Zhang H, Thakur BK, et al. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. Nat Cell Biol. 2015 Jun;17:816-26.
65. Park SJ, Hahn YS. Hepatocytes infected with hepatitis C virus change immunological features in the liver microenvironment. Clin Mol Hepatol. 2023 Jan;29:65-76.
66. Feng Y, Huang W, Wani M, Yu X, Ashraf M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. PLoS One. 2014 Feb 18;9:e88685.
67. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011 Apr;29:341-5.
68. Katakowski M, Buller B, Zheng X, Lu Y, Rogers T, Osobamiro O, et al. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. Cancer Lett. 2013 Jul 10;335:201-4.
69. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther. 2013 Jan;21:185-91.
70. Ke Y, Fan X, Rui H, Xinjun R, Dejia W, Chuanzhen Z, et al. Exosomes derived from RPE cells under oxidative stress mediate inflammation and apoptosis of normal RPE cells through Apaf1/caspase-9 axis. J Cell Biochem. 2020 Apr 10.
71. Shandilya S, Rani P, Onteru SK, Singh D. Natural ligand-receptor mediated loading of siRNA in milk derived exosomes. J Biotechnol. 2020 Jul 20;318:1-9.
72. Izco M, Blesa J, Schleef M, Schmeer M, Porcari R, Al-Shawi R, et al. Systemic Exosomal Delivery of shRNA Minicircles Prevents Parkinsonian Pathology. Mol Ther. 2019 Dec 4;27:2111-22.
73. Zhang Z, Dombroski JA, King MR. Engineering of Exosomes to Target Cancer Metastasis. Cell Mol Bioeng. 2019 Dec 23;13:1-16.
74. Fan Y, Li Y, Huang S, Xu H, Li H, Liu B. Resveratrol-primed exosomes strongly promote the recovery of motor function in SCI rats by activating autophagy and inhibiting apoptosis via the PI3K signaling pathway. Neurosci Lett. 2020 Sep 25;736:135262.
75. Elmas O, Erbas O, Yigitturk G. The efficacy of Aesculus hippocastanum seeds on diabetic nephropathy in a streptozotocin-induced diabetic rat model. Biomed Pharmacother. 2016 Oct;83:392-6.
76. Katsuda T, Tsuchiya R, Kosaka N, Yoshioka Y, Takagaki K, Oki K, et al. Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. Sci Rep. 2013;3:1197.
77. Ha DH, Kim HK, Lee J, Kwon HH, Park GH, Yang SH, et al. Mesenchymal Stem/Stromal Cell-Derived Exosomes for Immunomodulatory Therapeutics and Skin Regeneration. Cells. 2020 May 7;9:1157.
78. Zhu Y, Wang Y, Zhao B, Niu X, Hu B, Li Q, et al. Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. Stem Cell Res Ther. 2017 Mar 9;8:64.
79. Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, et al. Mesenchymal Stromal Cell Exosomes Ameliorate Experimental Bronchopulmonary Dysplasia and Restore Lung Function through Macrophage Immunomodulation. Am J Respir Crit Care Med. 2018 Jan 1;197:104-16.
80. Ti D, Hao H, Tong C, Liu J, Dong L, Zheng J, et al. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. J Transl Med. 2015 Sep 19;13:308.
81. Li J, Xue H, Li T, Chu X, Xin D, Xiong Y, et al. Exosomes derived from mesenchymal stem cells attenuate the progression of atherosclerosis in ApoE-/- mice via miR-let7 mediated infiltration and polarization of M2 macrophage. Biochem Biophys Res Commun. 2019 Mar 19;510:565-72.
82. Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. Cardiovasc Res. 2019 Jun 1;115:1205-16.
83. Heo JS, Choi Y, Kim HO. Adipose-Derived Mesenchymal Stem Cells Promote M2 Macrophage Phenotype through Exosomes. Stem Cells Int. 2019 Nov 5;2019:7921760.
84. Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, et al. Exosomes From Adipose-Derived Stem Cells Attenuate Adipose Inflammation and Obesity Through Polarizing M2 Macrophages and Beiging in White Adipose Tissue. Diabetes. 2018 Feb;67:235-47.
85. Nojehdehi S, Soudi S, Hesampour A, Rasouli S, Soleimani M, Hashemi SM. Immunomodulatory effects of mesenchymal stem cell-derived exosomes on experimental type-1 autoimmune diabetes. J Cell Biochem. 2018 Nov;119:9433-43.
86. Hu S, Li Z, Cores J, Huang K, Su T, Dinh PU, et al. Needle-Free Injection of Exosomes Derived from Human Dermal Fibroblast Spheroids Ameliorates Skin Photoaging. ACS Nano. 2019 Oct 22;13:11273-82.
87. Bai Y, Han YD, Yan XL, Ren J, Zeng Q, Li XD, et al. Adipose mesenchymal stem cell-derived exosomes stimulated by hydrogen peroxide enhanced skin flap recovery in ischemia-reperfusion injury. Biochem Biophys Res Commun. 2018 Jun 2;500:310-7.
88. Shin KO, Ha DH, Kim JO, Crumrine DA, Meyer JM, Wakefield JS, et al. Exosomes from Human Adipose Tissue-Derived Mesenchymal Stem Cells Promote Epidermal Barrier Repair by Inducing de Novo Synthesis of Ceramides in Atopic Dermatitis. Cells. 2020 Mar 10;9:680.
89. Alzahrani FA. Melatonin improves therapeutic potential of mesenchymal stem cells-derived exosomes against renal ischemia-reperfusion injury in rats. Am J Transl Res.

2019 May 15;11:2887-907.

90. Bier A, Berenstein P, Kronfeld N, Morgoulis D, Ziv-Av A, Goldstein H, et al. Placenta-derived mesenchymal stromal cells and their exosomes exert therapeutic effects in Duchenne muscular dystrophy. Biomaterials. 2018 Aug;174:67-78.
91. Chaubey S, Thueson S, Ponnalagu D, Alam MA, Gheorghe CP, Aghai Z, et al. Early gestational mesenchymal stem cell secretome attenuates experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. Stem Cell Res Ther. 2018 Jun 26;9:173.
92. Fan B, Li C, Szalad A, Wang L, Pan W, Zhang R, et al. Mesenchymal stromal cell-derived exosomes ameliorate peripheral neuropathy in a mouse model of diabetes. Diabetologia. 2020 Feb;63:431-43.
93. Shao L, Zhang Y, Lan B, Wang J, Zhang Z, Zhang L, et al. MiRNA-Sequence Indicates That Mesenchymal Stem Cells and Exosomes Have Similar Mechanism to Enhance Cardiac Repair. Biomed Res Int. 2017;2017:4150705.
94. Liu J, Chen T, Lei P, Tang X, Huang P. Exosomes Released by Bone Marrow Mesenchymal Stem Cells Attenuate Lung Injury Induced by Intestinal Ischemia Reperfusion via the TLR4/NF-κB Pathway. Int J Med Sci. 2019 Aug 14;16:1238-44.
95. Wang L, Gu Z, Zhao X, Yang N, Wang F, Deng A, et al. Extracellular Vesicles Released from Human Umbilical Cord-Derived Mesenchymal Stromal Cells Prevent Life-Threatening Acute Graft-Versus-Host Disease in a Mouse Model of Allogeneic Hematopoietic Stem Cell Transplantation. Stem Cells Dev. 2016 Dec 15;25:1874-83.
96. Long Q, Upadhya D, Hattiangady B, Kim DK, An SY, Shuai B, et al. Intranasal MSC-derived A1-exosomes ease inflammation, and prevent abnormal neurogenesis and memory dysfunction after status epilepticus. Proc Natl Acad Sci U S A. 2017 Apr 25;114:E3536-45.
97. Alessio N, Brigida AL, Peluso G, Antonucci N, Galderisi U, Siniscalco D. Stem Cell-Derived Exosomes in Autism Spectrum Disorder. Int J Environ Res Public Health. 2020 Feb 4;17:944.
98. Thomi G, Surbek D, Haesler V, Joerger-Messerli M, Schoeberlein A. Exosomes derived from umbilical cord mesenchymal stem cells reduce microglia-mediated neuroinflammation in perinatal brain injury. Stem Cell Res Ther. 2019 Mar 21;10:105.
99. Shen Z, Huang W, Liu J, Tian J, Wang S, Rui K. Effects of Mesenchymal Stem Cell-Derived Exosomes on Autoimmune Diseases. Front Immunol. 2021 Sep 27;12:749192.
100. Li Z, Chen X, Tao J, Shi A, Zhang J, Yu P. Exosomes Regulate NLRP3 Inflammasome in Diseases. Front Cell Dev Biol. 2022 Jan 3;9:802509.
101. Shigemoto-Kuroda T, Oh JY, Kim DK, Jeong HJ, Park SY, Lee HJ, et al. MSC-derived Extracellular Vesicles Attenuate Immune Responses in Two Autoimmune Murine Models: Type 1 Diabetes and Uveoretinitis. Stem Cell Reports. 2017 May 9;8:1214-25.
102. Nakano M, Nagaishi K, Konari N, Saito Y, Chikenji T, Mizue Y, et al. Bone marrow-derived mesenchymal stem cells improve diabetes-induced cognitive impairment by exosome transfer into damaged neurons and astrocytes. Sci Rep. 2016 Apr 22;6:24805.
103. Soundara Rajan T, Giacoppo S, Diomede F, Bramanti P, Trubiani O, Mazzon E. Human periodontal ligament stem cells secretome from multiple sclerosis patients suppresses NALP3 inflammasome activation in experimental autoimmune encephalomyelitis. Int J Immunopathol Pharmacol. 2017 Sep;30:238-52.
104. Mokarizadeh A, Delirezh N, Morshedi A, Mosayebi G, Farshid AA, Mardani K. Microvesicles derived from mesenchymal stem cells: potent organelles for induction of tolerogenic signaling. Immunol Lett. 2012 Sep;147:47-54.
105. Li Z, Liu F, He X, Yang X, Shan F, Feng J. Exosomes derived from mesenchymal stem cells attenuate inflammation and demyelination of the central nervous system in EAE rats by regulating the polarization of microglia. Int Immunopharmacol. 2019 Feb;67:268-80.
106. Rui K, Hong Y, Zhu Q, Shi X, Xiao F, Fu H, et al. Olfactory ecto-mesenchymal stem cell-derived exosomes ameliorate murine Sjögren's syndrome by modulating the function of myeloid-derived suppressor cells. Cell Mol Immunol. 2021 Feb;18:440-51.
107. Han C, Zhou J, Liu B, Liang C, Pan X, Zhang Y, et al. Delivery of miR-675 by stem cell-derived exosomes encapsulated in silk fibroin hydrogel prevents aging-induced vascular dysfunction in mouse hindlimb. Mater Sci Eng C Mater Biol Appl. 2019 Jun;99:322-32.
108. Wang Z, Yan K, Ge G, Zhang D, Bai J, Guo X, et al. Exosomes derived from miR-155-5p-overexpressing synovial mesenchymal stem cells prevent osteoarthritis via enhancing proliferation and migration, attenuating apoptosis, and modulating extracellular matrix secretion in chondrocytes. Cell Biol Toxicol. 2021 Feb;37:85-96.
109. Kul Y, Erbaş O. Exosomes: Classification, Isolation, and Therapeutic Applications in Various Diseases. JEB Med Sci 2022;3:6-12.
110. Ding Y, Cao F, Sun H, Wang Y, Liu S, Wu Y, et al. Exosomes derived from human umbilical cord mesenchymal stromal cells deliver exogenous miR-145-5p to inhibit pancreatic ductal adenocarcinoma progression. Cancer Lett. 2019 Feb 1;442:351-61.
111. Yu T, Zhao C, Hou S, Zhou W, Wang B, Chen Y. Exosomes secreted from miRNA-29b-modified mesenchymal stem cells repaired spinal cord injury in rats. Braz J Med Biol Res. 2019 Dec 5;52:e8735.
112. Hortu I, Ozceltik G, Karadadas E, Erbas O, Yigitturk G, Ulukus M. The Role of Ankaferd Blood Stopper and Oxytocin as Potential Therapeutic Agents in Endometriosis: A Rat Model. Curr Med Sci. 2020 Jun;40:556-62.
113. Shen H, Yao X, Li H, Li X, Zhang T, Sun Q, et al. Role of Exosomes Derived from miR-133b Modified MSCs in an Experimental Rat Model of Intracerebral Hemorrhage. J Mol Neurosci. 2018 Mar;64:421-30.
114. Sun Y, Liu G, Zhang K, Cao Q, Liu T, Li J. Mesenchymal stem cells-derived exosomes for drug delivery. Stem Cell

Res Ther. 2021 Oct 30;12:561.
115. Liu J, Yan Z, Yang F, Huang Y, Yu Y, Zhou L, et al. Exosomes Derived from Human Umbilical Cord Mesenchymal Stem Cells Accelerate Cutaneous Wound Healing by Enhancing Angiogenesis through Delivering Angiopoietin-2. Stem Cell Rev Rep. 2021 Apr;17:305-17.
116. Villamizar O, Waters SA, Scott T, Grepo N, Jaffe A, Morris KV. Mesenchymal Stem Cell exosome delivered Zinc Finger Protein activation of cystic fibrosis transmembrane conductance regulator. J Extracell Vesicles. 2021 Jan;10:e12053.
117. Li S, Yi M, Dong B, Jiao Y, Luo S, Wu K. The roles of exosomes in cancer drug resistance and its therapeutic application. Clin Transl Med. 2020 Dec;10:e257.
118. Muralidharan-Chari V, Clancy JW, Sedgwick A, D'Souza-Schorey C. Microvesicles: mediators of extracellular communication during cancer progression. J Cell Sci. 2010 May 15;123:1603-11.
119. Shifrin DA Jr, Demory Beckler M, Coffey RJ, Tyska MJ. Extracellular vesicles: communication, coercion, and conditioning. Mol Biol Cell. 2013 May;24:1253-9.
120. Yigitturk G, Erbas O, Karabay Yavasoglu NU, Acikgoz E, Buhur A, Gokhan A, et al. The neuro-restorative effect of adipose-derived mesenchymal stem cell transplantation on a mouse model of diabetic neuropathy. Neurol Res. 2022 Feb;44:156-64
121. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013 Feb 18;200:373-83.
122. Al-Nedawi K, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. Cell Cycle. 2009 Jul 1;8:2014-8.
123. Li B, Antonyak MA, Zhang J, Cerione RA. RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells. Oncogene. 2012 Nov 8;31:4740-9.
124. Abels ER, Breakefield XO. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. Cell Mol Neurobiol. 2016 Apr;36:301-12.
125. Yáñez-Mó M, Siljander PR, Andreu Z, Zavec AB, Borràs FE, Buzas EI, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicles. 2015 May 14;4:27066.
126. Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study. Bioscience. 2015 Aug 1;65:783-97.
127. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. Transplantation. 1968 Mar;6:230-47.
128. Wang YY, Li XZ, Wang LB. Therapeutic implications of mesenchymal stem cells in acute lung injury/acute respiratory distress syndrome. Stem Cell Res Ther. 2013 May 2;4:45.
129. Chen J, Li C, Chen L. The Role of Microvesicles Derived from Mesenchymal Stem Cells in Lung Diseases. Biomed Res Int. 2015;2015:985814.
130. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. Ann Thorac Surg. 2002 Jun;73:1919-25.
131. Safgöl Ö, Erbaş O. Exosomes derived from mesenchymal stem cells: Their content, obtaining methods, and therapeutic effects. D J Med Sci 2023;9:24-38.
132. Fierabracci A, Del Fattore A, Luciano R, Muraca M, Teti A, Muraca M. Recent advances in mesenchymal stem cell immunomodulation: the role of microvesicles. Cell Transplant. 2015;24:133-49.
133. Quesenberry PJ, Aliotta JM. The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. Stem Cell Rev. 2008 Sep;4:137-47.
134. Camussi G, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. Biochem Soc Trans. 2013 Feb 1;41:283-7.
135. Merjaneh M, Langlois A, Larochelle S, Cloutier CB, Ricard-Blum S, Moulin VJ. Pro-angiogenic capacities of microvesicles produced by skin wound myofibroblasts. Angiogenesis. 2017 Aug;20:385-98.
136. Hur YH, Feng S, Wilson KF, Cerione RA, Antonyak MA. Embryonic Stem Cell-Derived Extracellular Vesicles Maintain ESC Stemness by Activating FAK. Dev Cell. 2021 Feb 8;56:277-91.e6.
137. He C, Jaffar Ali D, Aseervatham G SB, Sun B, Xiao Z. Microvesicles - promising tiny players' of cancer stem cells targeted liver cancer treatments: The interesting interactions and therapeutic aspects. Pharmacol Res. 2021 Jul;169:105609.
138. Antonyak MA, Cerione RA. Microvesicles as mediators of intercellular communication in cancer. Methods Mol Biol. 2014;1165:147-73.
139. Antonyak MA, Li B, Boroughs LK, Johnson JL, Druso JE, Bryant KL, et al. Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. Proc Natl Acad Sci U S A. 2011 Mar 22;108:4852-7.
140. Kreger BT, Johansen ER, Cerione RA, Antonyak MA. The Enrichment of Survivin in Exosomes from Breast Cancer Cells Treated with Paclitaxel Promotes Cell Survival and Chemoresistance. Cancers (Basel). 2016 Dec 9;8:111.
141. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018 Apr;19:213-28.
142. Costa Verdera H, Gitz-Francois JJ, Schiffelers RM, Vader P. Cellular uptake of extracellular vesicles is mediated by clathrin-independent endocytosis and macropinocytosis. J Control Release. 2017 Nov 28;266:100-8.
143. Bordeleau F, Chan B, Antonyak MA, Lampi MC, Cerione RA, Reinhart-King CA. Microvesicles released from tumor cells disrupt epithelial cell morphology and contractility. J Biomech. 2016 May 24;49:1272-9.
144. Steinbichler TB, Savic D, Dudás J, Kvitsaridze I, Skvortsov S, Riechelmann H, et al. Cancer stem cells and their unique role in metastatic spread. Semin Cancer Biol. 2020 Feb;60:148-56.

145. Desrochers LM, Bordeleau F, Reinhart-King CA, Cerione RA, Antonyak MA. Microvesicles provide a mechanism for intercellular communication by embryonic stem cells during embryo implantation. Nat Commun. 2016 Jun 15;7:11958.
146. Gong J, Jaiswal R, Dalla P, Luk F, Bebawy M. Microparticles in cancer: A review of recent developments and the potential for clinical application. Semin Cell Dev Biol. 2015 Apr;40:35-40.
147. Nandy SB, Lakshmanaswamy R. Cancer Stem Cells and Metastasis. Prog Mol Biol Transl Sci. 2017;151:137-76.
148. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta. 2014 Aug;1846:75-87.
149. Kotmakçı M, Bozok Çetintaş V. Extracellular Vesicles as Natural Nanosized Delivery Systems for Small-Molecule Drugs and Genetic Material: Steps towards the Future Nanomedicines. J Pharm Pharm Sci. 2015;18:396-413.
150. Ha D, Yang N, Nadithe V. Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharm Sin B. 2016 Jul;6:287-96.
151. Li Y, Li S, Wang J, Liu G. CRISPR/Cas Systems towards Next-Generation Biosensing. Trends Biotechnol. 2019 Jul;37:730-43.
152. Fang T, Lv H, Wu F, Wang C, Li T, Lv G, et al. Musashi 2 contributes to the stemness and chemoresistance of liver cancer stem cells via LIN28A activation. Cancer Lett. 2017 Jan 1;384:50-9.
153. Tickner JA, Richard DJ, O'Byrne KJ. EV, Microvesicles/ MicroRNAs and Stem Cells in Cancer. Adv Exp Med Biol. 2018;1056:123-35.
154. Pala HG, Pala EE, Artunc Ulkumen B, Aktug H, Yavasoglu A, Korkmaz HA, et al. The protective effect of granulocyte colony-stimulating factor on endometrium and ovary in a rat model of diabetes mellitus. Gynecol Obstet Invest. 2014;78:94-100.
155. Zeringer E, Barta T, Li M, Vlassov AV. Strategies for isolation of exosomes. Cold Spring Harb Protoc. 2015 Apr 1;2015:319-23.
156. Simpson RJ, Lim JW, Moritz RL, Mathivanan S. Exosomes: proteomic insights and diagnostic potential. Expert Rev Proteomics. 2009 Jun;6:267-83.
157. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov. 2014 Jun;4:650-61.
158. Brock G, Castellanos-Rizaldos E, Hu L, Cottichia C, Skog J. Liquid biopsy for cancer screening, patient stratification and monitoring. Transl Cancer Res. 2015 Jun;4:280-90.