REVIEW ARTICLE

Roles of Mesenchymal Stem Cell-derived Extracellular Vesicles in Cancer: Development and Target Therapy

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Received date: Nov 5, 2024; Revised date: Dec 11, 2024; Accepted date: Dec 26, 2024

Abstract

Extracellular vesicles (EVs) are membrane structures that enclose proteins, lipids, RNAs, metabolites, growth factors, and cytokines. EVs derived from mesenchymal stem cells (MSCs) can either stimulate or inhibit tumor growth in various malignancies through paracrine signaling. Tumor-associated MSCs (TA-MSCs), often described as "wounds that never heal," actively participate in the development, propagation, and metastasis of tumors, impacting the immunological state of the tumor microenvironment. For instance, TA-MSCs can alter immune cell recruitment and cytokine production, leading to a pro-tumorigenic environment. Consequently, both the tumor and its microenvironment undergo functional alterations, the cargo of exosomes is modified, and an abnormal tumor-associated MSC phenotype is acquired. MSC-EVs contain exosome microRNA with both tumor-inhibitory and tumor-supportive effects. For example, MSC-EVs have been shown to deliver tumor-suppressive microRNAs that inhibit cancer cell proliferation and induce apoptosis. This review outlines the criteria for the modification, isolation, and characterization of exosomes, as well as their application in cancer, providing insights for clinical use. By understanding these mechanisms, we can better harness MSC-EVs for therapeutic purposes.

KEYWORDS: mesenchymal stem cell, extracellular vesicle, exosome, cancer therapy, drug delivery

Indones Biomed J. 2025; 17(1): 1-21

Introduction

Extracellular vesicles (EVs) serve as adaptable cell-to-cell transporters by enclosing proteins, lipids, RNAs, metabolites, growth factors, and cytokines in a nanoscale bilayer-enclosed membrane structure.(1) EVs can be secreted by any cell during both healthy and diseased processes, most notably cancer.(2) As paracrine mediators, EVs have been demonstrated as intercellular shuttle to transport biomolecules including lipids, carbohydrates, protein, and RNA between immune cells, fibroblasts, stromal cells, endothelial cells, and tumor cells, promoting communication throughout the tumor microenvironment. Consequently, EVs play a role in immunomodulation, metastasis, cancer etiology, and progression. The presence of EVs in biological fluids and oncological states are correlated, therefore EVs can be proposed as a useful diagnostic tool to be a more precise yet less invasive to detect tumor.(3)

There is a lot of interest in using mesenchymal stem cells (MSC)-EVs as a cell-free therapy as a result of the studies of their positive effects on inflammation and tissue repair.(4,5) Compared to cellular therapies, MSC-EV-based therapy offers a number of benefits. As a therapeutic option, EVs shouldn't be as vulnerable to unfavorable alterations

brought on by injection into the inflammatory milieu of damaged tissue as MSCs are. Moreover, EVs cannot duplicate themselves, thus injecting them poses a lesser safety concern. Unlike cells, EVs can also be genetically modified to carry the necessary therapeutic payload quite simply and safely. Compared to existing cellular therapies, they are less expensive and need less effort during production and storage. Finally, the intravenous injection method of EV distribution carries a lesser risk of vascular blockages because EVs are smaller than MSC.(6)

Despite the growing interest in MSC-EVs, there is still a lack of clarity regarding their dual role in tumor progression and inhibition. This review was conducted to elucidate the mechanisms by which MSC-EVs influence tumor growth and the tumor microenvironment, providing a detailed analysis of their cargo and its effects. By compiling and analyzing current research, this review seeks to identify the criteria for the modification, isolation, and characterization of EVs, and to explore their potential therapeutic applications in cancer treatment. Ultimately, this review aimed to bridge the gap in knowledge and offer insights that could lead to the development of more effective cancer therapies utilizing MSC-EVs.

EVs Biogenesis, Classification, Release and Uptake

EVs are released by all prokaryotes and eukaryotes cells, as a normal part of their physiology and in response to acquired disorders, including endothelial cells, cancer cells, and pathogens.(7) Exosome biogenesis involves multiple pathways and aids in the sorting of protein and RNA cargo to produce exosomes with specific biochemical makeup. Exosomes originate from endocytes, is a membrane-bound compartment found inside eukaryotic cells.(8) An endosome was made up by three distinct compartments: Early, late, and recycling endosomes. The shape and position of early endosomes change from tube-like to spherical as they mature into the late endosome state, moving from the outside cytoplasm to toward the nucleus. Furthermore, the late endosome's lumen forms intraluminal vesicles (ILV) through the endosomal membrane's inward budding, named as multivesicular bodies (MVBs).(9) MVBs have two possible outcomes: they can either merge with the plasma membrane or with lysosomes. After merging with the lysosome, an MVB's contents are hydrolyzed inside the lysosome. Alternatively, the MVB can fuse with the plasma membrane, resulting in the release of ILVs into the extracellular environment. However, not all ILVs are ultimately released into the extracellular space as exosome. (10) Inducible release or the trans-Golgi network release become two major pathways where exosomes were released.

Exosome biogenesis process can be broken down into the following steps: Firstly, is the initiation, where the internal budding of the plasma membrane produces early endosomes. Secondly, endocytosis where the situation of early endosomes continue to invade, late endosomes are formed.(11) Then, the formation of multivesicles, the step where late endosomes were indicated by the emergence of ILVs within the massive multivesicle bodies (MSVs), subsequently fuse with the plasma membrane to generate exosomes (Figure 1). The final step is secretion where the generated exosomes are either discharged into the extracellular space or used to transport lysosomes for destruction.(12) Throughout the entire process, a number of proteins integrate into the invaginating membrane and the cytosolic components are consumed, becoming part of the ILVs.(13)

EV can be simply categorized as ectosomes and exosomes. Ectosomes, which consist of huge vesicles with a diameter ranging from ~50 nm to 1 μm, microparticles, and microvesicles, pinch off the plasma membrane surface through outward budding. Exosomes are endosome-derived EVs that range in diameter from around 40 to 160 nm. MVB to add the variety of exosome contents, are formed at the end by consecutive invagination of the plasma membrane. They may interact with numerous intracellular vesicles and organelles.(7) EVs can further classified into 3 subgroups: membrane-shedding EVs (microparticles), multivesicular body-derived EVs (exosomes), and apoptosis-derived EVs (apoptotic bodies) based on variations in size and biosynthesis (Figure 2).(14) Exosomes are relatively smaller vesicles (40–100 nm) than microparticles, which are also referred to as microvesicles. Apoptotic bodies are larger vesicles (800–5,000 nm). Bioactive compounds carried by EVs have a high degree of stability and have the ability to alter recipient cells' cell activities.(15)

Numerous distinct mechanisms are involved in the uptake of exosomes and EVs by suitable target cells. These comprise, among other things, EV fusion with the target cell plasma membrane, phagocytosis, macropinocytosis, lipid raft-mediated internalization, and receptor-mediated endocytosis.(16) Macropinocytosis refers to the non-specific absorption of extracellular fluid and solutes into large vesicles known as macropinosomes. Micropinocytosis is a form of endocytosis where cells engulf extracellular fluid and dissolved substances into very small vesicles

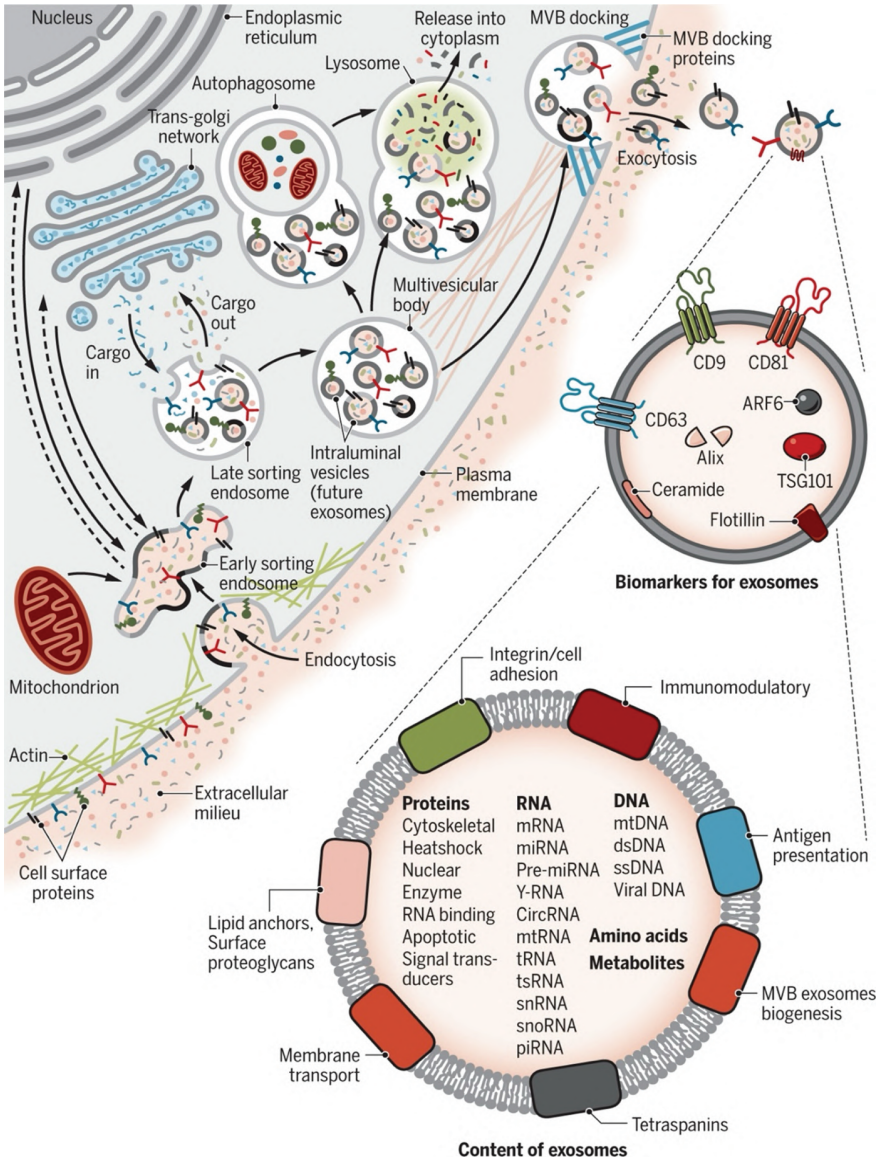

Figure 1. The Biogenesis and identification of exosomes.(7) Adapted with permission from American Association for the Advancement of Science).

including EVs. Phagocytosis focus on ingesting larger, solid particles, is as a process for EV incorporation mediated by the actin cytoskeleton. The integration of EVs through plasma membrane fusion requires the destabilization of the interacting lipid bilayers by a localized acidic pH and the surmounting of significant activation energy barriers.(17) One possible mechanism by which exosomes enter target cells is by receptor-mediated endocytosis. Therefore, a matching surface receptor on the exosome is necessary to facilitate the uptake by the cellular plasma membrane.(18) Glycolipids, different glycoproteins (such immunoglobulins and/or connective lectins), mucins, and heparan sulfate proteoglycans are specific components involved in this process. As a result, in contrast to the other EV uptake systems, receptor-mediated endocytosis represents a unique mechanism.(18)

Phospholipase D, phosphatidic acid, and sphingolipids are essential for the production of EVs.(19) By controlling Ras-associated binding protein (Rab35) and synaptosome-associated protein-23 (SNAP23), long non-coding (lncRNA) HOX antisense intergenic RNA (HOTAIR) is also known to enhance exosome secretion.(20) A lower pH in the microenvironment enhanced exosome secretion and receptor cell uptake (21), and p53 controlled tumor inhibition activation pathway 6 to trigger exosome secretion in response to stress (22). Moreover, exosome secretion was enhanced by heparin enzyme overexpression.(23) The potential for EV biosynthesis has gradually expanded for advancements in nanotechnology; however, numerous mechanisms still need to be explored further.

Three basic mechanisms are used by EVs to convey genetic information to receptor cells: direct fusion with the

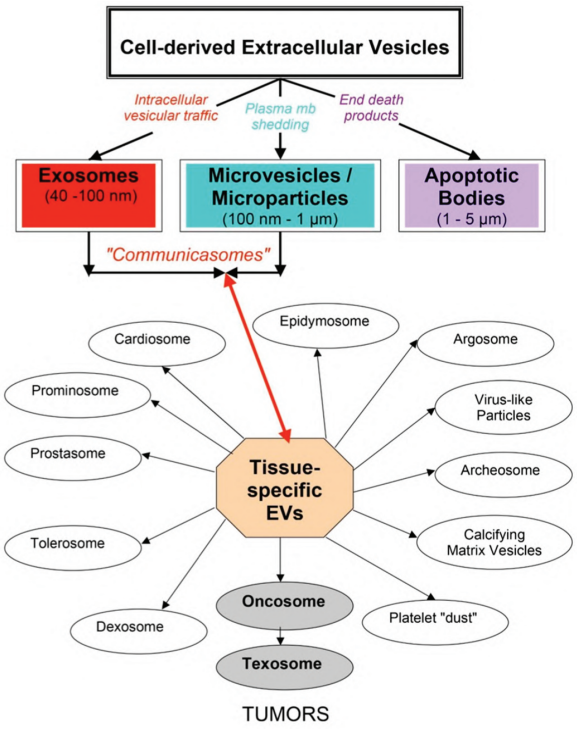

Figure 2. Complexity of eukaryotic EVs. EVs is mainly categorized into exosomes, microvesicles/microparticles and apoptotic bodies, and further classified into tissue-specific EVs. (14) (Adapted with permission from Frontiers Media SA).

plasma membrane, receptor-ligand contact, and endocytosis. The two primary types of endocytosis processes are clathrin-dependent and clathrin-independent. Phagocytosis, micropinocytosis, and endocytosis mediated by caveolin and lipid rafts are the subtypes of clathrin-independent routes.(7,16) On another side, the internalization of EV cargo was done via a three-step methods, which are: EVs target the acceptor cell, then enter the acceptor cells and internalize, and continue to the delivery of EVs content to the acceptor cell. The majority of publications described EV entrance and internalization-mediated cargo delivery into cells. On the contrary, internalization is not necessary to induce exosome-mediated cellular responses; instead, it is just necessary for receptor membrane binding to occur.(24)

EVs Components

A lipid bilayer membrane encloses exosomes because they develop by budding from early endosomes. Large concentrations of ceramide, sphingomyelin, and cholesterol are seen in exosome membranes.(25) According to some studies, lipid components including prostaglandins and phosphatidylserine can be crucial for exosome action. (26,27) EVs, especially exosomes, are produced by MSCs. Under various pathological conditions, EVs primarily consist of proteins, lipids, metabolites, and nucleic acids, which can reflect both the body's metabolic state and the functionality of the parental cells.(28) They may therefore be useful for clinical diagnostics and therapeutic benefits on a variety of diseases, including diabetes, autoimmune and neurodegenerative disorder, wound healing, liver diseases, renal diseases, and heart repair.(29) Additionally, EVs are implicated in immunological responses, neural transmission, antigen presentation, organ development, reproduction, and among other physiological processes.(30) Following internalization, recipient cells react to chemicals and gene expression carried by EVs, potentially altering their activities.(31) EVs therefore holds a prospect as a novel, cell-free approach for illness diagnosis and treatment. Therefore, they have sparked interests across a wide range of scientific domains.(32)

Exosomes lack proteins from the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus, but contain cytosolic proteins and those from the plasma or endosomal membrane.(32) During exosome production, the endosomal sorting complex required for transport machinery (ESCRT) complex forms small necks at the plasma membrane. It is believed that the filaments, tubes, conical funnels, and flat spirals within the ESCRT complex regulate membrane remodeling, a process that requires ATP for assembly and disassembly.(33)

A large number of transport proteins, including tubulin, actin, and actin-binding molecules, as well as other proteins linked to certain secreting cell functions, are found too in exosomes.(34) Major histocompatibility complex (MHC) class I molecules (35) and heat shock proteins (HSP), particularly HSP70 and HSP90 (34), are present in almost all exosomes. Involved in antigen presentation, HSP70 and HSP90 have the ability to attach antigenic peptides to MHC class I molecules.(34) Tetraspanins, including as CD9, CD63, CD81, and CD82, are highly concentrated in exosomes and not commonly seen in other microvesicles. Tetraspanins interact with other transmembrane proteins to facilitate antigen presentation and adherence. Through their interactions with integrins, CD9 and CD82 prevent tumor cells from migrating and invading.(36)

Exosomal small non-coding RNAs called miRNAs attach to mRNAs in the 3'-UTR (3'-untranslated region), destabilizing and fragmenting the mRNAs and reducing the amount of the encoded protein that is expressed.(37,38) Because of its route of delivery, miRNA is not easily broken down by extracellular ribonucleases.(39) The miRNA

carried by tumor-derived exosomes (TDE) represents the dysregulated miRNA profile of cancer cells. TDEs have the ability to travel to several cell types and the pre-metastatic niche, where they can have a broad impact on target cells' gene expression.(40) Exosomes can transform pre-miRNAs into mature-miRNAs while just conveying miRNA; this enables direct interaction with the recipient cell's transcriptome without the need for additional processing of these miRNAs.(41) Long non-coding RNAs are the second main class of RNA found in exosomes. They can disrupt gene expression through a variety of mechanisms, including direct annealing with genomic DNA and altering histone complexes.(42)

Overall, exosomes have the potential to be employed as therapeutic agents, drug delivery vehicles, and disease diagnostic tools, due to their unique properties.(43) Exosomes have been dubbed the fingerprint of the parent cell (44), because they contain distinct bioactive chemicals that reflect the elements, physiological state, and personality of the original cell (45). A number of investigations have demonstrated that the miRNAs found in the exosomes generated by cancer cells are the same as those found in the original cancer cells. Furthermore, exosomes and liposomes share a similar structure, which protects the contents of exosomes from the outside world and preserves the integrity of the cargo data. Exosomes can be isolated from patients non-invasively because they are present in bodily fluids. (46) Collectively, these characteristics lend credence to the concept that exosomes may serve as trustworthy diagnostic biomarkers for infectious, metabolic, neurological, and cancer disorders. They could also be utilized to determine a patient's current state of health (47) and assist medical professionals in making accurate diagnoses (48). Certain cell types and different physiological and pathological situations influence the exosomal components. Since the cargo is selectively sorted into exosomes, the exosomal content may differ from that of the original cells.(49)

EVs Isolation and Characterization

The increasing use of MSC-EVs as a therapeutic followed by increased demand for clinically appropriate EV separation and quantification techniques. A wide range of methods have been developed recently to identify, describe, and measure EVs. However, due to their tiny size and physiochemical variety, effectively isolating, characterizing, and quantifying these membrane structures remains a tough challenge. EV characteristics including density, size, and surface composition are the basis for common isolation techniques that divide EVs from the remainder of the cellular compartment. Less labor-intensive isolation techniques higher EV yields than more labor-intensive ones, but less purity of the EVs produced is lower. To guarantee the maximum EV yield and purity, the International Society for Extracellular Vesicles advises combining various isolation techniques.

For exosome separation, a number of recognized methods have been used, including size exclusion chromatography, density gradients, precipitation, filtration, and differential ultracentrifugation.(1,50) In exosome research, conventional ultracentrifugation has emerged as a dependable method. The process involves a sequence of centrifugation cycles with different centrifugal forces and durations to separate exosomes based on their size variations and density. A variety of commercial tools have recently been released to isolate exosomes for various uses. (51) These kits are better than ultracentrifugation because less time are required and less sensitive to technique, however they work better with smaller sample volumes. It is necessary to take into account the relative effectiveness of these various techniques in terms of recovery and specificity.(49) In the pre-clinical investigations evaluating the therapeutic potential of MSC-EVs in an *in vivo* tissue healing, differential ultracentrifugation was also the most frequently employed one for EVs isolation. With this method, cells and big cellular debris are removed using a series of differential centrifugation processes, and EVs are precipitated quickly. Smaller EVs (sEVs) are pelleted, while larger particles stay in the supernatant.(52) This technique yields medium purity of EVs when it comes to EV isolation. The main drawbacks of this approach are that it is currently inappropriate for usage in a clinical context due to its time-consuming nature and the need for expensive equipment. Moreover, the high centrifugation speed may impair the integrity of the EVs and contaminate the isolated EV population with proteins.

Density gradient centrifugation, which operates on the basis of various floating densities, is an alternate technique for separating the sEVs. After preloading a centrifuge tube with the sample and adding varying densities of sucrose or iodixanol solutions, ultracentrifugation is performed. Because of their distinct flotation densities, EVs float, which improves their ability to be separated from contaminants.(53) This process yields EVs with a comparatively high purity as a result. Like differential ultrahigh-speed centrifugation, thise process is labor-intensive and expensive. A significant chance of vesicle loss and damage because of the several

centrifugation stages is obtained. Additionally, size-exclusion liquid chromatography (SEC) can separate EVs from proteins based on their size; in the case of MSC-EVs, can impair their therapeutic effectiveness.

Solutions containing sucrose and iodixanol may also adversely affect the functionality of isolated EVs, and later pass through SEC, a polymer column with porous stationary phase. Proteins and other smaller particles will elute later because the holes in the polymer slow them down.(54) This method's ease of scaling up is another major benefit, particularly for the potential therapeutic application of MSC-EVs in the future.

The labor-intensive nature of this isolation process and the likelihood of sample contamination by lipoproteins and protein aggregates are its drawbacks.(52) Polyethylene glycol (PEG) is used as one technique for precipitation-based EV isolation with high yield and recovery. PEG, a precipitant that excludes water, is introduced to the sample, and then the particles are concentrated by centrifugation and an incubation period. EVs can be separated using immuno/affinity capture techniques according to the expression of their surface proteins. Therefore, in order to isolate EVs, prior knowledge about the markers expressed on them is required. For the enrichment, tetraspanin family members that are expressed on EV membranes are utilized. To separate the EVs with high purity, antibody-coated magnetic beads are frequently used further.(55)

Although a number of characterization and quantification techniques have been developed, no single method is able to accurately assess EVs. As a result, many methods are typically used to assess EV attributes. One technique to ascertain the size distribution and concentration of the EVs is nanoparticle tracking analysis (NTA).(56) NTA is a commonly used technique for quantifying EVs, however it is highly sensitive to contamination from non-EV particles. For the quantification of samples with lesser purity, this could be troublesome. Another technique to measure EVs in suspension is dynamic light scattering (DLS). DLS monitors the motion of particles undergoing Brownian motion in suspension, much like NTA does.(57)

The methods most frequently employed to describe the microstructure of MSC-EVs are scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. While TEM is more frequently employed to provide a 2D image of the EV with interior structural information, SEM scans the topography of the EV surface. The ability to integrate immunogold labeling with SEM and TEM-based imaging is a benefit.(6)

It has been demonstrated that exosomes separated from patient biofluids carry cargo unique to malignancy that reflects changed cellular or tissue states.(58,59) These results have given rise to the hypothesis that, in the context of liquid biopsies, the molecular composition of exosomes may offer special chances for learning about the existence, molecular profile, and behavior of cancer. Exosomes can thus be employed as biomarkers in liquid biopsies to track tumor load and therapy effectiveness in real time.(60,61) Although there is a growing interest in understanding the mechanism of action of MSC-EVs, the field is still in its early stages when it comes to identifying the specific molecules that are responsible for their therapeutic effects. Figure 3 provides a concise overview of the components that contribute to the positive outcomes of MSC-EVs. MSC-EVs are believed to govern essential processes in tissue repair, including apoptosis, cell proliferation, angiogenesis, and inflammation.(6)

EVs Delivery Strategies

The biophysical and mechanical properties of EVs are crucial in two essential elements of drug or treatment delivery: cellular absorption and tissue transport. By taking into account the mechanical qualities and physical interactions with biological systems, we can enhance the effectiveness of using EVs for treatment.

Multiple studies have demonstrated that biophysical characteristics of cells have an effect on their interactions with nanoparticles. The dimensions, morphology, and flexibility of micro-/nano-scaled particles impact their capacity to be transported into cells. The rate of endocytosis and the overall amount of nanoparticles taken up depend on the size of the particles in a non-monotonic way. For spherical gold nanoparticles, a diameter of approximately 50 nm is near to ideal for achieving maximum uptake. Concurrently, the presence of a high aspect ratio in their structure decreases the efficiency of nanoparticle uptake.(62) Furthermore, recent research has demonstrated a correlation between the mechanical characteristics of nanoparticles and their ability to be absorbed. Soft nanoparticles are more efficiently taken up by both tumor and non-tumor cells compared to stiff nanoparticles.(63) The alginate core can undergo crosslinking or remain uncrosslinked, leading to the formation of nanolipogels (NLGs) with increased stiffness or decreased stiffness, respectively. The hypothesized process suggests that more pliable NLGs are internalized into cells via fusion, whereas more rigid NLGs

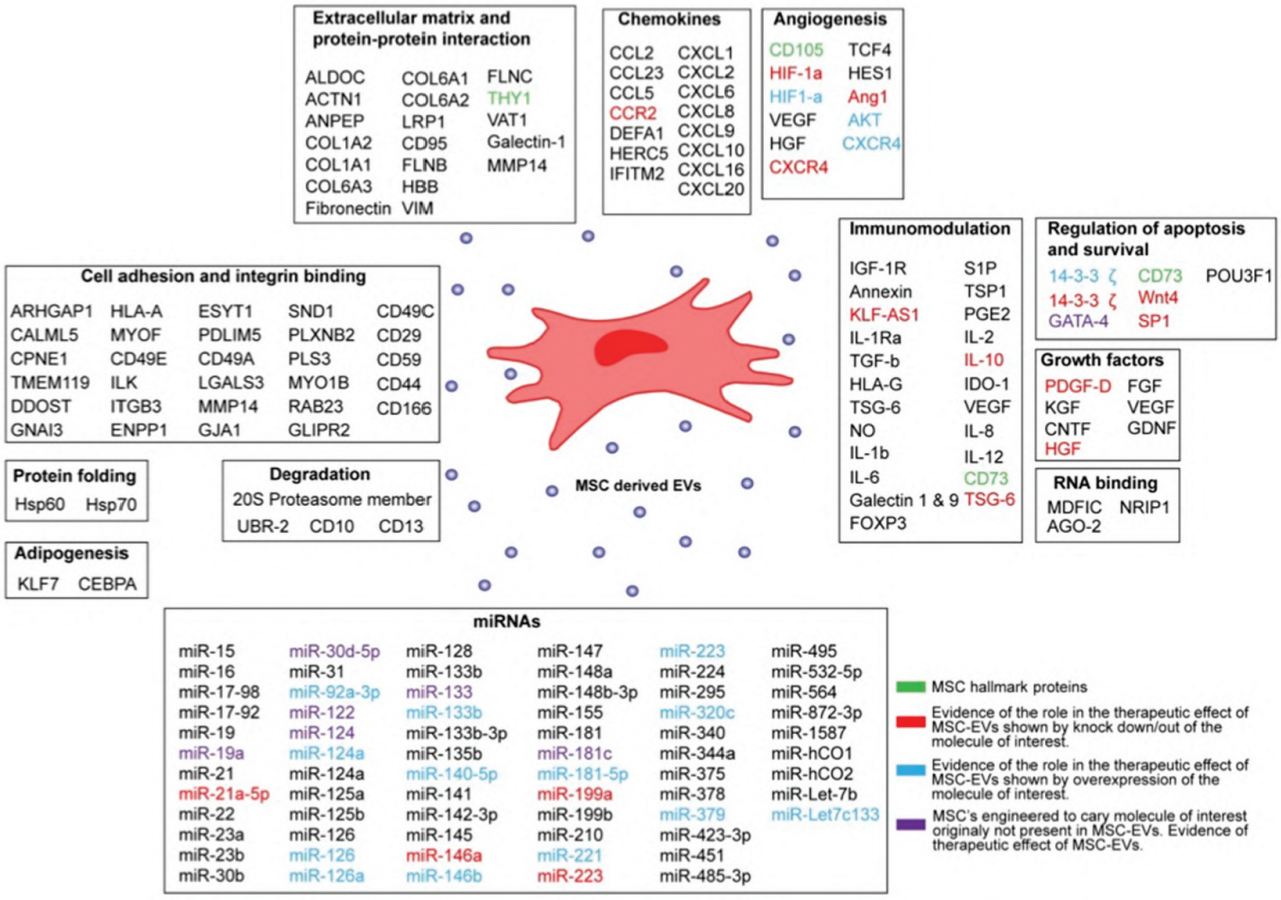

Figure 3. Schematic representation of the components of MSC-derived EVs for EVs characterization.(6) (Adapted with permission from Ivyspring International Publisher).

are internalized through endocytosis. However, a different study observed a contrasting pattern when utilizing lipid-coated polylactic-co-glycolic acid (PLGA) nanoparticles. The elasticity of the nanoparticles was altered by varying the amount of water included. Interestingly, it was found that the stiffer PLGA-based nanoparticles showed an increase in cellular absorption, which is the opposite of some similar studies.(47) This suggests a potential mechanism of how cells take in particles, which is influenced by the elasticity of the particles. This mechanism may result in a two-phase reaction. In other words, the fusion pathway is limited to particles with low stiffness, while particles with high stiffness rely on endocytosis.

To successfully reach their target cells, EVs must physically enter certain regions of interest. Transporting cargo deep within tissue often necessitates features that enable movement across compact, extracellular matrix (ECM)-rich microenvironments with narrow pores. EV particles can frequently exceed the size of these pores, hence posing a challenge at the scale of length. There are several significant factors to consider when handling this issue. Tissue microenvironments has the characteristic of being inelastic, with the ability to store and release energy, and can be restructured when subjected to external stress. (48) In gels with small pores that exhibit simply elastic behavior, EVs experience physical confinement. However, in gels that undergo stress-relaxation, EVs can spread rapidly as the pores can widen due to relaxation induced by stress.(64) EVs can undergo a decrease in size if they include specific membrane channels, such as aquaporins (AQP), that allow water to exit. Additionally, EVs can also experience an increase in pore size. AQP are proteins found in cell membranes that facilitate the exchange of fluids. When the expression of AQP1 is suppressed, the diffusion of EVs through stress-relaxing gels is significantly reduced. This indicates that the deformation of EV, resulting in a decrease in volume due to the expulsion of fluids, plays a crucial role in the transport of EV in the interstitial space. Hence, it is crucial to take into account the lack of flexibility in the target tissue's ECM and the presence of membrane channels on EVs while aiming to enhance their distribution through compact tissues with narrow openings.(65)

Various artificial drug delivery technologies, such as liposomes, polymers, and biomimetic particles, as well as sustained release systems like hydrogels and artificial cells, have been extensively researched for their biological qualities in living organisms.(66,67) Similar to other nano-/micro-sized materials administered, EV-based delivery systems undergo the following process: circulation through the bloodstream and interception by the reticuloendothelial system (RES); crossing the barrier of vascular endothelial cells and ECM to reach the specific disease sites, such as tumors; uptake by target cells and avoiding degradation in lysosomes, leading to localization within the cell or nucleus; and eventual degradation of components or excretion.(68) One important criterion for evaluating the capacity of a particle to carry cargo is its ability to mislead or bypass the disruption of the RES in living organisms and achieve subcellular transfer.

In order to address the issue in EV-based drug delivery systems, researchers have initially investigated the transportation of EVs through the bloodstream. Various chemical or biochemical trackers, such as fluorescent proteins (69), lipophilic dyes (70), conjugated probes (71), and engineered particles (72) have been employed in murine models to determine the pharmacokinetics of EVs. Consequently, the half-lives of various EVs have a biphasic pattern. The distribution of EVs at the organ level, including the liver, spleen, kidney, and lung, is predicted to occur approximately 3-4 hours after intravenous injection.(73,74) Exosomes have the ability to reduce their clearance by the RES by up to 23%, which is a significant improvement compared to artificial particles that are typically cleared by the liver with up to 90% efficiency.(75,76) The fundamental reason is that the presence of CD47 on the surface of exosomes acts as a signal that tells macrophages not to engulf them, thereby preventing phagocytosis. By utilizing this characteristic, the membrane of EVs containing CD47 has been isolated and employed to envelop biomimetic vesicles. This novel technique has demonstrated efficacy in preventing the engulfment of particles by macrophages, hence extending their presence in the bloodstream.(77)

Exosomes in Cancer Development, Metastasis and Immunity

Exosomes facilitate the transfer of information between cancer cells, and also to stromal cells which materialize the information to a pro-tumor microenvironment creation. In return, cancer cells utilize exosomes released by stromal cells to enhance their own proliferation and invasion. Tumor-derived exosomes were found to enhance the growth and formation of new blood vessels in endothelial cells. (78,79)

Stromal cells exert influence on the destiny of tumor cells through the transmission of exosomes. Researchers discovered that stromal cells surrounding breast cancer cells produce exosomes containing RNA RN7SL1, which is not protected by a membrane. This RNA activates a receptor called retinoic acid inducible gene I (RIG-1), which recognizes viral RNA patterns. As a result, an inflammatory response is triggered, leading to the growth of the tumor. (80) Cancer-associated fibroblast-derived exosomes (CAF-DEs) that are rich in a disintegrin and metalloproteinase 10 (ADAM10) promote cancer cell movement and sustain the stem cell characteristics of cancer cells through Notch signaling. Furthermore, CAF-DEs transported metabolic substances such as amino acids, lipids, and tricarboxylic acid (TCA)-cycle intermediates. Prostate and pancreatic malignancies were found to boost glycolysis and glutamine-dependent reductive carboxylation in cancer cells after exposure to CAF-DEs. This led to the promotion of tumor growth in settings where there was a lack of nutrients or when the cells were under stress due to nutrient scarcity.

Abundant data has substantiated the significant involvement of MSC-EXs in the processes of angiogenesis, tumor development, and metastasis. The most often utilized sources of exosomes are human umbilical cord MSCs (hUC-MSCs) and human umbilical cord Wharton's jelly MSCs (hWJ-MSCs). The impact of natural MSC-EVs on malignancies remains a subject of debate, with conflicting views on whether they have a beneficial or detrimental effect. According to a recent study, the dual effect appears to be associated with several aspects such as the origin of MSC-EXs, the dosage and timing of MSC injection, the kind of cancer, and other variables.(81)

Exosomes play a significant function in transmitting information inside the tumor microenvironment, which also take important part in tumor metastasis. Introducing exogenous exosomes produced from hUC-MSC Exosomes can enhance the proliferation and movement of gastric cancer cells by stimulating the Akt pathway.(82) Furthermore, MSC-EVs that originate from the bone marrow of mice and humans have the ability to cause breast cancer cells to migrate to the bone marrow and persist as cancer stem cells (CSCs) in a condition of dormancy for many years.(83,84)

The death of a significant majority of cancer patients is mostly caused by metastases, despite notable advancements

in surgical methods, radiation, chemotherapy, and targeted medicines, including immuno-therapy.(85) While there has been a significant decrease in the spread of cancer to other parts of the body, total eradication of the tumor is rarely achieved. This condition is caused by drug resistance, which occurs when intracellular pathways adapt or when survival-supporting autocrine and paracrine pathways are activated, together with the expression of several secreted proteins by drug-sensitive tumor cells following therapy.(86) Metastasis can happen when cancer cells are released from the main tumor into body cavities, which is the case for ovarian and CNS cancers. It can also occur through the hematogenous and lymphatic capillaries of the circulatory system.(2) Metastases of certain tumors exhibit a specific preference for particular organs, in addition to lymph nodes. For instance, prostate cancer tends to spread primarily to the bones, while pancreatic cancer and uveal melanoma have a tendency to metastasize to the liver. On the other hand, cancers like melanoma, breast cancer, and lung cancer have the ability to colonize multiple organs.

The initial stages involve disengagement from the ECM, infiltration into the adjacent tissue, and degradation of the basement membrane through proteolysis. Following intravasation, the survival of circulating tumor cells (CTCs) is accomplished through the formation of clusters, adherence to platelets, and evasion of the immune system. Afterwards, they are captured in distant small blood vessels and can escape by either moving through the connections between endothelial cells or by penetrating a single endothelial cell.(87) CTCs have the ability to establish themselves in the tumors they originated from. This mechanism favors the growth of cancer cell populations that are more aggressive than those seen in the original tumor.(88) The subsequent phases following extravasation include colonization and outgrowth inside the parenchyma of distant organs. A prevalent occurrence in the metastatic process is the establishment of disseminated tumor cells (DTCs) into a state of latency (dormancy), which can last for a period ranging from several months to several decades.(89)

The term "metastatic niche" refers to the creation and marking of a favorable milieu in distant organs that allows for the survival and proliferation of DTCs. This microenvironment acts as a "landing dock" for the DTCs. The niche is initiated through the interaction between tumor cells released factors, hematopoietic progenitor cells (HPC) recruitment, myeloid cells and BM-MSC. This process allows the engraftment of DTCs, which are then supported in their growth by endothelial precursor cells (EPC) and angiogenic factors.(90)

The invasion metastasis cascade denotes the process by which macrometastases are formed in distant organs. Tumor cells detach from the stroma and go into the bloodstream. Upon arrival at their pre-metastatic niche, tumor cells can either become dormant as DTCs or multiply to create small clusters of cells called micrometastases, which eventually develop into larger metastatic tumors known as macrometastases. Metastasis is a highly inefficient process, as only a minuscule fraction of tumor cells, namely 0.01%, that enter the bloodstream are capable of multiplying in distant organs. Epithelial to mesenchymal transition (EMT) often triggers the metastatic process. EMT refers to the process by which epithelial tumor cells acquire mesenchymal properties in the presence of CAFs, or cancer-associated fibroblasts, inside the tumor stroma. During the process of EMT, tumor cells undergo a loss of polarity and cell-cell junctions. As a result, they enter a state of reduced proliferation but with enhanced capacities to migrate and invade surrounding tissues.(91)

Exosomes have a role in the emergence of therapeutic resistance in tumor cells through various methods. Tumor-derived exosomes have the ability to transmit proteins and miRNAs associated with multi-drug resistance (MDR) to target cells.(92) Furthermore, exosomes have a role in tumor resistance by facilitating the removal of drugs from cells. The sequestration of cytotoxic medicines within melanosomes is a contributing factor to the treatment resistance observed in malignant melanomas.(93) In addition, exosomes have the ability to mitigate the impact of antibody medicines by regulating their interaction with tumor cells. Lymphoma exosomes contain CD20, which can attach to therapeutic anti-CD20 antibodies and shield target cells from antibody-mediated assault.(94) Exosomes derived from breast cancer cells that have high levels of HER2 expression contain functional HER2 and are capable of binding to the HER2-targeting antibody trastuzumab, hence suppressing its action.(95) Exosomes released by stromal cells also have a role in the development of treatment resistance in tumors. Exosomes produced from bone marrow MSC (BM-MSCs) stimulate the development of bortezomib-resistant multiple myeloma cells by activating various pathways that promote cell survival.(96) Hence, exosomes discharged by cancer cells and stromal cells possess the capability to influence the responsiveness of cancer cells to specific treatments.(97)

Endothelial cells play a role in the process of vascularization, which involves supplying cancer cells with oxygen and nutrients, both of which are limited in tumors. Cytokines, such as VEGF and basic FGF (bFGF), facilitate communication between endothelium and cancer

cells. Cancer cell-derived EVs include a range of chemicals, including miRNAs, that stimulate angiogenesis.(78) As an illustration, miR-9, which is linked with extracellular vesicles formed from cancer cells, decreased the levels of suppressor of cytokine signaling 5 (SOCS5). miR-210 is released in EVs formed from cancer cells in order to control the activity of neutral sphingomyelinase 2 (nSMase2) (98), hence stimulating the process of angiogenesis (99). The expression of the angiogenic inhibitor ephrin-A3, which is a target gene of miR-210, is reduced after the transfer EVs from cancer cells. miR-210 controls the process of forming new blood vessels in endothelial cells (100) and maintains the haemostasis of iron in cancer (101). It is increased in response to hypoxic conditions (100,101). Moreover, the modulation of nSMase2 expression has an impact on the generation of EVs, which in turn influences the ability to metastasize (99). EVs have been found to include numerous angiogenic factors (78); however, the specific roles of these factors have not been fully understood. In order to comprehend the role of angiogenic EVs in cancer development, it is imperative to elucidate the function of each component and demonstrate their combined impact.

Immune evasion is a prominent characteristic of cancer, and numerous research have explored the ways via which tumor cells avoid detection by the body's immune system. Tumor cells release EVs that can enhance or inhibit the immune response against cancer.(102,103) Indeed, tumor-derived EVs include different tumor antigens, including melan A, mesothelin, and carcinoembryonic antigen (CEA). These EVs have the ability to boost immunological activation. Moreover, there is a growing body of research indicating that EVs originating from tumors have the ability to inhibit the immune system. Tumor-derived EVs include a diverse range of immunoregulatory molecules, including FasL, TRAIL, and galactin-9, which facilitate the evasion of the immune system by cancer cells.(104) Collectively, the evidence suggests that tumor-derived EVs with immunological activity play a crucial role in facilitating communication within the tumor microenvironment related to cancer immunology. Hence, the EVs originating from the tumor and their contents could serve as vital objectives for cancer immunotherapy.(105)

MSC-EVs in Cancer Studies

Mounting data suggests that exosomes have significant functions in cancer. Exosomes transport cancer-causing proteins and genetic material to alter the behavior of recipient cells, and they have significant involvement in the development, growth, spread, and resistance to treatment of tumors. Exosomes have the ability to influence multiple types of recipient cells. Exosome uptake can lead to a long-lasting and effective alteration of the cells that receive them. This section will focus on the functions of exosomes in cancer and the underlying molecular pathways.(97)

Both normal and malignant cells rely on local and distal cellular communication for vital processes.(106) Exosomes, which are involved in intercellular communication, have significant involvement in various crucial oncogenic processes such as tumor spread, treatment resistance, and immunological responses. The roles of exosomes are dictated by the particular payload they transport. Exosomes, along with their distinct cargo comprising of proteins, metabolites, and nucleic acids, can offer insights into the potential regulatory factors that contribute to the advancement of tumors.(61)

The impact of gene overexpression on the anti-tumor activities of MSCs has also been examined. *In vitro*, MSCs-derived exosomes that express TRAIL were found to trigger apoptosis in cancer cells.(107) Another study demonstrated that exosomes obtained from MSCs that overexpress miR-122 increase the sensitivity of hepatocellular carcinoma (HCC) to chemotherapeutic drugs, including sorafenib. This leads to an enhancement of sorafenib's anticancer effects both in laboratory settings (*in vitro*) and in living organisms (*in vivo*).(108) Exosomes derived from MSCs, which have been modified to produce a short interfering RNA targeting GRP78, a protein that is overproduced in cancer cells resistant to sorafenib, effectively restore the sensitivity of HCC to sorafenib both in laboratory tests and in living organisms.(109) MSCs-derived exosomes that overexpress miR-119a were found to inhibit the growth, invasion, and migration of glioma cells.(110) The one that overexpress miR-16-5p hinder the proliferation, migration, and invasion of colorectal cancer cells (CRCs) *in vitro*. Additionally, they enhance the death of these cells by reducing the expression of integrin α2. Treatment with exosomes derived from miR-124a-overexpressing MSCs reduces the proliferation of glioma stem cell lines *in vitro*. In addition, administering exosomes derived from miR-124a-overexpressing MSCs to mice with implanted GSC267 glioblastoma cells enhances the animals's survival rate.(111) Furthermore, it has been demonstrated that the movement and infiltration of prostate cancer cells can be suppressed in laboratory settings by exosomes derived from miR-143-overexpressing MSCs through the reduction of trefoil factor 3.(112) A different research investigation found that exosomes derived from

MSCs overexpressing miR-101-3p were able to inhibit the growth, movement, and invasion of oral cancer cells. This was achieved by specifically targeting a protein called collagen type X alpha 1 chain.(113)

MSCs have a significant impact in facilitating the advancement of tumors in cancer. On one hand, they establish a structure for securing tumor cells in the shape of tumor stroma and release substances that promote tumor development.(114) On the other side, MSCs found in the tumor microenvironment have the ability to transform into M2-type macrophages, myeloid-derived suppressor cells (MDSC), or M2-type microphages when exposed to cytokines or chemokines.(115,116) During the re-programming of the tumor microenvironment, MSCs engage with neighboring cells and transmit signals to facilitate the coordinated changes initiated by the tumor. (117) Upon investigation of the mechanisms involved in the interactions between MSCs and other cells in the tumor microenvironment, it was found that MSCs release various bioactive substances that have the ability to significantly modify the essential functions of nearby cells. These functions include cell survival, programmed cell death, cell maturation, and cell specialization.(118,119) The paracrine activity of MSCs, which is based on the release of secreted factors in the extracellular environment, is now widely acknowledged as a cell-free approach. These substances are present in the conditioned medium (CM) of MSCs. (120) However, recent investigations have demonstrated that these CM not only contain soluble secretions, but also include particulate fractions that possess biological activity typically associated with MSCs. This suggests that MSCs may use a unique biological mechanism, which involves a small particle-based nano-communication system, to transmit information to different recipient cells.(120,121)

In the future, a crucial task in cancer research will be to collect all the EVs originating from a certain kind of tissue to “mirrors” the physiology state of the cells, for example in cancer cell and categorize them for comparison with the vesicles from their normal counterpart cells. This analysis provides a deeper understanding of the crucial factors that govern various behaviors of cancer cells, particularly in relation to their key biological functions. Additionally, it highlights the potential of EVs for the diagnosis and treatment of human cancers. Advancements in understanding the specific cargo composition of cancer cells-derived EVs have the potential to revolutionize early cancer diagnosis and offer new therapeutic options. This could significantly improve the chances of effectively treating this life-threatening disease.(14)

EVs-based Drug Delivery Systems for Cancer Treatment

Although chemotherapy has made significant advancements in cancer treatment, it is susceptible to quick elimination from the body, limited absorption, inadequate delivery to tumor sites, non-specific toxicity, and subsequent systemic side effects. This often leads to the development of tumor resistance.(122) In order to address these difficulties, a wide range of artificial nanodelivery systems have been created, including those that have received clinical approval.(123) Artificial drug carriers have several advantages, including the ability to decrease renal clearance and enhance site-specific delivery through shape. They can also improve tumor targeting through ligand-based approaches. Furthermore, these carriers allow for the simultaneous delivery of multiple therapeutic agents, protection from enzymatic degradation, evasion of the immune system, sequential release of drugs in different stages, activation in response to specific stimuli, and theranostic capabilities. However, most of these capabilities are not currently being used in clinical settings, mainly because the manufacturing process to achieve multi-functionality is complex and expensive. Liposomes are the most extensive group of nanoparticles that have been approved for therapeutic use. They are composed of a basic lipid bilayer that encloses a watery compartment. Liposomes are very adaptable carriers for delivering medications, as they can accommodate both hydrophobic drugs in their lipid membrane and hydrophilic drugs in their inner space. Moreover, EVS can serve as effective vehicles for transporting both hydrophilic and hydrophobic medicinal medicines. EVs offer potential alternatives to manufactured nanoparticles due to their inherent organotropic and tumor-targeting.(124) For example, it has been demonstrated that integrins associated with EVs have a role in guiding the specific colonization of tissues by connecting with target cells. These ITGs attach to receptors present in the extracellular matrix, such as laminin or other suitable ITGs.(125)

EVs have the ability to carry several medicinal substances, including chemotherapeutic drugs and nucleic acids like mRNA, miRNAs, small interfering RNAs (siRNAs), and short nucleolar RNAs (snoRNAs).(126) When compared to chemotherapy, delivering nucleic acids into target cells is more difficult because of certain factors. These factors include limited absorption by the cells, which is caused by the negative charge and big size of the nucleic acids, and vulnerability to enzymatic breakdown in the

blood and extracellular space.(127) While viral and non-viral cationic synthetic nanoparticles can effectively carry RNA in laboratory settings, there are significant safety concerns associated with their usage in living organisms.(128) For instance, the use of cationic lipid and polymer-based nanoparticles can cause cell shrinkage, cytoplasmic vacuolization, and immunotoxicity.(129) EVs are naturally occurring vehicles that can transport endogenous bioactive nucleic acids to specific cells. Hence, EVs have the potential to serve as a highly effective method for delivering exogenous small molecule and RNA-based medicines. The subsequent section will examine diverse approaches for drug loading before to and after EV isolation. It is important to mention that generalizing findings from drug-loading research is challenging due to the significant influence of factors such as the source of EVs, the technology used for isolation, the therapeutic chemical being loaded, and the specific loading protocol.

For the purpose of comparison, liposomes that possess similar characteristics to EV-based delivery systems, such as size, drug quantity, drug loading technique, and designed targeting molecules, should be employed.(130) For instance, a study contrasted synthetic fusogenic liposomes carrying cholesterol-siRNA conjugates with mouse melanoma-derived EVs that displayed the same conjugates.(131) They both displayed a negative surface charge, exhibited a comparable size distribution, and showed the ability to incorporate chol-siRNA. The administration of fusogenic liposomes resulted in the inhibition of certain genes, whereas EVs did not exhibit the same effect. Cells internalized EVs at a lesser degree when compared to liposomes. When EVs were exposed to an endolysosomal agent, genes were suppressed, suggesting that the attachment of siRNA was too strong, causing the cargo to become trapped in the endolysosomal pathway.(131) This work emphasizes the significance of conducting direct comparisons and demonstrates that the advantage of synthetic drug carriers over EVs and vice versa may vary greatly depending on the specific circumstances, such as the method used for loading drugs.

EVs show great potential for enhancing the administration of medicinal medicines because of their inherent characteristics, such as tissue specificity. EVs have been enhanced to optimize the potential for drug delivery by including targeting ligands, stimuli-responsive components, and immunoevasive elements. The application of long-developed engineering methodologies in the field of synthetic cancer nanomedicine could offer numerous benefits, leading to drug delivery systems that combine both biological and external features. Utilizing many naturally occurring EV surface chemicals could potentially be a more effective approach for achieving organ-specific targeting. Nevertheless, this particular method requires the separation of specific EV subpopulations that exhibit advantageous transport characteristics, a task that has proven difficult using existing methodologies.(132)

Although scientists and engineers have made efforts to utilize the distinct characteristics of EVs for the creation of intelligent drug delivery systems that offer significant advantages in terms of targeting, safety, and pharmacokinetics compared to synthetic nanocarriers, the practical application of EVs in clinical settings is still difficult. Due to the inherent complexity of EVs, differences in size, and natural variances experienced during their manufacturing, the risks associated with the production process are higher compared to completely synthetic production systems.(133) EVs can serve as carrier systems for several medicine delivery applications. When EVs are given to rats through the bloodstream, they have been proven to transport functional cargo more effectively than traditional delivery techniques, while also reducing the risk of immune clearance. Nevertheless, further assessment in clinically significant systems and a direct, quantitative comparison with liposome-based alternatives are necessary to thoroughly evaluate the risk-benefit ratio. The successful translation of EVs relies on the presence of economically feasible methods for producing them on a large scale, as well as techniques for isolating and characterizing them with high sensitivity to detect variations between batches and understand their biological impacts. Additionally, it is important to have widely applicable methods for loading drugs into EVs.(134)

Engineering Exosomes for Cancer Therapy

EVs can undergo chemical or biological modifications to expand, modify, or improve their therapeutic potential. Two overarching methodologies have been employed to introduce a diverse array of nanoparticles, reporter systems, targeted peptides, pharmaceutics, and functional RNA molecules. Modifying the parent cells will affect EVs, for instance by genetic or metabolic engineering, as well as by the incorporation of external materials into the EVs they secrete. Furthermore, EVs can undergo direct functionalization through techniques such as hydrophobic insertion, covalent surface chemistry, and membrane permeabilization.(135)

Detailed explanation and figure description regarding some methods to engineer EVs including genetic engineering, membrane modification, encapsulation and hybridization, is already explained in our previous review article.(136)

Genetic engineering was inevitably employed to modify EVs for therapeutic purposes, as it is unquestionably the best established strategy for cell manipulation. When mRNA is injected into a cell, it can be enclosed in EVs. These EVs can then merge with a specific target cell, causing the production of transgenic proteins.(137,138) Moreover, the control of gene expression can also occur through the transportation of EVs that contain an abundance of non-coding RNA sequences, such as miRNA or small interfering RNA (siRNA).(98) Nevertheless, there are other concerns that must be acknowledged and meticulously managed throughout these investigations. It has been proposed that the observed alterations attributed to the RNA enclosed in EVs may really be caused by other components of the vesicles that stimulate an increase in the production of naturally occurring miRNA molecules. Moreover, miRNA can be encapsulated and delivered to targeted cells via large protein complexes, lipoproteins, or protein-oligonucleotide conjugates which may be co-extracted with EVs during the purification process.(139) Hence, it is crucial to employ meticulous EV purification techniques (140), and carefully choose appropriate models that do not natively express the target miRNA (141).

The rise of EVs as significant agents in the field of physiology and pathology has created promising prospects in the field of nanomedicine.(1) Modification tactics offer a promising avenue to enhance the therapeutic applications of EVs beyond their intended use. EVs naturally facilitate horizontal gene transfer; and this can be utilized to modify gene expression via genetic manipulation or direct loading. Due to the complexity, some factors need to be consider to modify the cargo, without compromise the function including the cargo itself, intended application, and other pertinent factors. Every application will present distinct biological inquiries, which will subsequently determine the specific technical aspects of the modification approach. Utilizing a customized method for modifying EV provides the highest likelihood of achieving success, but it also poses significant hurdles for researchers in this sector.

Prior to engineering exosomes originating from MSCs, it is important to acknowledge the potential role of exosomes in promoting cancer progression. Exosomes formed from BM-MSC in patients with multiple myeloma promoted the advancement of multiple myeloma by including elevated quantities of oncogenic proteins, cytokines, and adhesion molecules compared to exosomes from healthy cells.(142) Exosomes generated from bone marrow MSCs stimulated tumor growth in living organisms by enhancing the formation of new blood vessels. Further investigation is needed to explore the contributory function of exosomes produced from MSCs in the advancement of cancer.

Exosomes have also been modified to enhance the process of phagocytosis, specifically targeting cancer cells and facilitating their engulfment by macrophages. The majority of cancer cells exhibit the presence of CD47 on their surface, which serves as a signal to prevent their ingestion by immune cells. When CD47 binds to signal regulatory protein α (SIRPα) on innate immune cells like macrophages and dendritic cells, it triggers the do not eat me signal, allowing tumors to avoid being engulfed by phagocytosis. (143) A separate study discovered that exosomes containing SIRPα had a stronger therapeutic index against tumor growth in CT26.CL25 and HT29 tumor-bearing mice, as compared to the same dose of ferritin-SIRPα.(144)

There are two specific aspects that can be enhanced in cancer treatment: targeting and effectiveness. Exosomes possess inherent ability to accurately target and home in on tumor sites, which may be attributed to their presentation of several cell-derived surface molecules. Moreover, exosomes have the ability to specifically gather at the site of cancer by altering their outer layer to display targeting molecules that can attach to receptors on cancer cells. The co-administration of therapeutic medicines with exosomes demonstrates enhanced cytotoxicity and improved efficiency in inducing apoptosis in cancer cells. Hence, engineered exosomes exhibit potential in the field of cancer therapy.(145)

Several medications are unable to penetrate the blood-brain barrier (BBB), which limits the effectiveness of numerous treatments for metastatic disease. Hence, an investigation was conducted to determine if exosomes produced from brain endothelial cells have the capability to transport a medication across the BBB. These findings demonstrated that the exosomes effectively transported medicines via BBB and into the brain. Upon application to a brain cancer model, it was observed that the treated group exhibited a lower quantity of labeled cancer cells in the brain compared to the control group. This demonstrates that modified exosomes have a high level of effectiveness in transporting cancer medications via BBB, making them a possible therapy option for brain tumors or metastases.(146)

Given the diminutive dimensions of exosomes, researchers often modify the donor cell and subsequently extract exosomes that carry the desired gene or medication.

(147) Exosomes have been engineered to transport a variety of substances. Gene delivery can be accomplished using non-viral or viral techniques, and various approaches have been explored to enhance the optimal loading of exosomes. Exosomes are released from the cell via the endosomal pathway, which viruses exploit to their advantage.(148) Through the manipulation of this mechanism, exosomes can be modified to transport a specific gene or medicine by utilizing a virus to load the exosomes.

Prior research has thoroughly examined the possibility of utilizing native exosomes as a potential cancer vaccine. However, these investigations did not involve any genetic modification of the exosomes. A recombinant adenovirus was utilized to integrate interleukin (IL)-18 into exosomes. When examined in the context of colon cancer, these exosomes were discovered to have the ability to enhance the proliferation of PBMCs and stimulate the secretion of Th1 cytokines. Additionally, they were found to attract T and DC cells in *vitro*.(149) A separate research team examined the impact of IL-2 and its ability to combat tumors.(150) The EL-4 cells, which had been previously modified to increase the production of ovalbumin, were subjected to another transfection process to further enhance the expression of IL-2. The exosomes that were modified by engineering techniques were subsequently separated from these cells and administered via injection into mice that had tumors. The modified exosomes were able to stimulate a targeted Th1 polarized immune response and CTL more effectively, leading to a notable suppression of tumor development. (150,151)

These advancements are notable among the numerous proof-of-concept, in vitro investigations into fluorescently labeled EVs. Although visibility is unquestionably beneficial, especially in live organisms, the capacity to enhance EVs with targeting or stimuli-responsive features provides the opportunity to manipulate an intricate biological system. In order to advance the research and transform EVs from intriguing biological candidates into smart nanoscale medicines, it is crucial to sustain progress in these domains.

MSC-derived Exosomes in Cancer Therapies

MSCs exert their therapeutic effects through paracrine signaling rather than direct cell-to-cell interaction. Increasing data indicates that exosomes formed from MSCs have the ability to transmit proteins and RNAs to recipient cells, resulting in multiple consequences on the proliferation of different types of tumor cells. In addition, MSCs are the sole human cell type that possesses the ability to produce exosomes in large quantities for the purpose of drug administration. There are conflicting reports on the impact of MSC-derived exosomes on tumor growth, with some suggesting suppression and others suggesting promotion. In addition to controlling the destiny of tumor cells, exosomes generated from MSCs can be used to transport anticancer treatments. Cell-derived exosomes provide several advantages as therapeutic agents compared to cells or synthetic nanoparticles. These advantages include the ability to be modified, excellent biocompatibility and stability characteristics, and a greater ability to carry different substances. They can be altered by attaching certain ligands or proteins to their surface in order to transport the therapeutic payload to desired cells and tissues.(49)

The connection between MSCs and cancer cells is enigmatic, as there are conflicting studies about the impact of MSCs on tumor cells, with some suggesting a stimulating effect and others suggesting an inhibiting effect.(152) The impact of MSCs on cancer cells is diverse and contingent upon factors such as the kind of cancer, stage of cancer, microenvironment, mutations, and the acquired resistance status of the cancer cell lines.(153,154) Despite several papers demonstrating the tumor-promoting actions of MSCs, there are also several articles highlighting the tumor-inhibitory effects of MSCs or modified MSCs on different types of tumors. However, the exact mechanisms behind these effects remain unknown. There are two potential approaches for producing anticancer agents within the body by MSCs: the first involves injecting gene-modified MSCs intravenously, and the second involves transplanting MSC-loaded artificial scaffold matrix near the tumor subcutaneously. The inhibitory effects of MSCs are ascribed to their direct cellular contacts or the secretion of factors. (155) The hBM-MSCs have demonstrated strong anticancer effects on Kaposi's sarcoma, which is a neoplasm associated with AIDS and characterized by high levels of angiogenesis. BM-MSCs limit tumor growth by preventing the activation of Akt protein kinase through direct interaction with tumor cells.(81)

Figure 4 describes the physiological function of MSC-EVs in cancer with its pro- and anti-tumor features. The EVs can support tumor angiogenesis indirectly which support tumor growth *in vivo*, but exhibit a contradictive result *in vitro*.(156) Another studies showed how MSC-EV can inhibit angiogenesis, but on the other studies the MSC-EV also facilitate the chemoresistance induction. Initially, researchers employed several cancer cell lines and mice

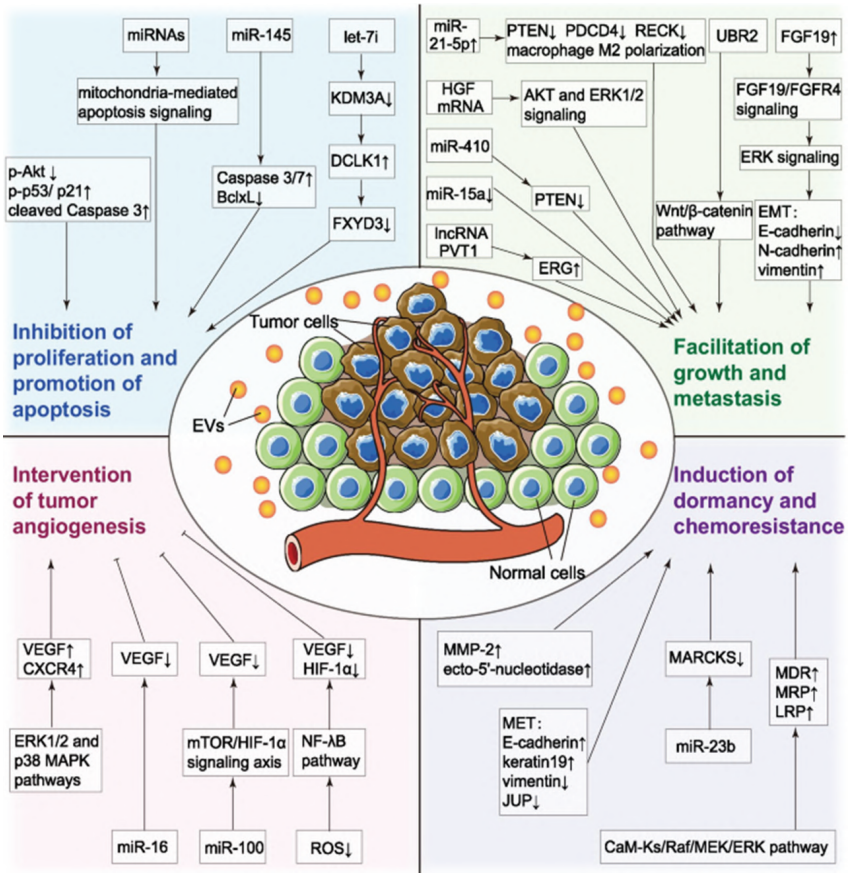

Figure 4. Physiological functions of MSC-derived EVs in cancers.(156) (Adapted with permission from Springer).

xenograft models to validate the regulatory functions of MSC-derived EVs in the cancer cell cycle, proliferation, and apoptosis. EVs derived from human BMSCs have been found to stimulate negative regulators of the cell cycle, resulting in apoptosis or necrosis and inhibition of tumor cell growth in hepatocellular carcinoma, ovarian cancer, and Kaposi's sarcoma.(157) Furthermore, the inhibitory and cell death-inducing effects of EVs obtained from human UCMSCs were seen in bladder cancer. The observed effects were associated with inhibited phosphorylation of AKT protein kinase and enhanced cleavage of Caspase 3.(158)

Recently, there is ongoing research on targeted drug delivery for malignancies with the aim of targeting specific subcellular compartments. Among the various approaches being explored, receptor-mediated endocytosis has the most potential.(159) Due to their negative charge and hydrophilic nature, miRNAs, which are promising agents for tumor treatment, face challenges in crossing cell membranes. Furthermore, they undergo rapid degradation once entering the body. Exosomes, being superior carriers, can effectively overcome this concern.(160) The exosomes of hUC-MSCs expressing miRNAs have been recognized as significant vehicles for gene or pharmacological therapy in related studies as described in Figure 5.(6)

The technique of gene modification is widely employed for the transfection of MSC-Exosomes with miRNA. Exosomes derived from BM-MSC that were engineered to have an increased expression of miR-126-3p. These exosomes were then co-cultured with pancreatic cancer cells. The study revealed that miR-126-3p effectively hindered the progression of pancreatic cancer by specifically targeting ADAM9. In a similar manner, in another study was able to co-cultured MSC-exosomes that were overexpressing miR-148b-3p with the breast cancer cell line MDA-MB-231. The researchers discovered that miR-148b-3p suppressed the growth, invasion, and movement of breast cancer cells, while simultaneously enhancing apoptosis. This effect was achieved by reducing the expression of TRIM59. A further investigation documented that the exosomal miR-205 originating from hBM-MSCs impeded the advancement of prostate malignancy by restraining RHPN2.(161) MSC-Exosomes that have been enriched with miR-185 were anticipated to function as a novel therapeutic approach for oral leukoplakia due to their ability to diminish inflammation, hinder cell proliferation and angiogenesis, and trigger cell apoptosis.(162) β-catenin, a crucial component of the Wnt/β-catenin signaling pathway, has a significant impact on tumor development. The inhibitory impact of

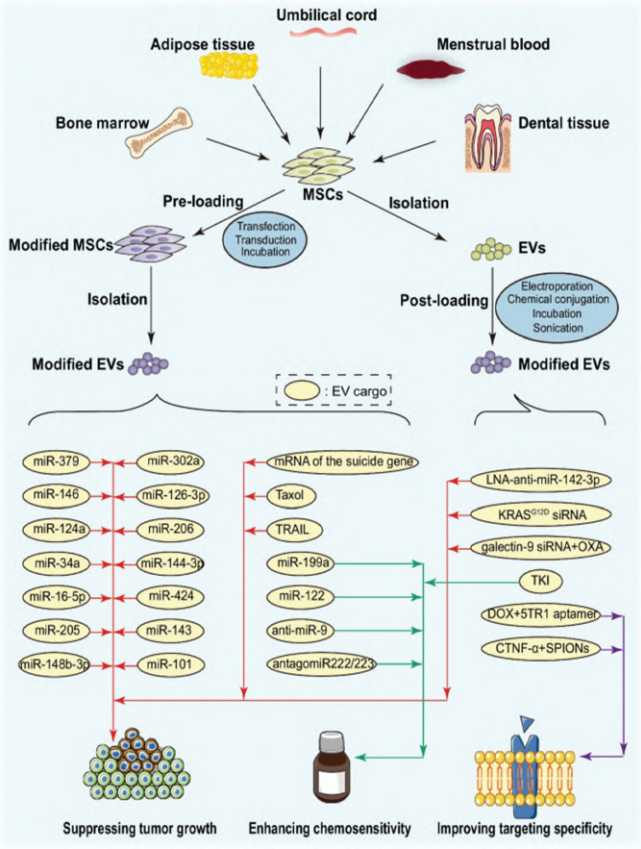

Figure 5. Applications of bioengineered MSC-derived EVs in cancer therapy.(6) (Adapted with permission from Springer).

miR-34c on β-catenin in nasopharyngeal carcinoma (NPC) was also proven.(84)

Because MSCs have a growing tendency to go towards tumors, the exosomes released from MSCs can be utilized in a tumor treatment strategy as a means to precisely and selectively target and transport anti-tumor substances to cancer cells. Furthermore, MSCs can be instructed to release healing substances, such as targeted proteins and miRs, using exosomes. This can effectively target and treat damaged or dysfunctional cells and sick tissues, meeting therapeutic needs. Nevertheless, the intricate nature of exosome interactions across several coordinating populations and a constantly evolving metabolic milieu necessitates meticulous discrimination and assessmen.(24)

Conclusion

In general, MSC-EVs can have various impacts on the growth of tumors and have the potential to be effective platforms for delivering anti-tumor drugs because of their great attraction to tumor cells. Current challenge is to find a better technique of purification and modification to express the determined benefit. It is crucial to have a clear understanding of component characterisation, immunological reactivity, and loading of exosomes without altering the inherent properties of the donor cell. Hence, it is imperative to establish appropriate criteria for the modification, isolation, and characterisation of exosomes in order to forward this promising breakthrough towards clinical use.

Authors Contribution

AM drafted the original manuscript and critically revised the manuscript manuscript. NMD edited and revised the manuscript. AW proposed and concepted the manuscript topic, and gave critical suggestions to the final draft. All authors have agreed with the final revisions of the manuscript.

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