Exosome as a target for cancer treatment

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ABSTRACT

Exosomes are small vesicles covered by a lipid bilayer, ranging in size from 50 nm to 90 nm, secreted by different cell types in the body under normal and pathological conditions. They are surrounded by cell-segregated membrane complexes and play a role in the pathological and physiological environments of target cells by transfer of different molecules such as microRNA (miRNA). Exosomes have been detected in many body fluids, such as in the amniotic fluid, urine, breast milk, blood, saliva, ascites, semen, and bile. They include proteins, lipids, and nucleic acids such as DNA, RNA, and miRNA, which have many functions in target cells under pathological and physiological conditions. They participate in pathological processes such as tumor growth and survival, autoimmunity, neurodegenerative disorders, infectious diseases, inflammation conditions, and others. Biomarkers in exosomes isolated from body fluids have allowed for a more precise and consistent diagnostic method than previous approaches. Exosomes can be used in a variety of intracellular functions, and with advances in molecular techniques they can be used in the treatment and diagnosis of many diseases, including cancer. These vesicles play a significant role in various stages of cancer. Tumor-derived exosomes have an important role in tumor growth, survival, and metastasis. In contrast, the use of stem cells in cancer treatment is a relatively new scientific area. We hope to address targeted use of miRNA-carrying exosomes in cancer therapy in this review paper.

INTRODUCTION

Extracellular vesicles (EVs) are lipid-bound particles secreted by cells or formed directly from the plasma membrane which allow communication between cells through their content. They include proteins, lipids, and nucleic acids such as DNA, RNA, and microRNA (miRNA), which have many functions in target cells under pathological and physiological conditions.[1] Identification of EVs by cells is based on ligand–receptor interaction.[2] These particles are divided into three primary classes based on size and release process, namely exosomes with less than 150 nm in diameter (smallest class), microvesicles/shedding particles (excreted by live cells), and apoptotic bodies (excreted by dying cells), which are larger than 100 nm in diameter.[3]

In this review, the authors will describe the properties of exosomes and their function. Exosomes, also referred to as intraluminal vesicles (ILVs), were initially introduced by Trams *et al* in the 1980s.[4] They are small vesicles covered by a lipid bilayer, ranging in size from 50 nm to 90 nm, secreted by different cell types in the body under normal and pathological conditions.[2] Exosomes are derived from the plasma membrane, while ILVs are formed within multivesicular bodies (MVBs). MVBs are then fused with or are directly derived from the membrane.[3 5] Exosomes have been detected in many body fluids, such as in the amniotic fluid, urine, breast milk, blood, saliva, ascites, semen, and bile.[6 7]

Exosomes are classified and identified by markers such as CD9, CD63, CD82, CD81, heat shock proteins (HSP), major histocompatibility complex, and lipid rafts such as flotillin-1 on the exosomal surface membrane.[8 9] Exosomes can be a carrier of >92,897 proteins, 584 lipids, 4934 miRNAs, and 27,642 messenger RNAs (mRNAs).[10] These vesicles have a variety of effects on target cells. Exosomes first bind to the target cell receptor, activating the signaling cascade in the cells. Exosomes may then either consciously or indirectly combine their cargo with target cells. The cells then release mRNA, miRNA, and functional proteins into the cytosol, resulting in a variety of biological processes. Different molecules, environmental conditions (low pH and oxygen), and mechanical stimulation all help to speed up exosome secretion.[11 12]

Exosomes also participate in pathological processes such as tumor growth and survival, autoimmunity, neurodegenerative disorders, infectious diseases, inflammation conditions, and others.[13 14] Tumor-derived exosomes (TEX) play an important role in tumor growth, survival, and metastasis.[15] TEX containing tumor-specific antigens is expressed in parental tumor cells.[16] These cells secrete more exosomes than normal cells and so the level of fluid exosomes in patients with cancer is elevated.[17] When TEX contacts its target cell, it causes phenotypic and functional changes in the recipient cell.[18] Leukemia blasts, like all tumors, form exosomes that are involved in the survival and proliferation of leukemia cells, resistance to apoptosis and chemotherapy drugs, angiogenesis, and migration.[19]

BIOGENESIS, SECRETION, AND UPTAKE OF EXOSOMES

There are three stages involved in exosome secretion: formation of ILVs in MVBs,

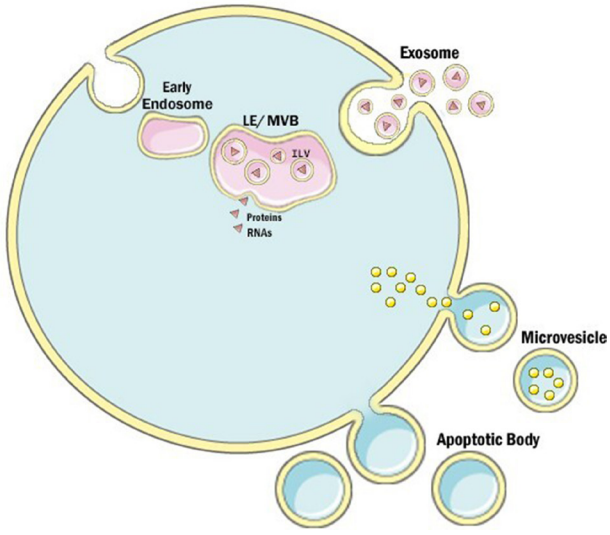

Figure 1 A glance at the biogenesis of exosomes in the cell. Inverted budding is a critical stage in the growth of MVB from late endosomes that contain ILVs. When MVBs fuse with the plasma membrane, ILVs from the inside become exosomes. ILVs, intraluminal vesicles; LE, late endosome; MVBs, multivesicular bodies.

transportation of MVBs to the plasma membrane, and merging of MVBs with the plasma membrane (figure 1).[3] Endosomes are formed by folding the plasma membrane inward. It is then assorted in the endoplasmic reticulum and processed into MVBs in the Golgi complex, and the cargo is packaged for secretion as exosomes. This is an endosomal secretion mechanism since these vesicles are generated by an endocytic source.[20] Endosomal vesicles consist of endocytic vesicles, early endosomes, late endosomes, and lysosomes.[21]

Internalization of the plasma membrane with proteins or other molecules leads to the development of endocytic vesicles, which then join early endosomes through clathrin-dependent or clathrin-independent pathways. Late endosomes vary in pH, form, protein context, and ability to integrate with vesicles from early endosomes. Late endosomes are nearly at the center of the cell, whereas early endosomes are close to the membrane. They have global outward, but early endosomes are cylindrical. Maturation of endosomes is necessary to form MVBs. Switching of their small GTPase markers, known as Rab, indicates conversion of early endosomes to the late ones. Studies have shown that a protein called SAND1 can repress Rab-5 on early endosomes while activating Rab-7 to form late endosomes. The key stage in the development of MVBs from late endosomes that produce ILVs is inverted budding.[20–22]

ILVs separate specific lipids, proteins, and cytosolic components.[7] ILVs inside MVBs become exosomes when MVBs fuse with the plasma membrane.[23] The endosomal sorting complex required for transport (ESCRT) machinery mechanism has the main role in the formation of exosome inside the endosome, but there are other pathways, including HSPs, tetraspanins, ceramides, phosphatidic acid, and cholesterol.[20 24 25] The ESCRT contains different complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, and the associated AAA ATPase Vps4 complex.[26] It is known that during exosome secretion MVBs fuse with the plasma membrane and release the exosome into the extracellular space. Rab GTPase is a regulator mechanism in MVB membrane fusion (figure 1).[27]

There are several mechanisms to absorb EVs such as exosomes by the target cells,[28] such as phagocytosis,[29] membrane fusion, micropinocytosis,[30] and clathrin-mediated endocytosis.[31]

DRUG RESISTANCE

Drug resistance remains one of the most significant obstacles in cancer treatment, manifesting itself through a variety of processes, such as decreased drug aggregation, increased efflux, increased biotransformation, drug compartmentalization, acquired genetic alteration of drug targets, or defects in cellular pathways.[32] A study has shown that exosomes induced chemoresistance in recipient cells.[33]

Koch *et al*[34] demonstrated that exosome biogenesis is modulated by the lysosome-related, organelle-associated ATP-binding cassette (ABC) transporter A3 (ABCA3). ABCA3 also mediated chemoresistance. Therefore, inhibiting this transporter increases lymphoma cell susceptibility to chemotherapy.

Galectin-3 (GAL-3) is a galactose-binding lectin that has different functions. Acute lymphoblastic leukemia (ALL) cells that co-cultured with stromal cells have high levels of GAL-3. GAL-3 of exosomes derived from these cells activates the nuclear factor kappa B (NF-kB) pathway in ALL cells and induces antiapoptotic effects in leukemic cells.[35] Interestingly, by binding $CD20^{+}$ exosomes in chronic lymphocytic leukemia to rituximab (anti-CD20 antibody), the function of this drug is reduced.[36]

Exosomes produced by chronic myeloid leukemia (CML) cells play a role in disease progression. Exosomes produced from imatinib-resistant CML cells can be internalized and induce drug resistance in susceptible CML cells. The level of miR-365 in exosomes derived from drug-resistant CML is higher compared with those from sensitive ones. MicroRNA induces chemotherapy resistance by inhibiting the expression of proapoptotic proteins such as BAX and cleaved caspase-3 in susceptible cells.[37]

In addition, exosomes derived from leukemic cells increase the level of proteins involved in chemoresistance in bone marrow stromal cells (BMSCs).[13] Exosomes obtained from patients' BMSCs also induce tumor cell resistance to bortezomib in multiple myeloma (MM) cells and promote survival of tumor cells by activating the pathways related to drug resistance and cell survival pathways, such as Notch1, signal transducer, activator of transcription 3 Akt 5, and NF-kB.[38]

IMMUNE SUPPRESSION

T lymphocyte and natural killer cell inactivation as well as T regulatory (Treg) cell differentiation are caused by vesicles originating from tumor cells.[39] TEX may have direct impact on the immune system, such as death of activated T cells in the presence of Fas ligand positive exosomes. Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand are molecules that induce apoptosis in activated T cells.[16] TEX has been shown in studies to play a role in Treg induction, expansion, and function, as well

as enhancement of Treg resistance to apoptosis through transforming growth factor-β and interleukin (IL) 10 mechanisms.[40]

ANGIOGENESIS

Angiogenesis is a term used to describe the process of formatting new blood vessels. This process occurs in physiological and pathological conditions.[41] Vascular endothelial growth factor (VEGF) is the main growth factor in angiogenesis.[42] The notch signaling pathway is activated downstream of VEGF signaling and negatively regulates VEGF-induced angiogenesis. The role of VEGF upregulation in hematological malignancies is debatable, but a study has presented conflicting results.[43]

Several clinical studies have shown that the level of angiogenesis is correlated with the stage of disease, prognosis, or response to therapy. Data also suggest that angiogenesis induction in hematological tumors has a pathophysiological correlation with disease progression.[44] The steps involved in angiogenesis include enzymatic degradation of the vessel's basement membrane, endothelial cell (EC) proliferation, migration, and tubulogenesis (EC tube formation) and maturation.[45] Exosomes produced by cells act as supportive mediators in the tumor microenvironment. The function of exosomes in the angiogenesis of hematological diseases has been explored in several studies. A study on CML reported that exosomes released by LAMA84 CML cells and patients' leukemia cells in the blood have a potential role in in vitro and in vivo angiogenesis. The study showed that adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) were significantly elevated in CML cells and that exosomes also stimulated human umbilical vein endothelial cells (HUVEC). In addition, exosomes of LAMA84 cells increased IL-8 expression and mitogen-activated protein kinase (MAPK) phosphorylation in HUVEC and therefore these factors cause an angiogenic effect.[46] Hypoxia is caused by the proliferation of malignant cells in MM. Exosomal miR-135b inhibited its target factor hypoxia-inducible factor 1 (FIH-1) in ECs and increased endothelial tube development under hypoxia through the hypoxia-inducible factors (HIF)–FIH signaling pathway.[47]

Exosomal miRNA from leukemic cells is also involved in angiogenesis. A research on K56 cells revealed that miR-92a, a member of the miR-17-92 cluster derived from K562 exosomes, could decrease the expression of the target gene integrin a5 and then augment endothelial migration and tube formation.[48] Furthermore, exosomal miR-210 of CML cells combines with the target gene ephrin-A3 and plays an essential role in the regulation of VEGF signaling and angiogenesis.[49] NB4 cells (acute promyelocytic leukemia cell line) generate EVs that contain promyelocytic leukemia/retinoic acid receptor alpha mRNAs, which fuse with ECs and compel them to express more tissue factor. Tissue factor as the starter of coagulation cascade protects the vessels. This factor is also involved in the development of malignancy through metastasis and angiogenesis. Tissue factor is directly (by altering growth regulator molecules) and indirectly (dependent on coagulation) involved in angiogenesis.[19 50 51]

ADVANTAGES OF EXOSOMES FOR DRUG DELIVERY

One of the applications of exosomes is their use as a drug delivery system. Exosomes derived from stem cells, such as immature dendritic cells or mesenchymal stem cells (MSCs), can be used as a drug delivery system with less immunogenic interactions without interacting with opsonin proteins, complement components, antibody molecules, and coagulation factors. During fusion with the cell membrane, the exosome's phospholipid bilayer membrane may pass the load within itself. For example, exosomes of dendritic cell origin are able to cross the cellular endocytic mechanism through interaction between exosome tetraspanin (CD9) and glycoprotein on the target cell surface. Some of the advantages of using exosomes as drug delivery include the ability to move across rigid biological membranes, the nano-sized structure, the ability to carry biological molecules to target cells, target specificity, non-synthetic origin, and its bilipid structure makes it resistant to degradation.[52] Also, due to their endogenous nature and special surface-building components, exosomes have longer half-life than liposomes. Exosomes have a wide range of applications in personalized medicine due to their ability to carry cargo without activating the immune system. For example, in order to effectively and purposefully transmit exosomes carrying antitumor miR, the donor cell can be manipulated to express the permeability of the platelet-derived growth factor receptor membrane combined with the GE11 peptide.[53]

Other advantages of exosomes include their small and homogenous size. This feature enables exosomes to escape phagocytosis as well as to easily pass through blood vessels around the tumor cells. Because exosomes originate in the patient's own cells, they can be used to safely transmit drugs to treat cancer.[54]

The reasons for using exosomes as natural nano-carriers for transmitting small interfering RNA (siRNA) and miRNA are their negative charge and hydrophilic characteristics, resulting in poor uptake by the target cell and their short lifespan in the bloodstream by nucleases.[55] Exosomes can regenerate cells and exchange genetics by communicating between cells and transmitting miRNA proteins and functional mRNAs. Numerous studies have shown that genetic information transmitted by exosomes can alter the activity and phenotype of the receptor cell.[56] Targeted synthesis of exosomes enables them to be used as anticarcinogenic therapeutic tools for targeted treatment of cancer.[57] Targeted exosomes for cancer treatment are created by the delivery of anticancer drugs and nucleic acids, such as siRNA, miRNA (miRNA suppressors or onco-miR inhibitors), and DNA.[58] Commercial liposomes are necessary in the successful siRNA and miRNA transfer to exosomes for therapeutic purposes (figure 2). Exosomes can inhibit cell proliferation and cause cell death in both in vivo and in vitro conditions by transferring siRNA or miRNA to the target cancer cell through gene silencing and/or gene regulation. The regulatory role of miRNA and its presence in lumen's exosomes are two important features that have led to the use of miRNA for diagnostic and therapeutic purposes.[55] Exosomes are responsible for transmitting miRNAs to near and far cells. Manipulated exosomes that link to the ligands, peptides, and antibody fragments are used for targeted delivery of miRNA and improve cancer

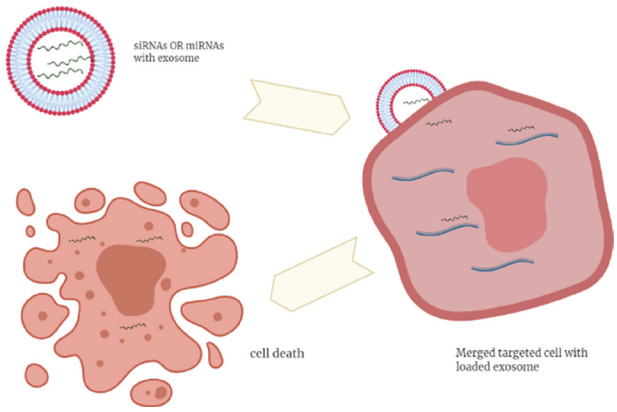

Figure 2 Schematic of the transmission of siRNA and miRNA by exosomes. Exosomes are used to deliver drugs and nucleic acids (such as small interfering RNA (siRNA), microRNA (miRNA) and spherical nucleic acids (SNAs) to the cells.

treatment.[57] The expression of onco-miRNA is one of the targeted therapies for cancer cells dependent on exosome-tumor suppressive miRNA. Exo-tumor suppressive miRNA, inhibition of proangiogenic mRNA, and eventual inhibition of tumor angiogenesis are all treatment choices.[55]

CLINICAL USE OF EXOSOMES

This section provides examples of the clinical use of exosomes in the targeted treatment of cancer. In the first study on the transmission of miRNA by exosomes, exosomes originating from HEK293 cells containing let-7a miRNA, let-7a miRNA was transferred to the tumor xenograft mouse model. The epithelial growth factor receptor (EGFR)-specific GE11 peptide or epithelial growth factor (EGF) was used to correct and improve the accumulation of exosomes in the target cell. Studies have shown that EGF-modified exosomes are suitable for transmitting anticancer drugs for targeted treatment of EGFR-positive cancer cells.[54 59] Exosomes derived from MSCs can transfer antitumor miRs. In a rat model with primary brain tumor, for example, injecting an exosome derived from MSCs carrying miR-146 into the tumor successfully reduces the development of glioma xenograft. Exosomes containing miR-302b are being used as a new therapy for lung cancer. These exosomes can inhibit lung cancer cell proliferation and migration through the transforming growth factor-β receptor II/ extracellular signal-regulated kinase (TGF-β RII/ERK) pathway.[53] An exosome containing miR-101 can amplify apoptosis in the gastric cancer cell by targeting anti-apoptotic myeloid cell leukemia-1.[57]

There are four ways to load miRNAs into exosomes: the sphingomyelinase 2-dependent pathway, the sumoylated heterogeneous nuclear ribonucleoprotein-dependent pathway, a signal sequence at the 3′ end miRNA, and the miRNA-induced silencing complex. The placement of miRNAs inside exosomes is controlled by the cell activation-dependent changes path of miRNA target abundance. Argonaute 2 is also involved in this process. In general, placement of miRNAs in exosomes depends on the sequences in miRNAs and protein complexes.[57] Allogeneic exosomes are formed by changing the content and the surface proteins, and these exosomes are exclusively ingested by processing cancer cells, not healthy cells. Therefore, one of the most important barriers to cell-based therapy will be solved.[60] Despite the attractiveness and novelty of targeted cancer therapy using engineered exosomes, this area is still in its infancy and is challenging and needs further study and research.[57 61] To further explore exo-miRNA, more research should be done on other malignancies and in larger study groups. By increasing knowledge about the therapeutic role of exosomes, personal treatment is possible. In addition, finding easier and cheaper ways to identify exosomes and miRNAs, additional markers on exosomes for easy detection, and recognizing miRNAs from the tumor microenvironment can facilitate their clinical use.[62]

CELL-FREE THERAPY OF CANCER BY STEM CELL EXOSOMES

Numerous studies have shown that the embryonic microenvironment can regenerate tumor cells from malignant to benign. However, there is still debate on the impact of adult stem cells on regeneration and changes in cancer cell phenotype.[56]

MSCs are multipotent and non-hematopoietic adult stem cells that are derived from bone marrow, umbilical cord, and placental or adipose tissue. These cells are used to treat cancer due to their anticancer activity.[63] By identifying and isolating exosomes from the MSC culture media and using them in research and clinics based on cell-free therapy, it is possible to use them as a treatment option in the future. In an intravenous mouse model, exosomes are tolerable without weight loss and adverse effects on renal and hepatic function.[64] The effect of produced exosomes on the target cancer cell and its biological pathways depends on both the specificity of the proteins on the membrane surface of the exoskeletons and the genetic information of healthy stem cells and tumor cells.[56] In addition, studies have shown that exosomes act as a cell-free vaccine and can be effective in treating cancer. Applications of microvesicles-human adult liver stem cells (MV-HLSC) include regenerative medicine and gene transfer tool.[65]

In 2016, Reza *et al*[66] examined the effect of human adipose MSC (hAMSC)-derived exosomes containing miRNA on A2780 and SKOV-3 ovarian cancer cells and concluded these exosomes could regulate cancer survival, cell cycle progression, and cytokine and cytokine-receptor expression. Exosomes derived from hAMSC-conditioned medium (CM) contain a wide range of miRNAs, including anticancer miRNAs as well as new and lesser known miRNAs. Cancer-derived exosomes have a distinct miRNA expression compared with hAMSC-CM-derived exosomes. hsa-miR-105, hsa-miR-214, hsa-miR-92, hsa-miR-21, hsa-miR-29, hsa-miR-9, and hsa-miR-222 are among these miRNAs.[66]

Studies have shown that exosomes derived from MSCs containing miR-23b, miR-451, miR-223, miR-24, miR-125b, miR-31, miR-214, and miR-122 can inhibit tumor growth and stimulate apoptosis through various pathways. miR-23b can promote and prolong the dormancy time of metastatic breast cancer cells by inhibiting the MARCKS gene, as a target gene and its product, which can improve cell cycle and cell motility.[65 67] A 2013 study found that MSC-derived exosomes containing miR-16 can inhibit tumor progression and angiogenesis by reducing VEGF expression in breast cancer cells. As a result, it is possible

to infer that MSC-derived exosomes can suppress VEGF expression in cancer cells. Lee and colleagues[65] concluded that MSC-derived exosomes regenerated tumor cell function by epigenetically transferring antiangiogenic miRNAs. Umbilical MSC-derived exosomes containing let-7f, miR-145, miR-199a, and miR-221 can inhibit RNA replication of hepatitis C virus.[55 64]

Exosomes derived from MSCs containing miRNA, according to Pakravan *et al*,[68] were able to modify the function of breast cancer cells in a paracrine manner. They were able to reduce VEGF expression using human bone marrow-derived MSCs containing miR-100 and the impact of miR-100 on the Mammalian target of rapamycin (mTOR)/HIF-1 signaling pathway and on balancing the signaling path.

Overexpression of miR-9 in glioblastoma multiforme (GBM) cells, as the deadliest and most common brain tumor in adults, leads to resistance in response to temozolomide. The resistance of GBM cells to temozolomide is due to the indirect effect of miR-9 on increasing P-glycoprotein expression. Munoz *et al*[69] used an MSC-derived exosome containing anti-miR-9 to suppress therapeutic resistance in GBM cells. The anti-miR-9 transition from the exosome to resistant GBM cells was expressed as a multidrug transporter during this test, and resistant GBM cells were sensitized to temozolomide, resulting in increased cell mortality and caspase activity. Glioma xenograft development can be greatly decreased by injecting MSC-derived exosomes containing miR-146 in an intratumoral rat model with a primary brain tumor.[70]

In ovarian cancer, using mesenchymal-derived exosomes, apoptosis can be induced in tumor cells by upregulation of apoptosis proteins (Bax, caspase-3, and caspase-29) and downregulation of Bcl-2 as an antiapoptosis.[57]

HLSC-derived exosomes (MV-HLSC) can inhibit the survival and proliferation of hepatoma tumors by transmitting selective genetic information such as miR451, miR223, miR24, miR125b, miR31, miR214, and miR122, resulting in the benign phenotype in cancer cells in vivo and in vitro. This effect is due to the modulation of signaling pathways that act differently in cancer cells than in normal cells. As noted earlier, the effect of produced exosomes on cancer cells depends on the specificity of both the proteins on the surface of the membrane and the genetic information carried. Therefore, it can be concluded that MV-HLSC is effective not only in hepatic tumors but also in lymphoblastoma and glioblastoma tumors.[56]

Exosomes derived from hAMSC containing hsa-miR-124-3p can reduce the expression of various cyclin-dependent kinases (CDKs), such as CDK2, CDK4, and CDK6, by stopping the cell cycle in the S phase of ovarian cancer A2780 cells. The relation between MSCs and cancer cells is not well understood.[66] Further research into the effects of MSC-derived exosomes on cancer cells, including growth enhancement or inhibition of cancer cell proliferation, is needed due to conflicting opinions.[65 68] The reason for these different effects may be due to the use of different model tumors, different sources of MSC (for instance, bone marrow-derived MSCs vs tissue-derived MSCs), the dose or timing of MSC injection, the performance heterogeneity of MSC preparations, and the putative involvement of various mechanisms such as chemokine signaling, vascular support, and immune modulation.[68]

CONCLUSION

Exosomes are important particles with remarkable roles, and their role as major players in intercellular and intracellular interactions is becoming increasingly clear. Biomarkers in exosomes isolated from body fluids have allowed for a more precise and consistent diagnostic method than previous approaches. Exosomes can be used in a variety of intracellular functions, and with advances in molecular techniques they can be used in the treatment and diagnosis of many diseases, including cancer.

Also, given the role of miRNA and other molecules within the exosome, experiments in this direction and the use of exosomes are still much discussed and should be studied and tested in vivo and in vitro for targeting tumors and surrounding cells. Exosomes can also be used for selective delivery of new nucleic acid drugs or conventional tumor therapy drugs. Further review of the tolerability and effectiveness of cancer exosomes in diagnosis and treatment should be done.

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REFERENCES

1 Sáenz-Cuesta M, Osorio-Querejeta I, Otaegui D. Extracellular vesicles in multiple sclerosis: what are they telling us? *Front Cell Neurosci* 2014;8:100.
2 Valadi H, Ekström K, Bossios A, *et al*. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol* 2007;9:654.
3 Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci* 2018;75:193–208.
4 Trams EG, Lauter CJ, Salem N, *et al*. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. *Biochim Biophys Acta* 1981;645:63–70.
5 Ruiz-López L, Blancas I, Garrido JM, *et al*. The role of exosomes on colorectal cancer: a review. *J Gastroenterol Hepatol* 2018;33:792–9.
6 Simpson RJ, Lim JW, Moritz RL, *et al*. Exosomes: proteomic insights and diagnostic potential. *Expert Rev Proteomics* 2009;6:267–83.
7 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.
8 Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* 2010;73:1907–20.
9 Xie Y, Chen Y, Zhang L, *et al*. The roles of bone-derived exosomes and exosomal microRNAs in regulating bone remodelling. *J Cell Mol Med* 2017;21:1033–41.

10 Suchorska WM, Lach MS. The role of exosomes in tumor progression and metastasis (review). *Oncol Rep* 2016;35:1237–44.
11 Deregibus MC, Cantaluppi V, Calogero R, *et al*. Endothelial progenitor cell derived microvesicles activate an angiogenic program in endothelial cells by a horizontal transfer of mRNA. *Blood* 2007;110:2440–8.
12 Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *Bonekey Rep* 2014;3:481.
13 Boyiadzis M, Whiteside TL. The emerging roles of tumor-derived exosomes in hematological malignancies. *Leukemia* 2017;31:1259.
14 De Toro J, Herschlik L, Waldner C, *et al*. Emerging roles of exosomes in normal and pathological conditions: new insights for diagnosis and therapeutic applications. *Front Immunol* 2015;6:203.
15 Alipoor SD, Mortaz E, Varahram M, *et al*. The potential biomarkers and immunological effects of tumor-derived exosomes in lung cancer. *Front Immunol* 2018;9:819.
16 Yang C, Robbins PD. The roles of tumor-derived exosomes in cancer pathogenesis. *Clin Dev Immunol* 2011;2011:842849.
17 Whiteside TL. Tumor-Derived exosomes and their role in cancer progression. *Adv Clin Chem* 2016;74:103–41.
18 Quesenberry PJ, Aliotta JM. Cellular phenotype switching and microvesicles. *Adv Drug Deliv Rev* 2010;62:1141–8.
19 Zhou J, Wang S, Sun K, *et al*. The emerging roles of exosomes in leukemogeneis. *Oncotarget* 2016;7:50698.
20 Barile L, Vassalli G. Exosomes: therapy delivery tools and biomarkers of diseases. *Pharmacol Ther* 2017;174:63–78.
21 Keller S, Sanderson MP, Stoeck A, *et al*. Exosomes: from biogenesis and secretion to biological function. *Immunol Lett* 2006;107:102–8.
22 Poteryaev D, Datta S, Ackema K, *et al*. Identification of the switch in early-to-late endosome transition. *Cell* 2010;141:497–508.
23 Yáñez-Mó M, Siljander PR-M, Andreu Z, *et al*. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles* 2015;4:27066.
24 Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol* 2014;29:116–25.
25 Elkin SR, Lakoduk AM, Schmid SL. Endocytic pathways and endosomal trafficking: a primer. *Wien Med Wochenschr* 2016;166:196–204.
26 Christ L, Raiborg C, Wenzel EM, *et al*. Cellular functions and molecular mechanisms of the ESCRT membrane-scission machinery. *Trends Biochem Sci* 2017;42:42–56.
27 Bellingham SA, Guo BB, Coleman BM, *et al*. Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Front Physiol* 2012;3:124.
28 Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. *J Extracell Vesicles* 2014;3:24641.
29 Feng D, Zhao W-L, Ye Y-Y, *et al*. Cellular internalization of exosomes occurs through phagocytosis. *Traffic* 2010;11:675–87.
30 Fitzner D, Schnaars M, van Rossum D, *et al*. Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J Cell Sci* 2011;124:447–58.
31 Tian T, Zhu Y-L, Zhou Y-Y, *et al*. Exosome uptake through clathrin-mediated endocytosis and macropinocytosis and mediating miR-21 delivery. *J Biol Chem* 2014;289:22258–67.
32 Cesi G, Walbrecq G, Margue C, *et al*. Transferring intercellular signals and traits between cancer cells: extracellular vesicles as "homing pigeons". *Cell Commun Signal* 2016;14:13.
33 Zhang C, Ji Q, Yang Y, *et al*. Exosome: function and role in cancer metastasis and drug resistance. *Technol Cancer Res Treat* 2018;17:1533033818763450.
34 Koch R, Aung T, Vogel D, *et al*. Nuclear trapping through inhibition of exosomal export by indomethacin increases cytostatic efficacy of doxorubicin and pixantrone. *Clin Cancer Res* 2016;22:395–404.
35 Fei F, Joo EJ, Tarighat SS, *et al*. B-cell precursor acute lymphoblastic leukemia and stromal cells communicate through galectin-3. *Oncotarget* 2015;6:11378.
36 Paggetti J, Haderk F, Seiffert M, *et al*. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood* 2015;126:1106–17.
37 Min Q-H, Wang X-Z, Zhang J, *et al*. Exosomes derived from imatinib-resistant chronic myeloid leukemia cells mediate a horizontal transfer of drug-resistant trait by delivering miR-365. *Exp Cell Res* 2018;362:386–93.
38 Wang J, Hendrix A, Hernot S, *et al*. Bone marrow stromal cell-derived exosomes as communicators in drug resistance in multiple myeloma cells. *Blood* 2014;124:555–66.
39 Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol* 2013;200:373–83.
40 Szajnik M, Czystowska M, Szczepanski MJ, *et al*. Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg). *PLoS One* 2010;5:e11469.
41 Shibuya M. Vascular endothelial growth factor receptor-1 (VEGFR-1/FLT-1): a dual regulator for angiogenesis. *Angiogenesis* 2006;9:225–30.
42 Moens S, Goveia J, Stapor PC, *et al*. The multifaceted activity of VEGF in angiogenesis–implications for therapy responses. *Cytokine Growth Factor Rev* 2014;25:473–82.
43 Chand R, Chandra H, Chandra S, *et al*. Role of microvessel density and vascular endothelial growth factor in angiogenesis of hematological malignancies. *Bone Marrow Res* 2016;2016:5043483.
44 Ribatti D. Is angiogenesis essential for the progression of hematological malignancies or is it an epiphenomenon? *Leukemia* 2009;23:433–4.
45 Rajabi M, Mousa S. The role of angiogenesis in cancer treatment. *Biomedicines* 2017;5:34.
46 Taverna S, Flugy A, Saieva L, *et al*. Role of exosomes released by chronic myelogenous leukemia cells in angiogenesis. *Int J Cancer* 2012;130:2033–43.
47 Umezu T, Tadokoro H, Azuma K, *et al*. Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. *Blood* 2014;124:3748–57.
48 Umezu T, Ohyashiki K, Kuroda M, *et al*. Leukemia cell to endothelial cell communication via exosomal miRNAs. *Oncogene* 2013;32:2747–55.
49 Tadokoro H, Umezu T, Ohyashiki K, *et al*. Exosomes derived from hypoxic leukemia cells enhance tube formation in endothelial cells. *J Biol Chem* 2013;288:34343–51.
50 Bluff JE, Brown NJ, Reed MWR, *et al*. Tissue factor, angiogenesis and tumour progression. *Breast Cancer Res* 2008;10:204.
51 Ku M, Wall M, MacKinnon RN, *et al*. Src family kinases and their role in hematological malignancies. *Leuk Lymphoma* 2015;56:577–86.
52 Srivastava A, Filant J, Moxley KM, *et al*. Exosomes: a role for naturally occurring nanovesicles in cancer growth, diagnosis and treatment. *Curr Gene Ther* 2015;15:182–92.
53 Tomasetti M, Lee W, Santarelli L, *et al*. Exosome-derived microRNAs in cancer metabolism: possible implications in cancer diagnostics and therapy. *Exp Mol Med* 2017;49:e285-e.
54 Srivastava A, Babu A, Filant J, *et al*. Exploitation of exosomes as nanocarriers for gene-, chemo-, and immune-therapy of cancer. *J Biomed Nanotechnol* 2016;12:1159–73.
55 Dilsiz N. Role of exosomes and exosomal microRNAs in cancer. *Future Sci OA* 2020;6:FSO465.
56 Fonsato V, Collino F, Herrera MB, *et al*. Human liver stem cell-derived microvesicles inhibit hepatoma growth in SCID mice by delivering antitumor microRNAs. *Stem Cells* 2012;30:1985–98.
57 Wang M, Yu F, Ding H, *et al*. Emerging function and clinical values of exosomal microRNAs in cancer. *Mol Ther Nucleic Acids* 2019;16:791.
58 Liu Q, Peng F, Chen J. The role of exosomal microRNAs in the tumor microenvironment of breast cancer. *Int J Mol Sci* 2019;20:3884.
59 Jeong K, Yu YJ, You JY, *et al*. Exosome-Mediated microRNA-497 delivery for anti-cancer therapy in a microfluidic 3D lung cancer model. *Lab Chip* 2020;20:548–57.
60 Gilligan K, Dwyer R. Engineering exosomes for cancer therapy. *Int J Mol Sci* 2017;18:1122.
61 Jiang X-C, Gao J-Q. Exosomes as novel bio-carriers for gene and drug delivery. *Int J Pharm* 2017;521:167–75.
62 Ingenito F, Roscigno G, Affinito A, *et al*. The role of Exo-miRNAs in cancer: a focus on therapeutic and diagnostic applications. *Int J Mol Sci* 2019;20:4687.
63 Rani S, Ryan AE, Griffin MD, *et al*. Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol Ther* 2015;23:812–23.
64 Pashoutan Sarvar D, Shamsasenjan K, Akbarzadehlaleh P. Mesenchymal stem cell-derived exosomes: new opportunity in cell-free therapy. *Adv Pharm Bull* 2016;6:293.
65 Lee J-K, Park S-R, Jung B-K, *et al*. Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. *PLoS One* 2013;8:e84256.
66 Reza AMMT, Choi Y-J, Yasuda H, *et al*. Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells. *Sci Rep* 2016;6:38498.
67 Cheng L, Zhang K, Wu S, *et al*. Focus on mesenchymal stem cell-derived exosomes: opportunities and challenges in cell-free therapy. *Stem Cells Int* 2017;2017:6305295.
68 Pakravan K, Babashah S, Sadeghizadeh M, *et al*. MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1α/VEGF signaling axis in breast cancer cells. *Cellular Oncology* 2017;40:457–70.

69 Munoz JL, Bliss SA, Greco SJ, *et al*. Delivery of functional anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. *Mol Ther Nucleic Acids* 2013;2:e126.

70 Salido-Guadarrama I, Romero-Cordoba S, Peralta-Zaragoza O, *et al*. Micrornas transported by exosomes in body fluids as mediators of intercellular communication in cancer. *Onco Targets Ther* 2014;7:1327.