

FUNCTIONS AND APPLICATIONS OF EXOSOMES

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Abstract: The cells continuously secrete a large number of different types of microvesicles including macro- and micro-molecules into the extracellular fluids. One of them are exosomes, which are nano-sized vesicles capable of transferring the DNAs, microRNAs, non-coding RNAs and lipids with or without direct cell to cell contact, so representing the novel way of intracellular communication. In this review, we tried to summarize the exosomal structure, composition, formation, and isolation. We also discussed their active role in pathogenesis. The potential use of exosomes for diagnostic and therapeutic purposes in various diseases is also discussed. Exosomes are perceived to be used as carriers between different locations of body. Exosomes are secreted by all types of cells and are also found abundantly in the body fluids such as: saliva, blood, urine and breast milk. The major role of exosomes is to carry the information by delivering various effectors or signaling molecules between specific cells. This review summarizes current knowledge about the exosomes, their functions, biological and therapeutic use as well as emerging exosomes based therapies that could not be applied before.

Keywords: exosome, microvesicles, microRNA, dendritic cells, drug

Exosomes are the micro-sized vesicles with diameter range of 40–100 nm and have received a lot of attention of scientists over past few decades (1, 2). The word “exosomes” was first used by Rose Johnstone in 1970, who found exosomes from the sheep reticulocytes (3). Laulagnier et al. exfoliated these vesicles from cell lines with ectoenzyme activity (4). Till 1990, these exosomes were considered as best as by-products of cell homeostasis. The revolution in exosomes was observed when it was found that β -cells release functional antigen-transforming exosomes (5).

FORMATION OF EXOSOMES

Exosomes are formed by the endocytic cellular pathway consisting of three different stages: (i) plasma membrane invagination form the endocytic vesicles; (ii) in second stage, inward budding of endosomal membrane starts, which gives rise the multi-vesicular bodies (MVB's); (iii) in third and last stage, MVB's fuse with the plasma membrane and releases the vesicular contents (exosome) (6). The membrane proteins, which undergo the endosomal pathway, exhibit the same stages. Different types of lipidic molecules are known for their involvement in

exosome formation and release like phosphatidic acid and ceramides (7).

Size of the exosomes are dependent on their site of origin as well as lipid bilayer structure in cell (8).

COMPOSITION OF EXOSOMES

Exosomes are unique due to its protein and lipid contents, which provide additional hint for their identification. Exosomes mostly contain fusion proteins and transport proteins (annexins and flotilin), heat shock proteins (HSP) (HSP70), CD's proteins (CD9, CD81), as well as phospholipases and other lipid related proteins (9). All these proteins can be used as positive markers. More than 4400 different proteins can be identified in association with exosomes by mass spectrophotometer and these proteins serve as cargo for intracellular communication (10). Along with proteins, exosomes are also enriched with lipids like cholesterol, sphingolipids, phosphoglycerides, ceramides and short and long saturated fatty acid chains (11). Research indicates that exosomes serve to deliver the prostaglandins to the target cells (9). It has also been investigated that exosomes (Fig. 2) have saccharide

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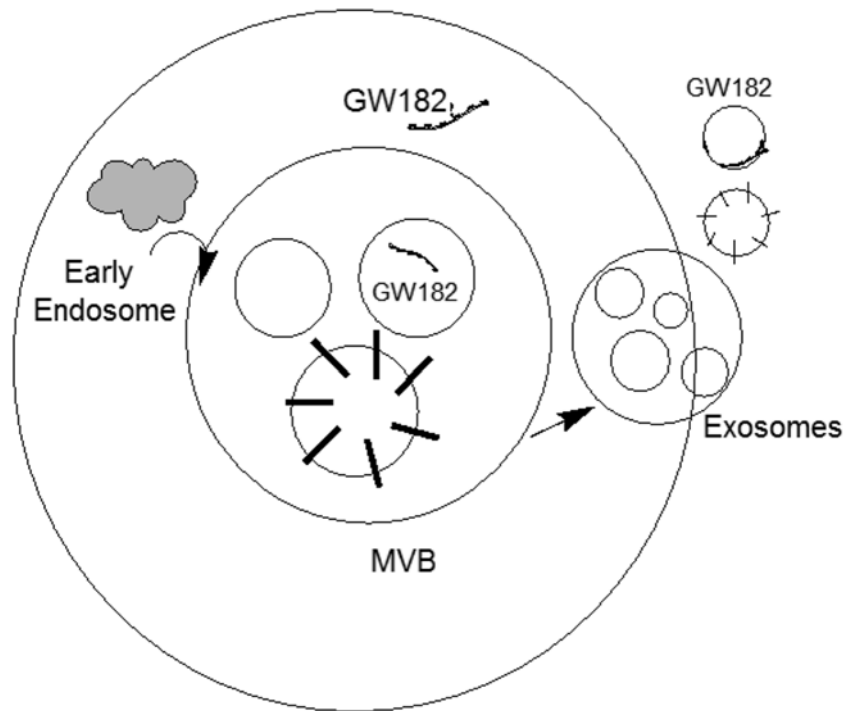


Figure 1. Schematic diagram of exosome production (6)

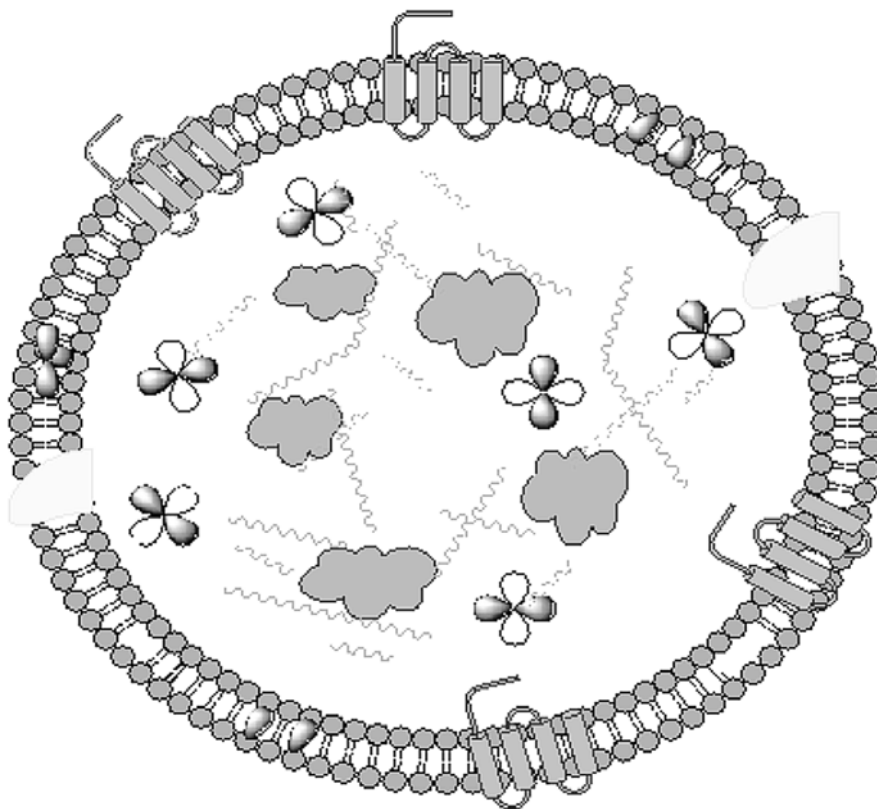


Figure 2. Representation of mid-size exosome (blobs = proteins, ribbon = RNAs) (12)

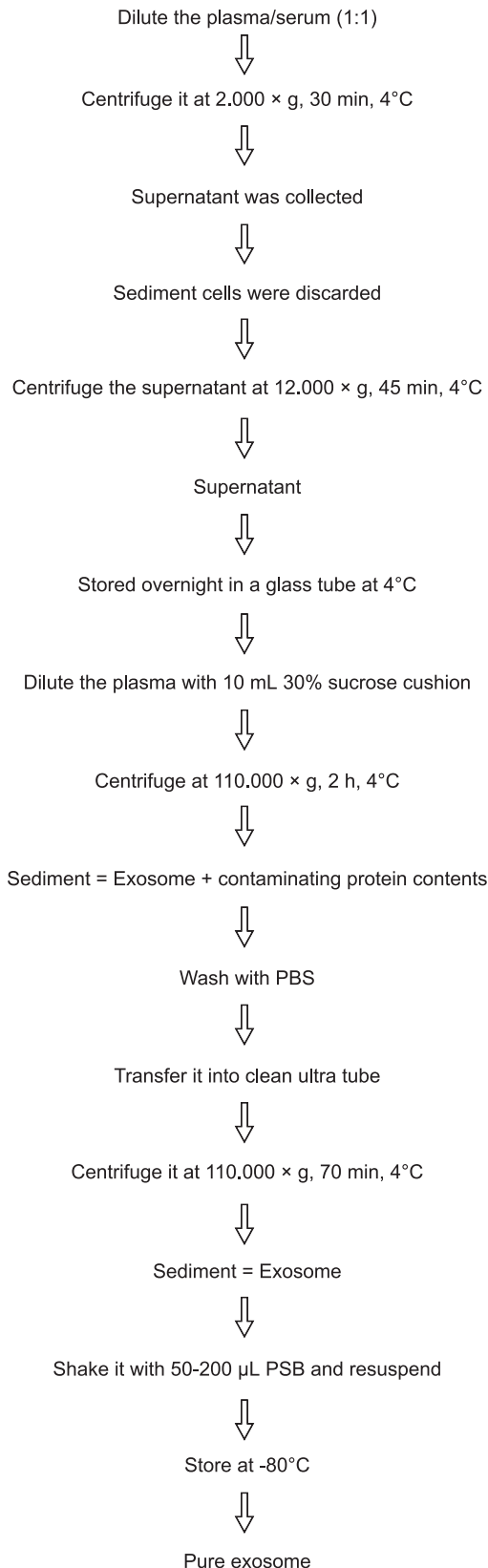


Figure 3. Isolation of exosome by ultra-centrifugation method from plasma/serum (15)

groups in their structure and are enriched with man-nose and glycan (12). It has also been reported that exosomes possess miRNA in a significant amount (13). The cargo function of exosomal RNA is entirely different than that of normal cell RNA (14).

EXOSOMES ISOLATION

Exosomes are isolated by ultracentrifugation method. The exosomes isolation is based on the size of exosomes (15). Exosomes extraction from blood or cell-culture media is complicated because a large number of micro-sized particles are present in media having the same size range as that of exosomes (16). Schematic representation for the isolation of exosome is given in Figure 3.

BIOLOGICAL FUNCTIONS OF EXOSOMES

Multiple cell lines that release the exosomes *in vitro* like neuronal cells, fibroblast cells, adipocytes, intestinal epithelial cells and tumor cell lines have been described. *In vivo*, exosomes are found to be present in many biological fluids like: synovial fluid, breast milk, blood, urine and saliva, amniotic liquid and malignant effusions of ascites. In blood serum, exosomes are almost present in a quantity of 3,000,000 per microliter (17). The first reported biological function of exosomes is as proteins, which are expelled out from reticulocytes during the process of maturation in erythrocytes. Authors believed that particles, which sediment from plasma at 10,000 × g, are circular in nature and name them as exosomes (18). Further research showed that exosome secretion is just like an excretion process to get rid of unnecessary proteins and RNA (4). With the passage of time, more research was being conducted on exosomes discovering that exosomes are found to be secreted by many cell types. On the basis of their origin, exosomes perform a variety of functions. Extensive studies have been done on the facilitator effect of exosomes in immune response (1) and its antigen presenting role has also been extensively reported (19). Exosomal role in coagulation, inflammation and angiogenesis were also reported (20). After the activation, platelets secrete exosomes as well as other shedding microvesicles (21). In this case, exosomes did not perform any role in coagulation. It has been reported that exosomes are involved in dictyostelium cells migration by using chemo-attractant signals (22). Another group of researchers studied the level of miRNA in exosomes of human breast milk for several months during lactation (2). They reported that certain miRNA's like miR-155 and miR-181a, that play

an important role during immune regulation and were present in high concentration during first six months of lactation, were significantly reduced afterward (7). Recent studies demonstrate that the exosomes are not only involved in triggering downstream signaling but they also specifically target the recipient cells and exchange proteins. Exosomes also deliver the specific nucleic acids and work as cargo (23). The unique function of exosomes is cell to cell communication, especially between the far distance cells in the body. Similarly, exosomes play a unique role in spreading various pathogens like virus and prion from one cell to another (24).

EXOSOMES IN DIAGNOSTICS

For last few years, much research has been done on diagnostic aspect of exosomes and it was discovered that almost all the body fluids (blood, saliva, milk, and urine) contained exosomes. Because of unique structure of the exosomes, which possess proteins, lipids and RNAs, it may be useful for the diagnostic purposes (25). In late 1970s, microvesicles (MVs) were derived from the cancer cells in person suffering from Hodgkin's disease (14). Since that day to-date, considerable efforts have been done to use the MVs as diagnostic tool (Table 1). It was reported that MVs levels were elevated in serum, urine and blood in the cancer patient (26). However, microvesicular components may provide important information regarding a disease. For example, mucin bearing MVs are used as diagnostic marker for the diagnosis of adenocarcinoma (27). A proteomic investigation of urine identified eight proteins, which act as an important diagnostic tool in bladder cancer

(28). Thus it can be said that protein portion of the exosomes are the useful tool for the diagnosis of the diseases. In addition, recent studies have showed that cancer patients exhibit different patterns of RNA and miRNA. In cancer patients, RNA and miRNA have been found in circulating MVs form (9).

The PCR of miRNA is a sensitive and stable method for the diagnosis and detection of miRNA in patients' serum, which is a new promising approach to detect disease in early stages. Down-regulation of miR-92a in plasma is the biomarker of hepatocellular carcinoma and leukemia (12).

EXOSOMES AS TARGETED DRUG DELIVERY VEHICLES

Exosomes can be used as targeted drug delivery systems. Alvarez et al. first of all presented and proved this hypothesis (36) by using immature dendritic cells (DCs). They used DCs derived from the bone marrow of mouse as a source of exosomes and these exosomes were devoted as stimulatory molecules such as MHCII and CD80. They purified the exosomes by ultracentrifugation method and used as cargo for siRNA delivery both in *in vitro* and *in vivo* studies. They selected brain as a target tissue in body, because it is believed that blood brain barrier (BBB) is the major obstacle in drug delivery to central nervous system. Sealed functions of BBB are due to the capillary endothelial cells that are tightly sealed by junctions and regulate the barrier functions (19). For ensuring targeted delivery of exosomes, Ratajczak et al. (27) used the novel strategy by utilizing LAMP2B, an exosomal surface protein, that display the targeted peptide on its surface.

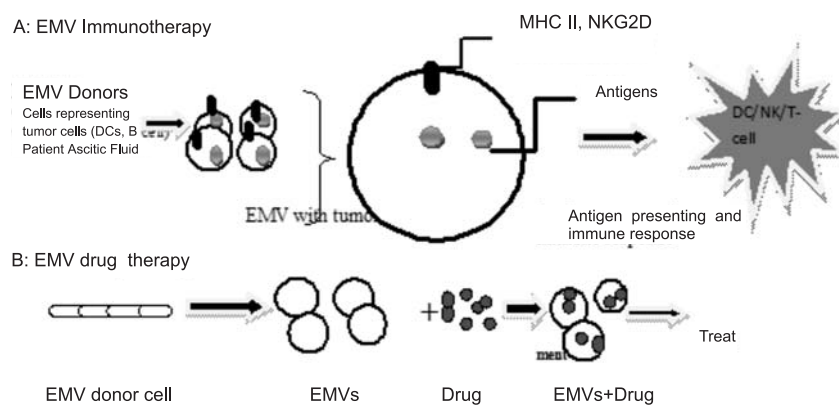


Figure 4. Extracellular membrane vesicle therapy (EMVs) **A:** EMV immunotherapy. Tumor antigen on the membrane surface from different sources was introduced *in vivo* to elicit targeted immune responses. **B:** EMV drug therapy. Drug packaged into/onto EMVs isolated from donor cells to minimize degradation and increase delivery to intended sites (18)

Table 1. Circulating exosomes as potential diagnostic markers for various diseases.

	Sample type	Marker	Disease	Ref.
Quantity	Plasma	PMPs level	Gastric cancer	(29)
	Serum	PMPs level	Prostate cancer	(30)
Protein expression	Ascites	CD24, EpCAM	Ovarian cancer	(31)
	Serum	Tissue factor	General cancer	(32)
	Plasma	Tissue factor	Breast cancer	(33)
	Pleural effusion	SNX25, BTG1	Mesothelioma	(34)
	Urine	Fetuin-A	Acute kidney injury	(9)
miRNA or mRNA expression	Serum	Glioblastoma	Glioblastoma	(10)
	Serum	MAGE-1, HER-2	Gastric cancer	(35)

TREATMENT OF BRAIN INFLAMMATORY DISEASE BY EXOSOMES ENCAPSULATED WITH DRUGS

Zhuang et al. used the encapsulated curcumin (Exo-cur) or JS1124 (Exo-JS1124) inhibitor of signal transducer and activator of transcription and delivered it into the microglia cells through intranasal route. They used lipopolysaccharide (LPS)-induced inflammatory model for the experimental mice to induce inflammation. They showed that mice treated with Exo-cur and Exo-JS1124 were protected from LPS-induced inflammation. They believed that exosomes were selectively taken by the microglia cells and subsequently induced the apoptosis of the microglia cells after its intranasal delivery (30).

EXOSOMES AS AN APPROACH FOR TREATING ARTHRITIS

Dendritic cells (DCs) and T-cells have been used for delivery of immunosuppressive cytokines for the treatment of various collagen induced inflammations in different mouse model (31). DCs are the antigen presenting cells that regulate the immune activity. Various factors are involved in stimulating or suppressing immune responses of DC. DCs have low level of MHC and other molecules such as ICAM-1, so they can suppress T-cell immune response. The immunosuppressive ability of DCs enhanced its genetic modification and genetically modified DCs showed dramatic control in the progression of autoimmune diseases like diabetes and arthritis (24). DCs with viral vectors expressing the immunosuppressive agents exert their effect more pronouncedly than T-cells or fibroblasts (8).

Due to the ability of genetic modification of DCs, they produce distal therapeutic effects specially when exosomes were delivered along with viral vectors (6). Immunosuppressive DCs-exosomes can modify the endogenous immune cells, such as APCs, so they may be responsible for anti-inflammatory effects (1).

EXOSOMES IN IMMUNOTHERAPY AND NERVOUS SYSTEM

Exosomes/MVs (EMVs) have cell to cell communication function for transfer of genetic material (4). The dramatic progress in the research of MVs for drug delivery is due to its low immunogenicity and unique delivering properties. With the help of genetic engineering, EMVs are used to transport the therapeutic drugs either by direct insertion or by loading onto the targeted gene (38). Exosomes also serve as an excellent therapeutic cargo due to its protection rendered to enclosed contents. Due to these possible advantages, EMVs mediated therapy is actively studied and is used in three different fields i.e., immunotherapy, RNA-interference (Fig. 4) and drug delivery (18). The most wide investigational portfolio of EMVs is in immunotherapy, which is an efficient way of cancer treatment through the preparation of vaccines containing antigen presenting cells to recognizes the tumor cells (20). It has been shown that B-lymphocytes secrete EMVs and these EMVs contain MHC-II, which can induces the cell response *in vitro* (19). These EMVs may be used as an emerging therapy in the treatment of various nervous system diseases. Sun et al. developed drug-loaded exosomes (35). They successfully loaded curcumin into EMVs, which significantly reduced the LPS-induced inflammation.

Table 2. Studies on the immunogenicity of tumor-derived exosomes and their vaccines.

Exosome source	Modification	Model	Results	Ref.
Mouse colon carcinoma and melanoma	Parent cells were heat treated	Mouse	Elevated level of Hsp-70, elicit Th 1 response	(1)
Mouse lung carcinoma	Parent cells were heat treated	Mouse	Activates DCs and T-cells and produce immune response	(32)
Human renal cancer	Parent cells were modified to release GPI-IL-12	<i>In vitro</i>	IL-12 promotes the release of IFN- α	(19)
Ascites from colorectal cancer	Exosomes were purified	Phase 1 clinical trials	GM-CSF induce beneficial tumor specific CTL response	(3)

ROLE OF EXOSOMES IN SENESCENCE AND AGING

Senescence is the cellular part of aging of tissues due to the irreversible growth arrest and other physiological changes occurring in cell morphology, cell behavior and function. The miRNAs are small non-coded RNAs, which regulate the gene expression and play an important role in biological processes. Recent developments have shown that exosomes contain miRNAs, which are released into variety of cells and play an important role in cell-to-cell communication and information transfer. Exosomes with miRNAs formed the complex cellular network senescence and contribute to aging (39).

ANTI-TUMORIGENIC ROLE OF TUMOR-DERIVED EXOSOMES

The protein portion of the exosomes reflects the cell type specificity for their cell of origin from which they are secreted. Particularly exosomes, which are derived from tumor may contain tumor-specific antigens on their surface as present in tumor cells (21). Tumor antigens such as carcinoembryonic antigen (CEA) (12) and mesothelin (13) are observed in tumor-derived exosomes. Due to this observation, it is suggested that tumor exosome-based cancer vaccines may be developed. Tumor-derived exosomes might be used as a tumor antigen source, which might be able to induce the CD8⁺ T-cell dependent anti-tumor effects in mice (39). Recently, it was reported that dendritic cells loaded with tumor exosomes elicited the CD8⁺ T-cell response against the tumor cells in malignant gliomas patient (40). For augmenting anti-tumor activity/immunity, tumor derived exosomes have been investigated for direct application (Table 2). Research showed that tumor-derived exosomes produced specific antitumor activity when its parent

cells were genetically modified. These genetically modified exosomes can express pro-inflammatory cytokines such as IL-2 (6). Heat shocked lymphoma cells, which releases the exosomes expressing MHC and other co-stimulatory molecules, induce efficient anti-tumor T cell immunity (40).

CONCLUSION

Increasing research efforts are being done on the exosomes from which we are gaining knowledge on the mechanism of their formation, secretion, *in vivo* pathways, and biological role of their nucleic acid, protein and lipid. With the emergence of exosomes/EMVs responsible for cell to cell communication, researchers gathered the information on their role, both on physiological and pathological functions as well as their use in different therapies. The most interesting aspect of exosomes is their use as vesicular carriers. They carry the large sized molecules such as RNA and proteins that influence gene expression. These microvesicles are similar to viruses and are capable of communication from one cell to another and easily pass the contents of cells across the cell membrane and deliver the macromolecules that are biologically active. Much research had been done on therapeutic applications of the exosomes. Further developments are aimed to ensure therapeutic functions and clinical potential of exosomes including their cargo property, targeting function and different sources of exosomes that enable tissue targeted applications of exosomes.

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Received: 13. 10. 2013