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Enigmatic Exosomes: Role in health and disease with significance in cancer

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Abstract

Exosomes are vesicles released by a number of cells during normal as well as disease conditions that play an important role in inter-cell communication. They are composed of cellular lipids, proteins along with mRNA, and micro RNA. Recently their role as potential biomarkers has gained a lot of attention. Exosomes can reveal information about the cell of origin and the condition of the cell as well. Their role as biomarkers in diseases like Alzheimer's, brain tumors, chronic kidney disease, salivary gland diseases, breast cancer has been already established. Role of tumor derived exosomes in cancer progression, metastasis and drug resistance is widely discussed at present times. In contrast, anti-tumorigenic characteristics have been observed by exosomes released from healthy immune cells. Anti-tumor therapies based on exosomes, for example, by blocking the formation of tumor-derived exosomes or having exosomes release therapeutic agents at specific sites is being explored. The use of exosomes from dendritic cells in tumor vaccination and its safety has been demonstrated in phase I studies as yet. Salivary exosomes can be relevant diagnostic, prognostic and predictive biomarkers in oral diseases especially oral cancer. Exosomes released from cells with intra cellular pathogens as in tuberculosis or toxoplasmosis may promote antigen presentation and activate macrophages thus playing a role in immune surveillance. Their role in forensic analysis is also being explored. On the other hand, exosome mediated drug expulsion has led to drug resistance thus hindering the therapy. This review brings a brief insight into the current knowledge of exosomes

Keywords: Biomarker, Body fluid, Cancer, Diagnosis, Drug resistance, Exosomes, Micro vesicles, Stromal remodeling, Therapy, treatment, Tumor microenvironment, Vaccine

Introduction

Exosomes are membrane bound vesicles carrying a large array of macromolecules like proteins, lipids, nucleic acids, viruses or any other pathogens derived from their originating cell [1]. These structures are formed by the fusion of external membrane of vesicular bodies with the plasma membrane and then released into extracellular matrix [2]. Exosomes vary from microvesicles in that the latter are larger sized measuring 200-1000 nm and also vary from apoptotic bodies which are 0.5-3μm [3]. Exosomes are nano sized with a diameter of 30-200 nm [3] or 40-100nm [4] and exhibit a density of 1.13-1.21g/mL in a sucrose gradient [4]. They may be sedimented at 100,000xg [5,6]. Exosomes sometimes show a 'cup shaped' or 'saucer like' morphology when viewed under electron microscopy [7,8]. Exosomes have emerged as mediators of cellular intercommunication both in health and disease conditions.

Exosomes are released by all types of cells hemopoietic and non-hemopoietic, normal as well as tumor cells [9]. The phenotype of the exosomes depends on the cell of origin [2,10]. They are released during normal physiologic as well as pathologic conditions. They are released by reticulocytes, dendritic cells, B and T lymphocytes, platelets, mast cells and macrophages. Epithelial cells, fibroblasts, astrocytes and neurons also secrete exosomes. Release or secretion of exosomes in these cells can be modulated by ligand cognition or stress condition [11,12]. Release of exosomes can also be triggered by other stimuli like ceramide, changes in membrane pH, hypoxia, and microbial attack [13].

Biosynthesis, composition and regulation of these vesicles have gained a lot of attention in recent times. Trams et al. was the first to coin the term exosomes to these membrane bound structures [14]. Live cells such as maturing reticulocytes were shown to release exosomes for the first time in early 1980s [15,16] and the authors proposed that this was probably a mechanism through which the cells discard their inert material [8,14].

Exosomes may be found in most of the body fluids like plasma, breast milk, saliva, tears and urine [17]. Tumor derived exosomes are present in supernatant of tumor cells, malignant effusions of tumor patients, broncho-alveolar lavage fluid, and cerebrospinal fluid as well [2]. These structures can travel via the body fluids and are capable of causing metastasis at a distant site [11]. The exchange of exosomal content between the parent cell and target cell may be observed locally or at distant site through cell to cell interactions [13].

All exosomes have a common set of molecules like, actin and actin-binding proteins, the heat shock proteins Hsp70 and Hsp90, and trimeric G proteins as well as tetraspanin family (CD9, CD63, CD81, CD82) [4] which help in biosynthesis, structure, function and transport. In addition, each exosome also contains specific cell components which are unique to its parent cell and its function [4]. For example, exosomes from dendritic cell show an increased expression of MHC class I and class-II peptides which play a vital role in activating T-cell response thus suppressing tumor growth [18].

"Exosomes mainly contain cellular lipids, like cholesterol, diglycerides, sphingolipids (including sphingomyelin and ceramide), phospholipids, glycerophospholipids (including phosphatidylcholine,

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phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol and polyglycerophospholipids (i.e., bisphosphate) [12]. Exosomes also seem to contain bioactive lipids, such as prostaglandins and leukotrienes, and active enzymes of lipid metabolism that may generate these lipids" [19,20].

Exosomes in Health

Exosomes are involved in normal physiologic processes like lactation, immune response and neuronal function and diseases like liver disease, neurodegenerative disease, and cancer [21-29]. Exosomes released from healthy normal cells of immune system appear to have antitumor characteristics. Use of exosomes from dendritic cells in tumor vaccination and its safety has been demonstrated in phase 1 studies and is proved in brain cancer therapy [30]. Exosomes have been used in immune therapy of cancer to improve the overall survival [31].

Exosomes and Infectious Diseases

Use of exosomes in the treatment of infections such as toxoplasmosis, diphtheria [32], tuberculosis [33] and atypical severe acute respiratory syndrome [34] has been reported. Exosomes released from cells with intra cellular pathogens as in tuberculosis or toxoplasmosis may promote antigen presentation and activate macrophages thus playing a role in immune surveillance [10].

Exosomes in allergic and autoimmune diseases

Exosomes-mediated vaccination for allergic diseases has also been reported. Development of mucosal tolerance during allergic disease and its protective effect induced by exosomes possibly mediated by plasmacytoid dendritic cells has been reported by few authors [35]. They further stated that the presence of microbes like *S. aureus* in the gut effects tolerogenic processing as a result of activated immune response [35]. Exosome based treatment of autoimmune diseases has been found to be useful in animal models.

Exosomes in salivary gland diseases

Exosomes isolated from saliva may be helpful in diagnosing as well as understanding the pathogenesis of various salivary gland pathologies like Sjogrens syndrome including cancer [36].

Exosomes in cancer

Interaction of tumor cells and surrounding microenvironment is essential for tumor establishment and progression. This may occur by following mechanisms (a) cell to cell and cell to matrix interactions, (b) local secretion of certain factors supporting the survival and tumor growth (interaction between cancer cells and cells in stroma, (c) direct cell to cell communication with tumor cells, i.e., trogocytosis, (d) development of specific niches inside the tumor microenvironment which may help in development of drug resistance, or (e) transformation of the tumor cells to cancer-initiating/stem cells [13]. Tumor derived exosomes usually exhibit protumorigenic role but some anti-tumorigenic properties have also been described. Role of exosomes in mechanisms such as metastasis, angiogenesis, hypoxia, Epithelial Mesenchymal Transition (EMT) signaling, Tumor Growth Factor-β (TGF β) signaling, and Wnt-β-catenin signaling collectively favors the tumor development and progression [13]. Tumor cells interact with the fibroblasts, endothelial cells and immune cells in the surrounding stroma. The amount of exosomes released has been found to be more with tumor cells than normal cells [37].

Anti-Tumorigenic Role of Exosomes

a) Immunogenic properties or tumor exosome based cancer vaccine

i) Indirect application: Exosome pulsed dendritic cells (DC), exosomes contain antigenic proteins specific to parental tumor cell. Thus these are used to pulse dendritic cells resulting in transfer of T-antigens to DC which result in cytotoxic T Cell dependent antitumor effects in mice and human ex vivo models [38,39]. Exosomes secreted by DC are known to induce T-cell mediated anti-tumor immune responses. Antigens in exosomes trigger immune response by activating T lymphocytes, natural killer cells, and dendritic cells. Potential role of exosomes in immuno therapy, vaccination [10], and gene therapy is widely explored in recent times [40].

ii) Direct application: Release of exosomes can be triggered by genetically modifying parent cells to express proinflammatory cytokines/ enriched chemokines such as IL 18,12,2 [41-43] or by subjecting the cells to stress conditions/heat shock proteins [44].

b) Inducing tumor cell apoptosis: Exosomes are shown to exhibit pro apoptotic properties on tumor cells directly. Increased Bax and decreased Bcl2 expression causing apoptosis of tumor cells was noticed in certain tumors [45].

Exosomes are abundantly present in tumor environment in patients with advanced cancer. However effective antitumor effects are rarely seen. Their role on tumor cells and their survival is yet to be ascertained.

Protumorigenic Role of Tumor Exosomes

a) **By inducing immunosuppression:** Tumor exosomes can either directly induce apoptosis of activated cytotoxic T cells or down regulate the T cell/NK cell functions in addition to direct killingthus promoting tumor growth [46]. Exosomes can influence the differentiation of myeloid cells into dendritic cells with subsequent formation of myeloid-derived suppressor cells and may be involved in TGFβ1 mediated suppression on T cells thus favoring the growth of tumor [47]. Transfer of genetic material between exosomes and bone marrow (BM) cells can influence the function of future population of BM cells [48]. Tumor exosomes support function of Regulatory T (Treg) cells by maintaining the number and enhancing resistance to apoptosis of Treg cells via TGF β and IL-10 dependent mechanism [49].

b) **By promoting tolerance to tumor specific antigens:** Tumor cells release exosomes which provide antigens to dendritic cells as well as influence the dendritic cells to differentiate into a suppressive tolerogenic phenotype thus suppressing antigen specific immune response [50].

c) **Facilitation to tumor invasion and metastasis:** Exosomes act as central mediators of the tumor microenvironment by expressing molecules involved in angiogenesis promotion, remodelingof stromal cells, remodeling of extracellular matrix through matrix metalloproteinases (MMPs), signaling pathway activation through growth receptor/ factor transfer, providing hypoxic environment for increased aggressiveness,chemoresistance and intercellular genetic exchange [51,52]. Exosomes help the tumor cells to survive immune surveillance and evasion thus promoting tumor growth and metastasis. They help in creating a suitable environment at a distant site which favors the seeding and growth of tumor cells [52].

Hypoxia promotes the survival and invasion of epithelial tumors [53]. The hypoxic niche within the tumor contains cells that are drug

resistant and may also promote Epithelial Mesenchymal Transition [54]. Hypoxia facilitates the release of many tumor-promoting factors that influence adjacent tissues in the tumor microenvironment with increased angiogenic and metastatic potential [55,56]. Therefore, treatment either directly targeting hypoxia or the factors favoring hypoxia is currently being widely investigated [57,58]. In breast cancer hypoxia induced more aggressive cell phenotype as observed by King et al. [59].

Tetraspanin and D6.1A enriched exosomes upregulate angiogenesis by causing proliferation and migration of endothelial cells as well as differentiation and maturation of the stem cells of endothelial origin [60,61]. Exosomes from mesenchymal stem cells (MSC) can induce release of vascular endothelial growth factor expression by cancer cells by upregulating the ERK1/2 pathway [62]. Exosomes released from platelets upregulate mRNA expression of angiogenic factors such as MMP-9 [63].

Epithelial mesenchymal transition is said to be an important feature of aggressive tumors [6]. Cells which have undergone EMT show increased plasticity and ability to migrate causing widespread metastasis [64]. Exosomes from these cells exert influence on surrounding tissue and may help in developing resistance [65]. Role of EMT and exosomes is largely explored at present times.

TGF β has a pivotal role in inducing EMT, tumor survival and progression [66,67]. Certain cancer cells secrete TGF β enriched exosomes capable of converting fibroblasts into myofibroblasts thus modifying the stroma which inturn is essential for tumor growth, angiogenesis and metastasis. Exosomal TGF β resulted in increased production of FGF2 than soluble TGF β [68]. Exosomes from damaged epithelial cells influence the fibroblasts causing fibrosis and initiating regenerative response in the surrounding stromal tissue by primarily delivering TGF-β1 mRNA to the site of fibrosis [57,69]. Supression of lymphocytic response to interleukin 2 by TGF β enriched exosomes was noticed by Clayton and his colleagues [70].

Wnt signaling plays a vital role in tissue development, and deviation in pathway leads to cancer development [71]. Early embryonic development requires β-catenin protein which acts as an intracellular signal transducer and also regulates the coordination of cell to cell adhesion and gene transcription [72]. Increased nuclear β-catenin, is observed in different tumors [73]. However, the mechanism is elusive.

"It was observed that IL-4 activated macrophage released exosomes which enhanced the invasion of breast cancer cells, due to uptake of miR-223 and disruption of the Mef2c-β-catenin pathway" [74].

Exosomal micro RNAs modulate the release of cyto/chemokines from the epithelial cells thus maintaining and regulating the innate immune response. While doing so, they may parcipate along side Toll-like receptors (TLRs), and their associated downstream signaling pathways, such as nuclear factor kappaB (NF-κB) and MAPK. These mechanisms may promote cancer survival and growth by evading immune surveillance [75].

Exosomes released from ovarian cancer cells composed of matrix metalloproteinases which were proteolytic in nature thus resulting in increased degradation of extracellular matrix and thus promoting tumor invasion into the stroma. Growth factors, some chemokines, and proteases which are essential for seeding and growth of tumor cells were found in CD44 which served as a reservoir [76-78]. Heat shock proteins like Hsp 90 secreted via exosomes can activate MMP-2 thus promoting the invasion of tumor cells [79]. Platelet derived exosomes in lung cancer cell lines have shown to promote tumor progression [63].

Exosomes from tumor cells influence the normal cells at the site of future metastasis by transferring miRNAs and other genes involved with cell adhesion, matrix degradation, angiogenesis, oxidative stress and so on to the target cells [13].

d) **Transport of RNAs and proteins for tumor survival and growth:** Tumor exosomes caused tumor progression mainly by the transferring RNA and proteins from tumor cells to other neighboring cells, which resulted in promoting angiogenesis and suppressing immune surveillance [30]. Exosomes from viral infected cells can transfer viral materials to non-infected cells in the target site thus altering the target genes as in case of Epstein–Barr virus (EBV) infected nasopharyngeal carcinoma. [80]. Valadi et al. found the presence of small RNAs and mRNAs from 1300 genes present in exosomes that were absent in the parental cell and suggested that these RNAs be referred to as exosomal shuttle RNAs (esRNAs) to differentiate them from circulating micro RNAs [81].

e) **Drug interference:** Resistance to chemotherapy, radiation therapy and targeted therapies has caused a major hinderance in effective cancer treatment. Several mechanisms have been proposed for the drug resistance. Exosomes help the tumor cells to expel the tumoricidal drugs or neutralize antibody-based drugs [11]. Drug resistance is multifactorial and leads to sustenance of tumor. It may be due to various mechanisms like the cancer cells opting a secondary salvage pathway when the primary one is shut [82], by preventing miRNA activation of tumor suppressor genes [83], by conversion of tumor cells into a more aggressive phenotype with increased plasticity by epithelial mesenchymal transition [84], fibrosis/desmoplasia in surrounding stroma leading to reduced drug penetration etc [85].

Exosome-released factor can promote (a) EMT cell morphology, resulting in stemness; (b) promote fibroblast-like cell formation that causes desmoplatic reaction (stromal reaction); (c) promote immune escape mechanisms; and (d) promote angiogenesis and metastasis. The miRNAs expelled by exosomes can regulate multiple signaling pathways that cumulatively promote resistant phenotypeof most tumors [13]. Tumor cells expel drugs into surrounding stroma using specialized transporters of the multidrug resistance (MDR)-ATP binding-cassette transporter (ABC transporters) system which is activated in different cancers [86].

Thus the regulatory and immunologic role of exosomes depends on its molecular phenotype and cell specificity and the environmental factors as well. Different tumor types and growth patterns may influence the peripheral circulation of exosomes. Despite being safe, clinical efficiency in humans is still questionable since results obtained are mainly on animal models. Thus a careful evaluation is needed [11].

Techniques for isolation, and identification of exosomes

Exosomes are isolated by multiple centrifugation and ultracentrifugation steps with a rotational force up to 100,000 × *g* for sedimentation. Centrifugation is sometimes combined with 0.1 μm to 0.22 μm filtration to separate these particles and to exclude larger particles and cellular debris [87]. To obtain less contaminated and purer forms, sucrose, iodixanol [88],deuterium oxide density gradients or proprietary reagents, such as ExoQuick, have been used [89,90].

Immunoaffinity capture methods with the use of magnetic beads coated with antibodies against exosome-specific surface marker, such as the tetraspanins, CD63 or CD82, can be utilised to obtain exosomes from tumor cells or patient serum [87].

Electron microscopy with negative staining [7], immunoblot procedures [1], mass spectrometry [91], various RNA isolation techniques like phenol-based techniques (TRIzol®), silica column (e.g.,

RNeasy® (Qiagen) or miRCURY™ (Exiqon) and combined phenol and silica column approaches (e.g., TRIzol® followed by RNeasy (Qiagen), miRNeasy (Qiagen) or mirVana™ (Ambion) have been utilized and compared [92-94].

"The RNA yield can be determined by spectrophotometric analysis at 260 nm, and a profile of the exosomal RNAs can be determined using the Agilent 2100 Bioanalyzer Lab-on-a-Chip instrument system (Agilent Technologies). Typical profiles of RNA extracted from exosomes contain a size distribution of 25–2000 nucleotides and are characteristically absent of ribosomal RNAs"[92]. Real-time reverse-transcription PCR assay, oligonucleotide microarray analysis [51], next-generation RNA sequencing [95-97] may detect even specific RNAs or micro RNAs.

Quantification of exosomal protein is challenging due to their nanosize. Currently enzyme-linked immunosorbent assays (ELISA) or by immunoblotting may be used for isolation. Cell lines expressing GFP tagged CD63 (a specific marker of exosomes),may produce exosomes with a marker that can be easily identified and quantified using fluorescent spectrometry [98]. New technologies which can track or identify these nanosized particles have been developed by Nanosight Ltd. [99].

The composition of exosomal proteins has been studied using immunoblotting [100], peptide mass spectroscopy mapping [5,6] and affinity extraction into magnetic beads, followed by phenotyping by flow cytometry [70].

Numerous studies have observed that the exosomal RNA to be quite different from the RNA in the parental cell, in that they have no ribosomal RNA [81, 95]. In contrast, the exosomal microRNA content was found to be similar to that in the original tumor, thus giving the idea of their possible use as diagnostic marker [12]. However, an abundance of certain micro RNAs that are absent or present at very low levels in the parental cells has recently been noticed [101, 102]. These shows that some microRNAs may be preferentially released. The biosynthesis and secretion of these vesicles is still vastly studied. Their role as reliable markers is still questionable.

On the contrary, Hannafon et al. found that microRNA expression signatures were nearly the same between TD-exosomes and tumor cells, with the exception of miR-1246, suggesting that these circulating TD-exosome micro RNAs could serve as an alternative to biopsy [12]. In addition, a database called miRandola has been designed to catalog all extracellular circulating microRNAs and at present contains 2312 entries with 581 unique mature microRNAs observed in circulation from 21 different types of samples [103].

Conclusion and Future directions

Exosomes play a very important role in health as well as disease. Tumor derived exosomes with their anti or pro-tumorigenic properties either suppress or promote cancer development through modulation of intercellular communication within the tumor microenvironment. Further research related to exosome secretion and isolation may allow the development of advanced diagnostic, preventive and therapeutic methods. Possible creation of synthetic exosomes and utilization of exosome mediated drug delivery targeting specific cancer cells may be future possibilities.

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