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**Review article**

# Exosomes: An oncogenic kit

***Swatantra Patel*** [1], ***Himanta Ghritlahare*** [2]

[1,2] *Postgraduate student, Department of Oral Pathology and Microbiology, Rishiraj College of Dental Sciences and Research Centre, Bhopal*

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ABSTRACT

Exosomes are nanometer-size vesicles manufactured with in late multivesicular endosomes and actively secreted into the extra-cellular environment. The major role of exosomes seems to be the transport of bioactive molecules between cells. Depending on the cells of origin, exosomes are implicated in the regulation of several cellular events.

Cancer derived exosomes (CCEs) are important players in the formation of the tumour microenvironment by enabling the escape of tumour cells into the immunological system and help initiating the inflammatory response. They also act in the differentiation of fibroblasts and mesenchymal cells into myofibroblasts, triggering the angiogenic process, enhancing the metastatic evolution of the tumour by promoting epithelial to mesenchymal transformation of tumour cells, and by preparing the tumour niche in the new anatomical location.

## Introduction

Exosomes are nanometer-sized vesicles, typically 30-100nm in diameter. They are manufactured within the endosomal system, as multivesicular bodies (MVB), which are ultimately secreted into the extracellular space. They exhibit a typical bi-layer lipid membrane, which is high in cholesterol, ceramide, and has phosphatidyl serine (PS) residues evenly distributed between outer and inner membranes; as such they may appear to the immune system as apoptosis-related particles[1].

Exosomes contain signal proteins and/or peptides, microRNAs, mRNAs, and lipids[2]. In exosomes from mast cells, mRNAs for more than 1,300 genes and more than 100 microRNAs were detected[3]. Some of the mRNAs from exosomes have been shown to be functional, and murine exosomes that were "taken up" by human cells resulted in the synthesis of mouse proteins[4]. How a molecule is determined to be excluded or included in exosomes and which molecules are functional after their cellular uptake are questions whose answers are still elusive; however, JAB1/CSN5, a component of the COP9 signalosome regulatory complex, may play a role in sorting ubiquitinated proteins into exosomes[4].

## FUNCTIONS OF EXOSOMES

The major role of exosomes seems to be the transport of bioactive molecules between cells[5], with consequences in targeted cell phenotypes, such as

* Corresponding author: **Dr.** ***Swatantra Patel, Postgraduate student, Department of Oral Pathology and Microbiology, Rishiraj College of Dental Sciences and Research Centre, Pipalner road, Gandhinagar, Bhopal 462 036, Mobile: 7805027886, Email: drswatantrapatel@gmail.com***

mRNA and miRNA related to the transfer of genetic, and sometimes epigenetic, information between cells. Additionally, and as described above, another exosome function includes lipid trafficking[6]. The presence of exosomes in healthy body fluids suggests a role of these vesicles in the normal physiology of the body, including communication in the immune system[7], tissue repair, and communication within the nervous system]. Exosomes have also been associated with infection[8] and several pathological conditions, such as in the progression of neurodegenerative disease, cardiovascular diseases and cancer[9] or, on the other side, in the protection against atherosclerosis[10].

The importance of exosomes in tumorigenesis is emphasized by the general increased content of these vesicles in biological fluids of cancer patients relatively to healthy controls, being observed an increased content of exosomes as the tumour progresses[9]. Interestingly, CCEs cause both anti-tumorigenic and pro-tumorigenic effects. Studies have shown that bladder cancer cell lines shed exosomes containing proteins important for tumour progression, and these exosomes inhibit tumour cell apoptosis through Akt and ERK pathways. On the other side, CCEs can transport tumour antigens to dendritic cells and induce immune responses. These differences between biological functions observed for exosomes most likely arise from differences in the cargo present either on the surface of the vesicle or internally[11].

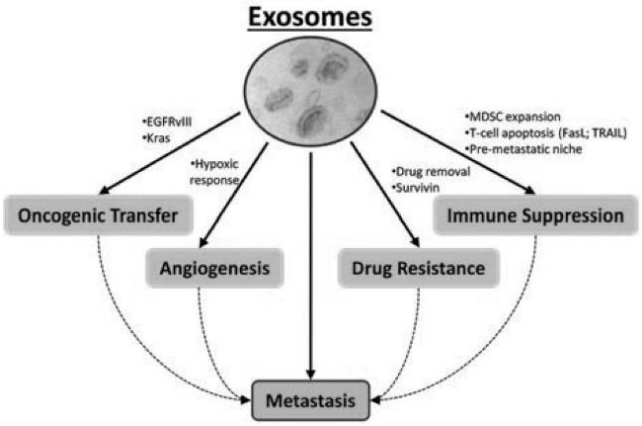
Functions of exosomes are not adequately characterized, including their functions in autocrine and paracrine signaling[2]. Because exosomes provide signals to distant cells, exosomes act as a newly described nanoparticle- based endocrine system. For cells affected by exosomes, cellular membranes and the surface molecules of exosomes interact in uptake of exosomes probably via class I and class II MHC molecules, ICAM-1, integrins, and tetraspanins on exosomal surfaces[12].

## CANCER EXOSOMES AND IMMUNE ACTIVATION

Cancer cells also produce exosomes, evident in culture and surprisingly abundant in malignant effusionsuch as peritoneal ascites of ovarian cancerand pleural fluid of mesothelioma[13]. In fact, aberrant signalling pathways, particularly those related to p53 response elements such as Steap3, may positively regulate exosome secretion, suggesting elevated exosome secretion as a property of malignancy and genotoxic stress[14].

In many respects, cancer-derived exosomes resemble those of apc origin in their biophysical and biochemical properties. As would be expected, cell type–specific differences are also present, the most significant of which are the expression of tumour-associated antigens, particularly those found in association with the cell membrane. Comparisons of whole-tumour-cell lysates with tumour exosomes reveal often striking enrichment within the exosomes of tumour antigens such as *HER2/neu*, melan-A, Silv, carcinoembryonic antigen[15], mesothelin, and others. Immunization of mice with dcs pulsed with cancer cell–derived exosomes demonstrate that it is possible to induce protective antitumour[13]immune responses using cancer-derived exosomes as a source of an antigen or antigens. Similarly, in an *ex vivo* human model system, exosomes taken from malignant effusions proved an effective source of tumour antigens for cross-presentation to CD8+ cytotoxic T cells by dcs. This aspect has since been explored in the context of phase i studies, albeit with one or more added factors for

enhancing or recruiting dc functions. To date, direct activation of T cells




by cancer exosomes has not been shown; rather the T-cell stimulatory function of cancer exosomes requires uptake and processing by professional apcs, which subsequently elicit T-cell activation.

Stress proteins expressed on the surface of cancer cell–derived exosomes may also have influence over other cell types, and are therefore not dc-selective nicely showed that Hsp70 present at the exosome surface (from colorectal cancer cell lines) could directly activate natural killer (nk) cells, supporting migration and cytotoxic functions. In contrast, sub-lines that produced exosomes lacking surface Hsp70 were poorly activating[16]. ExosomalHsp expression is a complex issue; and even when elevated exosomalHsp expression is apparent after stress, the elevation may not always correlate with enhanced immune function—a difference attributable to luminal as compared with surface expression of Hsp[17].

## CANCER EXOSOMES AND IMMUNE SUPPRESSION

We have cited several examples of cancer exosomes exerting a positive influence on the immune system, but these scenarios do not seem to be well reflected in the clinical setting. We know that patients with gross malignant ascites produce copious quantities of exosomes *in vivo*. Yet, regardless of the exosome content of such fluids, the disease more often than not pursues a progressive course[18]. Anecdotally, therefore, the concept of natural immune-activating cancer exosomes may be misleading, at least in an advanced disease setting. An alternative view suggests that the secretion of vesicles that would encourage immune-mediated destruction of the tumour is not in a cancer cell's interest[19].

Mounting evidence is indeed pointing to exosomes as major participants in immune evasion. Although the concept of tolerance induced by exosomes was well described in the context of acquired dietary antigens[19] and, more recently, in reproductive biology, transplantation, and respiratory allergens, several novel mechanisms (both direct and indirect) have recently been described in the context of cancer exosomes[20].

Among the earliest such reports is a description of melanoma-derived exosomes that were lethal to T cells. These cancer cells naturally express Fas ligand, and expel by the multivesicular endosomal route at least a proportion of this molecule in the form of exosomes. FasL–bearing exosomes, upon encountering activated (Fas-positive) T cells, can essentially crosslink T cell Fas and trigger apoptotic death[21]. Other influences of exosomally expressed members of the tumour necrosis factor superfamily may include downmodulation by ovarian cancer exosomes of the CD3-ζ chain. This molecule is an integral component of the T-cell receptor (tcr) complex, which is essential for competent signalling after tcr–mhc–peptide interactions. Melanoma exosomes expressing tumour necrosis factor α may also affect the CD3–tcr complex in a reactive oxygen species–mediated manner. Thus, cancer

exosomes can exert drastic effects to oppose one or more T-cell functions and, in some situations, may constitute an important mechanism by which tumours eliminate activatedT cells that may recognize and kill them[21].

However, apoptotic death of T cells is not a universal consequence of interactions with exosomes. The outcome depends both on T-cell status and on the molecular phenotype of the exosome. In chronic inflammatory disease, for example, exosomes may in fact attenuate T-cell apoptosis, prolonging theirsurvival inappropriately and adding to persistent inflammatory injury[22]. Other death-independent effects of cancer exosomes on the immune system have been reported. Liu *et al*., for example, pretreated mice with breast cancer exosomes before implanting tumours and documented accelerated tumour growth[23]. This accelerated growth was result of the negative influence of cancer exosomes on nk cell functions, inhibiting nk cell proliferation (in response to interleukin-2) and impairing subsequent cytotoxic functions. Similarly, studies by other researchers showed that human nk cells also become significantly functionally impaired following treatment with several cancer exosome types, manifested by down modulation of nkg2d, which is among the most important tumour-recognition molecules for nk cells. This molecule is also of importance for other lymphocyte subsets, such as CD8+ T cells, γδ–T cells, nk–T cells, and others. Cancer exosomes may therefore negatively modulate the functions of multiple branches of the immune system, with effects seemingly particularly focussed toward suppressing cytotoxic function[24].

## CANCER CELL DERIVED EXOSOMES

Some cancer-associated antigens are highly enriched on cancer cell exosomes. Other molecules, such as adhesion molecules, stress-proteins, lactadherin and lipid residues like PS, may facilitate the selective uptake of cancer exosomes by dendritic cells (DCs)[1]. Thus exosomes may act as a vehicle for delivering antigens to DCs, which can cross-present to T cells, and activate cytotoxic(CD8+) T-cell responses against the cancer antigens. However, the immune-activating function of cancer exosomes is controversial, and may not be true at all in situations of advanced/progressive disease. Several mechanisms have been described, showing cancer exosomes can actively interfere with correct immune function. Cancer exosomes can inhibit the differentiation of DCs (from precursor cells), and may trigger the development of TGFb-producing myeloid suppressive cells (MSC). Cancer exosomes may directly express functionally active TGFb, which can inhibit effector T-cell function while activating regulatory Tcells. Other molecules include the MHC-like ligands of NKG2D, which are expressed by cancer exosomes, and act to systemically switch off the NKG2D-dependant mechanism for NK and T cell activation. Some reports also describe Fas-positive exosomes, which can bind to Fas-ligand onactivated T cells, triggering their death[1].

## SUMMARY

Exosomes secreted by cancer cells are dynamic and highly complex, and the field as it stands remains somewhat controversial. It may be that, in early neoplastic lesions, cancer cells and the exosomes they produce have not yet acquired the potent suppressive molecules and mechanisms described here. Under such conditions, exosomes may play an

important role in disseminating relevant tumour rejection antigens to the immune system, assisting the immune response, through the activities of dcs. However, by its very existence, progressive disease has overcome or overwhelmed the immune response, and exosomes in these scenarios harbour multiple mechanisms for attenuating several branches of immunity.

Identifying the factor or factors responsible for this possible switch from immunogenic to immune-suppressive exosomes will be a major challenge, but will in turn offer exciting novel therapeutic opportunities for blocking tumour immune escape while retaining efficient tumour-antigen handling by the immune system.

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