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A prospective highlight on exosomal nanoshuttles and cancer immunotherapy and vaccination

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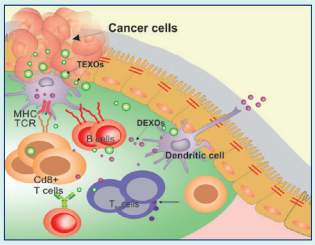
Abstract



Introduction: Exosomes (EXOs) and ectosomes (ECTOs) are nanoscale membranous extracellular vesicles (EVs) derived from different cells mediating various cellular communications. EXOs are liberated based on the exocytosis of multivesicular bodies, while ECTOs are ubiquitously released from the plasma membranes.

Methods: Here, in this paper, we go over the extracellular vesicular machineries and concisely highlight their clinical importance in solid tumors and their possible applications in cancer immunotherapy/vaccination.

Results: In various types of cancers, these vesicles play central roles delivering cancer cell messages to the target cells, as a result both of them seem to provide a novel useful means for diagnosis and therapy of malignancies. Dendritic cell-derived exosomes (DEXOs) are able to activate the tumor antigen-specific $CD8^+$ cytotoxic T-lymphocytes (CTLs) and hence induce antitumor responses in vivo. Within the tumor microenvironment (TME), however, tumor cells seem to generate exosomes (the so-called oncosoems) that may act in favor of tumor progression.

Conclusions: As complex systems, these vesicular micro-/nano-machines convey important cellular messages dependent upon the cells/tissue setting(s). In addition to their potential in diagnosis of cancers, they have been exploited for cancer immunotherapy/vaccination. However, such treatment strategies need to be carefully designed to attain desired clinical outcomes.

Introduction

The epigenetic and genetic reprogramming or modifications due to the genomic instability are thought to be the fundamental features of tumorigenesis. These modifications result in the expression of abnormal or mutated proteins. Therefore, the antigenic characteristics of the tumor cells can be perceived by the innate and cognate immune systems - a phenomenon known as immunosurveillance.[1] However, it should be pointed out that the cancer cells are able to adopt some crucial mechanisms to secure their survival, proliferation, progression and invasion even after chemo/immunotherapy. These strategies may manifest by emerging their own growth signals, resisting to growth-inhibitory signals, challenging apoptotic processes, preserving their replicative potentials, sustaining angiogenesis, and finally, migrating by metastatic invasion and colonizing into the neighboring tissues and organs.[2] In fact, cancer cells are able to escape the immunosurveillance functions of immune system through immunomodulation, immunoselection/immunoediting and immunosubversion.[3] In addition, solid tumors attain unique capability to create a permissive milieu – the so-called tumor microenvironment (TME) – to escape such immunosurveillance. TME is often associated with aberrant metabolisms (e.g., anomalous consumption of glucose and L-tryptophan), emergence of irregular microvasculature and modified interstitium with high pressure fluid that impose significant pathophysiologic barrier functions against cancer treatment modalities.[4,5] Further, within TME, tumor cells impose immunosuppressive functions via regulatory T (T_{reg}) cells and/or myeloid-derived suppressor cells and downregulation of major histocompatibility complex (MHC) expression.[1,6] The malignant cells, unlike normal cells, are able to escape the anoikis[7,8] during metastasis, while their death can hardly ever induce any immune responses against tumor cell derived antigens.

As one of intriguing mechanisms, cancer cells exploit membranous vesicles machineries for communication with the neighboring cells. These cellular communication

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machineries are known as exosomes (EXOs) and ectosomes (ECTOs), which are micro-/nano-scaled vesicles secreted from various cells to convey messages related to immune responses and signal transductions.[9]

Biogenesis of extracellular vesicles

The biogenesis of bioactive EXOs commences with the fusion of multivesicular bodies (MVBs) with the plasma membrane and release of intraluminal vesicles (ILVs) as EXOs.[10] This phenomenon, which was initially observed as a mechanism for the removal of transferrin receptor during maturation of reticulocytes, is now considered as an alternative secretory pathway of the endocytic network; readers are directed to see a previously published book chapter on "Biological membranes and barriers" for the vesicular trafficking.[11] In fact, the MVBs are intermediate cellular compartments originated from endosomes through invagination of the limiting endosomal membrane. The ILVs, which are not yet released to the extracellular space, can drive the formation of EXOs (50-100 nm in diameter) that are released on the exocytosis of MVBs. Unlike EXOs, the ECTOs (50-350 nm in diameter) are ubiquitous vesicles assembled at and released from the plasma membrane.[12] The fusion of liberated EVs with target cells is initiated through interaction of the external faces of cell membranes, which is mediated by fusogens such as syncytin-1. Both EXOs and ECTOs show rolling and membrane fusion potential with rapid dissolution and specific markers such as CD63 and CD61 for EXOs, and TyA and C1q for ECTOs.[12] Fig. 1 represents schematic illustration of various extracellular vesicles in communication with other cells such as B and T lymphocytes and the biogenesis of such vesicular machineries.

EXOs derived from dendritic cells (DCs) are known as dexosomes (DEXOs) that contain ligands capable of activating the natural killer (NK) cells. Immunomodulatory impacts of DEXOs provide possibility towards development of reprogramed DEXOs for the specific activation of immune system including invariant Natural killer T (NKT) cells and antigen-specific T and B lymphocytes.[13] As shown in Fig. 1 (panel A), compelling evidences have shown that tumor antigen-loaded DEXOs are able to activate the tumor antigen-specific CD8+ cytotoxic T-lymphocytes (CTLs) and hence induce antitumor responses in animal models and human clinical trials.[14] However, it appaers that there exist somewaht controversies upon the impacts

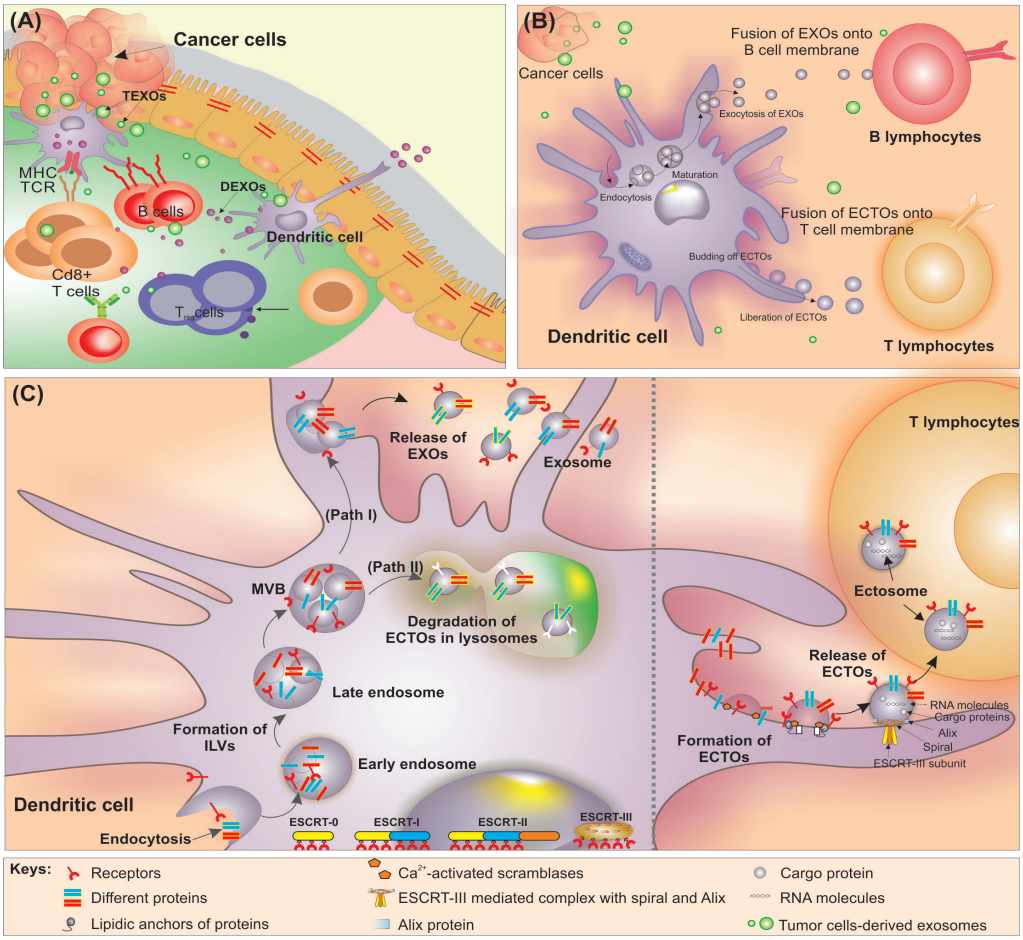

Fig. 1. Schematic illustration of extracellular vesicles in solid tumors. A) Tumor cells-derived exosomes (TEXOs) and dendritic cells-derived exosomes (DEXOs) in tumor microenvironment of small intestine cancer. B) Extracellular vesicles (EVs) communication with B and T lymphocytes. C) Biogenesis of exosomes (EXOs) and ectosomes (ECTOs) in dendritic cells.

of the EVs, perhaps becuase of the tumor cells-derived exosomes (TEXOs) that is favor of cancer progreesion. For the biogenesis of EXOs (Fig. 1C), transmembrane proteins should be endocytosed and transferred into the early endosomes. While the "endosomal sorting complex required for transport" (ESCRT) for the early endosomal sorting (ESCRT-0) involves ubiquitinated proteins, sorting of the late endosome by intraluminal vesicles (ILVs) and forming of the multivesicular bodies (MVBs) are mediated by ESCRT-I and –II. Then, as demonstrated in Fig. 1 (panels B and C), the formed MVBs can undergo either the liberation of ILVs (i.e., through exocytosis of EXOs to the extracellular space) or the degradation of ILVs (i.e., via fusion of MVBs with lysosomes).

For the formation of ECTOs (Fig. 1C), transmembrane proteins (e.g., tetraspanins, matrix metalloproteinase MT1-MMP, integrins, receptor agonists) are assembled in distinct membrane domains creating some kind of molecular raft – key to outward membrane budding. This occurs in association with lipidic anchors (e.g., myristoylation, palmitoylation) of proteins, and the Ca^{2+}-activated scramblases that randomize the distribution of lipids. Then, the cytoskeleton becomes limper and various cytosolic proteins and RNA molecules are sustained within the vesicles. The ECTOs are then pinched off, in which TSG101, a member of the ESCRT-I complex, mediates mobilization of ESCRT-III towards plasma membrane facilitating the assembly of a spiral form structure. This structure is disassembled by ATPase VPS4, and finally ECTOs are liberated, readers are directed to a comprehensive review published recently by Cocucci and Meldolesi.[12]

Clinical impacts of EVs

It should be articulated that the cancer-derived EVs encompass biological information and elements (e.g., receptors, enzymes, biomarkers, reactive oxygen species, genetic markers) as well as a number of key oncogenes and RNA molecules that can induce proneoplastic effects. The contents of normal and cancer cells-generated EVs show marked differences. Large TEXOs (the so-called oncosomes) were shown to mediate intercellular transfer of distinct classes of functional microRNA, namely, enhanced migration of cancer-associated fibroblasts (CAFs) by miR-1227. Among the proteins enriched in TEXOs, cytokeratin 18 (CK18) was reported as one of the most abundant oncomarkers found in the circulation and tissues of prostate cancer cases.[15] Through secretion of TEXOs, the migrating tumor cells happen to condition the distant sites to make them permissive for colonizing and thereby advancing the disease.[16] It should be pointed out that cancer-originated EVs display marked ability to elicit a rapid tissue growth, while other extracellular vesicles (e.g., DEXOs) can impose tumor suppressor potentials. Thus, it seems that the vesicular nanomachines, depending on the cell origin, are able to shuttle bioelements to the target cells, which can either promote or suppress the cancer-related phenotypes.[12] Of note, TEXOs enriched in patients' sera can be isolated and used as a reliable individual-specific source of antigens to load DCs. Such antigen-loaded DCs can be exploited as personalized anticancer vaccination modality.[17]

In addition to the cell-based vaccination and immunotherapy of cancer using re-programed individualized DCs, the DEXOs were shown to provide well-tolerated promising modalities for vaccination against malignancies.[18] Recently, in a phase I/II clinical trial, seven patients with advanced stage of squamous cell carcinoma of esophagus were treated with a vaccine comprised of monocyte-derived dendritic cells (moDCs) pulsed with SART1 peptide. It was found that the vaccine was able to induce SART1 peptide-specific cytotoxic T lymphocytes (CTLs) while the moDCs were able to liberate DEXOs capable of inducing SART1 peptide-specific CTLs.[19] However, the efficacy of DEXOs-based immunotherapy against cancer depends on the responsiveness of both B and T lymphocytes, which is in turn reliant upon the presence of both T- and B-cell DEXO-associated epitopes.[20] DEXOs contain various proteins and lipid necessary for biological functions of EVs. Among these components, immunorelevent molecules such as MHC molecules, costimulatory molecules, heat shock proteins (HSP), and peptide antigens are responsible for striking role of DEXOs in T cell ($CD8^+$ and $CD4^+$) dependent anti-tumor immune response stimulation. Several studies confirmed potential effects of EXOs loaded antigen to eradicate tumor *in vitro, ex vivo* and *in vivo*, in which addition of immune stimulation components (TLRs agonists, bacterial/viral peptides) may enhance the immune response. For instance, incorporation of the G protein of vesicular stomatitis virus into EXOs co-expressed with antigen was shown to improve (*a*) maturation of active DCs, (*b*) stimulation of antigen specific responses of CTLs, (*c*) upregulation of costimulatory molecules (CD80, CD86, CD40), (*d*) generation of IL12 as a DC effector, and (*e*) acceleration of antigen internalization by endocytosis following presenting by the MHC class I.[21]

Up until now, capitalizing on clinical potential of EVs, a number of clinical trials have been settled. The first clinical trial study supported by the Institute Gustave Roussy in France was performed using autologous EXOs pulsed with MAGE 3 peptides for the immunization of stage III/IV melanoma patients. In this study, fifteen HLA-$A1^+$, $B35^+$, HLA $DPO4^+$ metastatic melanoma patients expressing MAGE3 antigen on tumor cells were undergone the trail, and the autologous DEXOs pulsed with MHC class I-peptide or MHC class II –peptide were administered subcutaneously/intradermally in different dosages (4 times weekly). No major toxicity was observed in patients under such immunization modality. The results showed that, in contrast to insignificant increased percentage of peripheral blood lymphocyte and undetectable MAGE3 specific T lymphocyte response, nor T_h neither T_c induction of NK cell functions boosted in these patients.[22] Table 1 lists some of the EXOs-based clinical trials conducted for diagnosis and/or therapy of

Table 1. List of the exosome-based clinical trials conducted for diagnosis and/or therapy of different malignancies

Trial ID: Description	Cancer type	Intervention/Experiment	Sponsor
NCT01779583: Circulating Exosomes As Potential Prognostic And Predictive Biomarkers In Advanced Gastric Cancer Patients ("EXO-PPP Study")	Gastric Cancer	Molecular profile in tumor derived exosomes	Hospital Miguel Servet
NCT02393703: Interrogation of Exosome-mediated Intercellular Signaling in Patients With Pancreatic Cancer	Pancreatic Cancer	Exosomes purification for downstream applications (e.g., proteomics and RNA sequencing)	Memorial Sloan Kettering Cancer Center
NCT01668849: Edible Plant Exosome Ability to Prevent Oral Mucositis Associated With Chemoradiation Treatment of Head and Neck Cancer	Head and Neck Cancer; Oral Mucositis	Dietary supplement: grape extract	James Graham Brown Cancer Center
NCT02147418: Exosome Testing as a Screening Modality for Human Papillomavirus-Positive Oropharyngeal Squamous Cell Carcinoma	Oropharyngeal Cancer	Exosome protein signature outcome measure	New Mexico Cancer Care Alliance
NCT01294072: Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Colon Cancer	Dietary supplement: curcumin conjugated with plant exosomes	James Graham Brown Cancer Center
NCT02071719: Prediction of Response to Kinase Inhibitors Based on Protein Phosphorylation Profiles in Tumor Tissue From Advanced Renal Cell Cancer Patients	Renal Cell Cancer	Tumor exosomes from urine and serum	VU University Medical Center
NCT02310451: Study of Molecular Mechanisms Implicated in the Pathogenesis of Melanoma	Metastatic Melanoma	Blood test	Centre Hospitalier Universitaire de Nice
NCT02464930: Evaluation of MicroRNA Expression in Blood and Cytology for Detecting Barrett's Esophagus and Associated Neoplasia	Barrett's Esophagus; Gastroesophageal Reflux; Esophageal Adenocarcinoma	Sensitivity and specificity of tissue and serum microRNA expression	Midwest Biomedical Research Foundation
NCT01550523: Pilot Immunotherapy Trial for Recurrent Malignant Gliomas	Malignant Glioma of Brain	IGF-1R/AS ODN	Thomas Jefferson University

Data were obtained from clinicaltrials.gov website. All clinical trials listed were in recruiting phase.

various malignancies.

It should be pinpointed that an effective tumor specific immune response within TME needs activation of both innate and adaptive immune systems through cellular (i.e., induction of natural killer (NK) cells, cytotoxic $CD8^+$T cells and gamma delta T cells) and humoral such as antibody-dependent cellular cytotoxicity (ADCC) immune responses.[13] However, the penetration of whole monoclonal antibodies (mAbs) into TME seems to be largely size-dependent in large part due to high oncotic pressure within TME, and often a population of cancer cells in the core of solid tumors appears to remain untouched that can be the main cause of disease relapse. To overcome these anomalous pathophysiologic traits of solid tumors, novel cancer therapy strategies have been implemented including multifunctional nanomedicines,[23-39] multispecific antibody (Ab) scaffolds,[40-42] and various vaccination strategies such as edible vaccines.[43] It should be stated that the selection of effective mAbs for cancer immunotherapy appears to be very laborious and sophisticated,[44-46] while nanocarriers used in formulation of nanomedicines may induce inevitable toxic impacts nonspecifically.[47-53]

Final remarks

It appears that EXOs, TEXOs (small and large oncosomes) and DEXOs are important cellular micro-/nano-machineries that are involved in many cellular functions. Based on the cell origin, in malignancies, these EVs convey various messages to promote or to inhibit antitumor responses. Despite plethora of investigations on various EVs, it seems we still need to fully decode the main messages of these silently whispering vesicles and examine their potentials in diagnosis and treatment of diseases (in particular malignancies) in which the involved cells use such intricate bio-machineries for their communications. The conducted studies together with the growing body of evidence indicate that EXOs provide great potentials as a novel nanoscale cellular machineries for various diagnostic and therapeutic purposes. However, there exist some striking questions to be addressed. What if these EVs convey signals to suppress the immunosurveillance or danger signals to make TME much more permissive? Is it likely that they shuttle danger signals to neighboring cells/tissue? Do the malignant cells use such capacity for the migration and hence colonization into the neighboring cells/tissue? What are the main roles of lipid rafts , membranous caveolae and clathrin coated-pitsd in this process? Taken all, in the best scenario, we may capitalize on these cell-free vaccination system. And, if we exploit these paramount and worth pursuing nanoshuttles for cancer immunotherapy and vaccination, which issues need to be considered to improve the exosomal immunogenicity? To the best of our knowledge, these EVs need to be optimized in terms of (*a*) the antigen-loading efficiency, (*b*) the compositions, morphology and sizes, (*c*) the in vivo trafficking, and finally (*d*) the biological fates and impacts in the target cells.

Prospective Highlights

What is current knowledge?

√ Extracellular vesicles (exosomes) are important cellular micro-/nano-machineries that convey biological information.

√ Cancer-derived exosomes can favor the progression and development of cancer.

√ Dentric cells-derived exosomes may act against cancer progression and development.

What is new here?

√ The information of exosomes must be decoded in different cancers and patients to be used for personalized diagnosis and therapy.

√ Dentric cells-derived exosomes need to be carefully designed for cancer immunotherapy and vaccination.

√ For achievement of greater immunotherapy and vaccination, the antigen-loading efficiency of exosomes must be improved.

√ The compositions, morphology and sizes of exosomes may affect their biological impacts.

√ In vivo trafficking and bio-degradation of exosomes must be fully clarified.

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Ethical issues

There is none to be disclosed.

Competing interests

No competing interests to be declared.

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