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*Review Article*

# Exosomes in cancer therapy: a novel experimental strategy

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**Abstract:** Exosomes are small membrane vesicles of endocytic origin secreted by most cell types. They play important roles in intercellular communications and many physiological processes. DCs-derived exosomes can prime naïve T cells and activate NK cells to shrink the tumor. Tumor-derived exosomes carry a variety of tumor antigens that trigger the robust tumor antigen-specific immune response. Tumor-derived exosomes also contain metastasis or invasive-related molecules, which maybe potential targets for cancer immunotherapy. Effector T cells-derived exosomes possess cytotoxic activity of their original cells, thus cause tumor cells lysis. In this review, we summarized the recent advances on the biogenesis and composition of exosomes, the functions of anti-tumor immune response, and the promising applications on cancer immunotherapy of exosomes from different origins. Exosomes schlep efficient targets homing to tumor sites and tend to be a promising new tool of immunotherapy to fight cancer in a cell-free system.

**Keywords:** Exosomes, cancer immunotherapy, DCs, CTLs, CAR-T cells

## Introduction

Exosomes were fist described in the 1980s by Johnstone et al. They noted that vesicles shed from cultured monolayer cells retained enzymatic activity reminiscent of the parent cells [1]. Such small vesicles were formed by inward budding inside intracellular endosomes, leading to the formation of multivesicular bodies (MVBs), which could fuse with the plasma membrane (PM) and release exosomes out of the cell [2, 3]. Exosomes were lipid bilayer vesicles with 30-150 nm in diameter [4] and 1.13 g/ml-1.19 g/ml in buoyant density [5]. The proteins, TSG101, ALIX, CD63, and HSP70, are exosomal markers and commonly used to identify the presence of vesicles as true exosomes [6, 7].

Except the common marker proteins, exosomes also contain the specific molecules that reflect their cellular origins. Exosomes derived from professional antigen-presenting cells (APCs), such as B cells and dendritic cells (DCs), enriched in MHC/peptide complex and costimulatory molecules, therefore play an important role in anti-tumor response in immune stimulation and regulation [8-10]. Tumor-derived exosomes (Texs) carried antigens are important as a source of specific stimulus for the immune response against cancer [11]. In addition, the targeting specificity of cytotoxic T lymphocytes (CTLs) and chimeric antigen receptor engineered T (CAR-T) cells had preserved in CTLs and CAR-T cell-derived exosomes respectively [12].

Functions of exosomes in the biological process depend on the interaction between exosomes and the target cells [13]. Exosomes could directly fuse with the PM after binding to the target cells. By labeling exosomes with the lipophilic dye R18, scientists obtained direct evidence for fusion of exosomes with target cell membranes, in which self-quenching is relieved upon dilution as a consequence of fusion, resulting in an increase in the fluorescence of target cells [14]. In another way, several studies have demonstrated that exosomes also can internalized through special endocytic path-

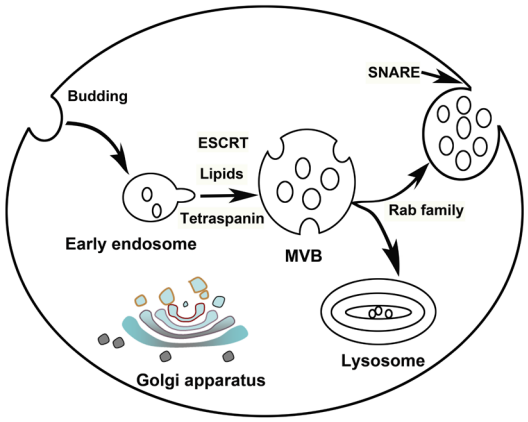


**Figure 1.** Schematic representation of the biogenesis and release of exosomes by eukaryotic cells. Exosomes firstly formed as multivesicular body (MVB) by budding into early endosomes. Several mechanisms including ESCRT, lipids and tetraspanin-dependent, are involved in MVB formation. If MVBs did not target for lysosomal degradation, they may mobilize and fuse with plasma membrane (PM) to release exosomes, depending on Rab family and soluble NSF-attachment protein receptor (SNARE) complexes respectively.

ways, which depend on the actin cytoskeleton, phosphatidylinositol 3-kinase activity, and dynamin-2 function [15-17]. Therefore, exosomes can deliver their contents to target cells, and prime its biological functions.

Despite therapeutic advances in recent decades, lacks of specificity and effectiveness remain the main drawbacks of clinical cancer treatment. Exosomes have a potential effect in anti-tumor immune response and may take a place in cancer therapeutic intervention. However, more information about exosomes should be uncovered before clinical application [18], which is so attractive that an increasing number of studies about it are underway. To gain further information on this aspect to conduct and promote application of exosomes, we summarize the biogenesis, composition, functions, and clinical trials in cancer immunotherapy of exosomes.

## Biogenesis and composition

Several molecules and complexes are involved in the formation of MVBs, which could fuse with the PM and release exosomes [19], including the Endosomal Sorting Complex Required for Transport (ESCRT) machinery [20], lipids (such as ceramide) [21] and the tetraspanins [22, 23] (**Figure 1**). Components of ESCRT machinery are necessary for MVB [20]. ESCRT machinery consists of a set of cytosolic protein complexes, known as ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, and several associated proteins, which conserved from yeast to mammals [24, 25]. ESCRT-0 complex, consisting of the hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), recognizes ubiquitylated cargo during the initial step of endosomal sorting. ESCRT-I and -II are involved in inducing membrane deformation into buds with sequestered cargo. ESCRT-III induces vesicle scission [26, 27], and the accessory proteins, especially the VPS4 ATPase, involves dissociation and recycling of the ESCRT machinery.

ESCRT-independent mechanisms also contribute to MVBs formations. Trajkovic et al. reported in 2008 that ceramide was involved in MVBs formation [21]. Then several labs started to confirm the function of exosomes secretion in vivo through neutral sphingomyelinase inhibition [28, 29]. In addition, four transmembrane domains of the tetraspanin family involve in cargoes clustering for MVBs formation. Van Niel et al. firstly found that CD63 was responsible for sorting melanosomal proteins into MVBs cargo in human melanoma cells, independent of ceramide and ESCRT [30]. TSPAN8 expression [31] and CD81 [32] were also suggested could alter both the containing of exosomes.

After biogenesis, MVBs may mobilize and fuse with the cell membrane to release their intraluminal vesicles as exosomes, if they did not target to lysosomal degradation [33, 34]. RAB family proteins, such as Rab11, Rab35, and Rab27 serve a function in MVB docking to the PM [35-37], which is required for eventual fusion of the two membranes, to allow the secretion of the exosomes. After docking of the two different intracellular compartments, soluble NSF-attachment protein receptor (SNARE) complexes play an important role in fusion of the lipid bilayers [38]. The SNARE proteins SNAP-23, VAMP-7 and VAMP-8 are involved in $Ca^{2+}$-regulated fusion of secretory lysosomes with the PM in different cell types [39-41].

In accordance with the biogenesis and secretion process of exosomes, the composition of exosomes includes both PM and cytosolic components, besides cytoskeleton proteins,

but very few from other intracellular organelles (nucleus, mitochondria, Golgi) [6]. Studies from different cell type revealed that exosomes contain about 4,563 proteins, 194 lipids, 1639 mRNA and 764 microRNA, demonstrated their complexity [42, 43]. Both ubiquitous and cell-specific proteins maybe target selectively to exosomes [44]. The former includes the components involved in exosomes biogenesis and, perhaps, in some unknown common exosomes functions cytoskeletal components, such as tubulin, actin and actin-binding proteins, as well as ANNEXINS and RAB protein family who participate in intracellular membrane fusions and transport [44]. Exosomes also contain proteins that involve in specific cell functions. Proteomics analysis of exosomes derived from DCs (Dexs) showed that it possesses MHC-I and -II molecules, which could potentially stimulate $CD8^+$ and $CD4^+$ T cells. Costimulatory molecules are also contained in Dexs, forming a unique molecular composition for strong immune-stimulatory functionality [6, 8, 45, 46]. As characterized by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF), MHC-I was found to be present together with the heat shock proteins HSC70 or HSP90 in exosomes derived from human mesothelioma cells, revealing that Texs may be involved in tumor immunity process [47].

In addition to proteins, exosomes also contain mRNAs and microRNAs (miRNAs), which can be taken up by neighboring or distant cells, subsequently repress target mRNAs of acceptor DCs and modulate recipient cells [48, 49]. Transfer of exosomes represents a novel mechanism of DC-to-DC communication and post-transcriptional regulation between DCs [14]. Exosomal miRNAs also play an important role in disease progression, and can stimulate angiogenesis and facilitate metastasis in cancers [50, 51]. Thus, exosomal miRNAs show potential for use as noninvasive biomarkers to indicate disease states [52, 53].

### DCs-derived exosomes

As the professional APC of the immune system, DCs play a critical role in initiating antigen-specific immune response and tolerance [54]. DCs are responsible to capture, process, and present antigens to naïve T cells, which are then activated, building an essential bridge between innate and adaptive immune responses [55, 56]. In cancer immunity, DCs are involved in the first step of immune response that aims to eliminate tumor cells through triggering tumor-specific cytotoxic lymphocyte [57]. Therefore, DCs induced from peripheral blood mononuclear cells (PBMCs) of cancer patients are cultured in vitro and pulsed by tumor lysate or tumor antigen peptide for cancer immunotherapy [58-61]. Provenge, the first DCs vaccine product approved by FDA, which was applying for immunotherapy for castration-resistant prostate cancer, represented a 4.1-month improvement in median survival (25.8 months in the sipuleucel-T group vs. 21.7 months in the placebo group) [62]. However, DCs vaccine is living cells. The cost is very high for storage and stability over longer periods. In addition, DCs are susceptible to immunosuppressive molecules and immune regulation in the tumor microenvironment [63].

Dexs carry many immune function-associated molecules of DCs, peptide/MHC complexes that trigger antigen specific T cell response [9, 10], and co-stimulatory molecules including CD80, CD83, CD86, that contribute to the T cell priming and activation [8]. Particularly, exosomes derived from tumor antigen peptide-stimulated DCs are able to prime tumor-specific CTLs in vivo, result in the tumor growth delayed, even eliminate tumor in some case of mouse models [8].

Scientists proposed several mechanisms to explain Dexs stimulate T cells via their MHC and costimulation molecules loading (**Figure 2**). Dexs activate T cells directly in vitro [9], however, direct Dex-to-T cell stimulation appears to be inefficient in priming naïve T cells and rarely occurs in vivo [64, 65]. Rather than direct Dex-to-T cell stimulation, it is much more efficient than transfer antigenic peptide/MHC complexes in Dexs to bystander APCs. Dexs are internalized into the endosomes and processed by APCs, followed the antigen presentation on the surface of APCs indirectly [34, 66-69]. Another way that Dexs mediated indirect antigen presentation is Dexs fuse with APCs surface after binding to target APCs, thereby transferring their peptide/MHC complexes to APCs surface immediately [67, 70]. Dexs incorporated by tumor cells could turn tumor cells into immunogenic targets for a possibly more effective

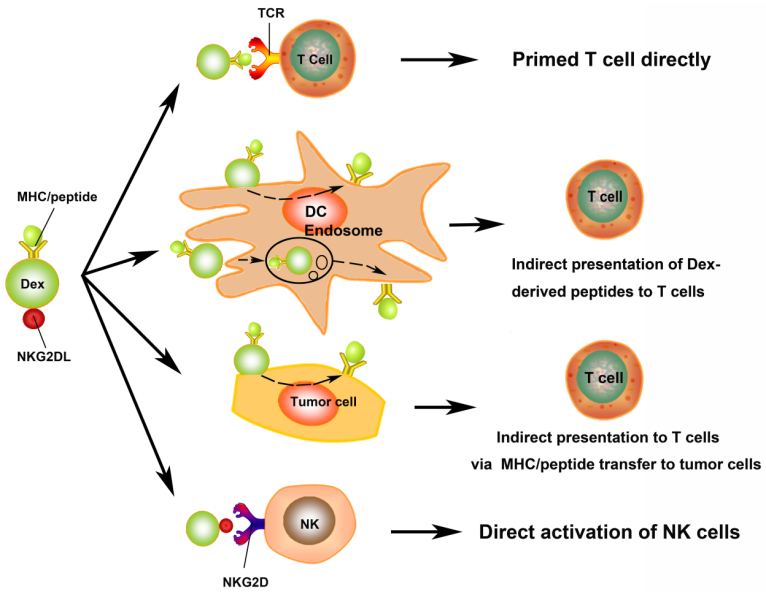


**Figure 2.** The role of exosomes derived from DC (Dexs) in anti-tumor immunity. Dexs may stimulate T cells via direct and indirect routes. The presence of MHC/peptide complex on the surface of Dexs stimulates T cells directly. Dexs activate T cells indirectly by bystander DCs via two mechanisms, one is Dexs fuse with PM of DCs and transfer MHC/peptide complexes to DCs surface immediately, the other one, Dexs are internalised into the endosomes and processed by APCs, following the antigen presentation on the surface of APCs indirectly. Dexs may also present MHC/peptide complexes to host T cells by MHC/peptide transfer to tumor cells. Dexs were shown to possess NKG2D ligand (NKG2DL), which can interact with NKG2D and activate NK cells.

response. In a recent study, treatment of human breast adenocarcinoma cells with Dexs was able to restimulate previously primed T cells, showing significantly higher percentages of interferon-γ (IFN-γ)-secreting induction than exposure to non-Dex-treated adenocarcinoma cells [8]. These studies suggested that Dexs maintain the essential immunostimulatory faculties of DCs, may become a promising tool for cancer immunotherapy.

In addition, Dexs have the bilayer membrane to keep the bioactivity stable in -80°C for at least 6 months with a phenotype and function preserved [71]. Used as a cell-free cancer immunotherapy tool, Dexs maybe more resistant to the immunosuppressive microenvironment in the tumor, which can down-regulate costimulatory molecules on DCs, therefore block T cell response [72]. Compared to DCs, Dexs surface possess natural killer (NK) cell lectin-like receptor subfamily K, member 1 ligands (NKG2D-L), which can engage with NKG2D expressed on NK cells, resulting in NK cells activation (**Figure 2**) [73]. Dexs express BCL2-associated athanogene 6 (BAG6, also known as BAT3) on their surface, which has been shown to enhance NK cell cytokine release [74]. Additionally, tumor necrosis factor (TNF) in Dexs induces NK cell-mediated IFN-γ production [75].

Because of their high potential and benefits for immunotherapy, Dexs have developed as clinical cell-free cancer vaccines. Two phase I clinical trials [76, 77] and one phase II trial [78] of Dexs in advanced cancer patients have been completed. The results suggested that Dexs are safe, and a mild T cell response and a potential increase in NK cell lysis ability observed. So combined Dexs with NK cells for cancer immunotherapy might be considerable.

### Tumor-derived exosomes

Texs bear MHC-I molecules, HSP70, and antigens, that are considered to be a source of specific stimulus for the immune response against cancer. Texs trigger anti-tumor responses more efficiently than irradiated tumor cells, apoptotic bodies, or lysate of cancer cells [11]. Stress-inducible exosomal HSP70 functions as an endogenous danger signal, promotes NK cell activation and cancer cell lysis via granzyme B [79]. Texs can efficiently deliver a variety of tumor antigens to DCs (**Figure 3**) and, thus, have employed as antigen carriers for cancer immunotherapy [80]. Rao et al. demonstrated that hepatocellular carcinoma (HCC) cell-derived exosomes, carried an array of HCC antigens, can elicit a stronger DC-mediated immune response than cell lysates in vitro and in vivo. In addition, HCC tumor microenvironment was improved by HCC cell-derived exosomes, demonstrated by increased numbers of T lymphocytes, elevated levels of IFN-γ, and decreased levels of interleukin-10 and tumor growth factor-β in the tumor (**Figure 3**) [81, 82].

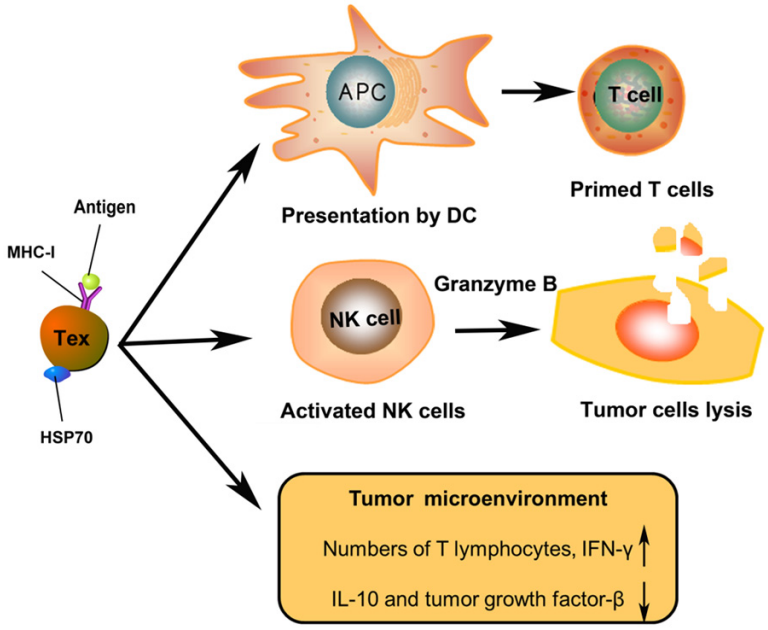


**Figure 3.** The role of tumor cells secreted exosomes (Texs) in anti-tumor immunity. Texs expressed tumor MHC/antigen complexes internalised by or fused with antigen-presenting cells (APCs) to prime T cells. Stress-inducible exosomal HSP70 functions as an endogenous danger signal, promotes NK cell activation and cancer cell lysis via granzyme B. Texs improve the HCC tumor microenvironment in vivo after infusion.

The same as Dexs, Texs may also be the potential candidate used as a cell-free tumor vaccine. However, the isolation of autologous Texs may require culture and expansion of patients' cancer cells in vitro, which may be inconvenient in clinical applications. Ascites-derived exosomes (Aexs) of advanced colorectal carcinoma (CRC) patients are considered be released from CRC cells [83]. Aexs contain MHC-I and -II molecules, co-stimulatory molecules, ICAMs, and the immunogenic carcinoembryonic antigen (CEA) of CRC, which maybe recognized by APCs. In a phase I clinical trial, Aexs were used to treat 40 advanced CRC patients with Aexs alone or Aexs plus GM-CSF [83]. Safety of Aexs on patients has confirmed, but no significant therapeutic effect observed when Aexs used alone. However, Aexs plus GM-CSF can activate $CD8^+$ CTL thus elicit CEA-specific antitumor immune response. It is suggested that Aexs in combination with GM-CSF maybe a potential method for cancer immunotherapy in the future [83]. Nevertheless, the immunogenic potential and anti-tumor efficiency of Aexs still need to investigate in clinical trials.

Studies unraveled that Texs possess immunosuppressive properties and directly modify tumor cells' intrinsic motility and invasiveness capacity. Texs has been shown to suppress T cell and NK cell response, stimulate myeloid-derived suppressor cells (MDSCs), resulting in facilitating tumor growth [84, 85]. Texs could induce T cell apoptosis that facilitate evasion of immune surveillance [86]. In particular, Texs directly enhance tumor cells' metastasis and invasiveness through some mechanisms [18]. Integrins distributed on the Texs surface, which bind to extracellular matrix (ECM) components, such as fibronectin, providing a substrate favoring cell adhesion and enhancing cell mobility [87]. Texs are involved in the biogenesis and activity of an invasive structure called invadopodia of tumor cells via the MVB-dependent delivery of metalloproteinases and other exosomes cargoes [88]. Cellular physiology of both surrounding and distant normal cells can also change by Texs to facilitate metastasis and growth of cancer cells [89, 90]. Therefore, it is vital to distinguish the immune-activated and immunosuppressive Texs, in order to remove the latter ones when technology is available, ensuring the curative effect of Tex vaccine. Alternatively, the immune-inhibitory effect of Texs successfully suppressed by combining Texs with appropriate, immune-stimulatory adjuvants, thus an anti-tumor response might be developed [91]. On the other hand, Texs included different molecules from donors' tumors that are involved in enhancing metastasis and invasiveness of tumor or inhibiting anti-tumor immune response. These molecules in Texs are useful markers to block as potential targets for cancer immunotherapy, but a great deal of additional research will be required to develop these therapies for clinical use.

### CTLs and CAR-T cells derived exosomes

Adoptive cell therapy (ACT) relieves tumors with infused CTLs. Mature DCs that pulsed by tumor lysates or tumor antigen peptides activate

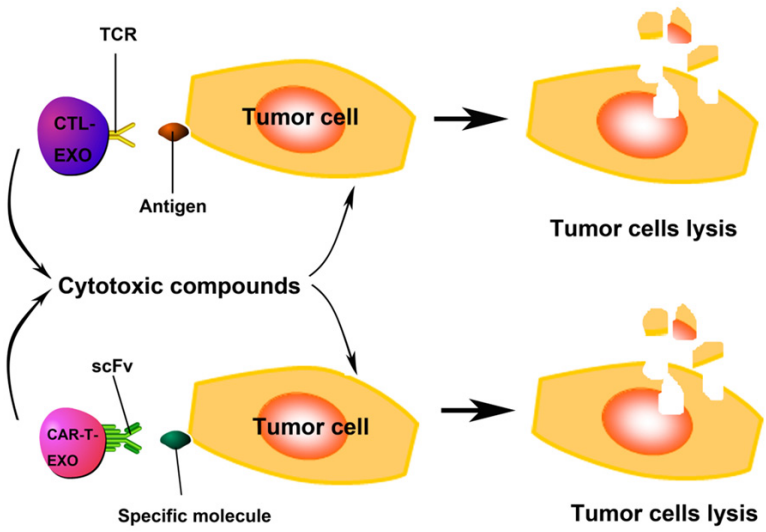


**Figure 4.** The role of exosomes derived from CTLs (CTL-EXO) and CAR-T cells (CAR-T-EXO) in anti-tumor immunity. The presence of TCR/CD3/CD8 on CTL-EXO enable CTL-EXO target antigen expressing tumor cells specificly, and cytotoxic compounds contained in CTL-EXO kill the target cells. CAR-T-EXO may possess antibody-derived single-chain variable fragment (scFv), which determines the targeting specificity of CAR-T cells, can recognize specific molecules on target cells (such as CD19 on B-cell neoplasms) and kill them by cytotoxic compounds release.

naïve T cells isolated from PBMC, trigger CTLs induction. Most cytotoxic T cells express T-cell receptors (TCRs) that can recognize specific antigens. TCRs bind to the MHC/antigen complex, and T cell destroys the target cell through releasing the cytotoxins perforin, granzymes, and granulysin (**Figure 4**) [92]. As early as in 1989, Peters et al. suggested that it's essential for human T cell-derived exosomes to participate in the process of CTLs and target cell interaction [12, 93, 94]. As specifically expressed on CTLs surface, the presence of CD3, CD8, and TCR on CTLs-derived exosomes suggested that exosomes could deliver cytotoxicity to targeted cells. Exosomes can interact with target cell by TCR binding to antigen/MHC-I complex, resulting in target cell death [12]. The cytotoxicity to target cells achieved by the cytotoxic compounds in the exosomes, including perforin, granzymes, and lysosomal enzymes [93]. In 2002, it was unraveled that the secretion of exosomes by CTLs accelerated by TCR activation and the TCR/CD3ζ complex existed on the surface membrane of exosomes derived from human CTLs [95].

Adoptive transfer of CAR-T cells suggests a promising new method in cancer immunotherapy. The targeting specificity of CAR-T cells are determined by an antibody-derived single-chain variable fragment (scFv) in the CAR structure. However, a major limitation of CAR-T cells application in clinic is poor therapeutic effects in solid tumors rather than in lymphoid malignancies. CAR-T cell-derived exosomes might possess antibody-derived scFv, being the promising alternative of cell therapy. The cell-to-cell contact between CAR-T cells or CTLs and tumor cells is necessary for the anticancer effect of CAR-T cells and CTLs. Unlike lymphoid malignancies, CAR-T cells and CTLs must penetrate stroma rich matrixes of the solid tumor to interact with tumor cells. However, tumor microenvironment may limit the activity of CAR-T cells and CTLs [96]. While cell-free exosomes are small nanometer-sized particles and were able to be located at specific antigen-targeted tumor in situ and to attack the tumor cells, since exosomes have the ability to cross biological barriers such as the blood-brain barrier (BBB) [97-99] and blood-tumor barrier (BTB) which was confirmed by the large quantity of Texs in body fluids [100]. The enhanced angiogenesis effect and leaky vasculature of the tumor are also attractive for intravenously injected exosomes to migrate to the tumor [100].

## Conclusion and prospects

Exosomes are easily available through ultracentrifugation process generally. Ultracentrifuges are widespread and straightforward to use, suggesting that the protocol of exosomes isolation and infusion can implement in clinical laboratories as a routine immunotherapy procedure for the cancer patients. But we now know that which isolated production is a mixture of exosomes and other extracellular vesicles (EVs). Here is impossible to distinguish them on the basis of a single property, such as size, structure, buoyant density, or presence of a given protein, so purification of exosomes is technically unavailable so far [7]. Novel isolation methods are required to enrichment of the

specific subtypes [18]. Therefore, better knowledge of specific markers of EVs subtypes is required. Extensive quantitative proteomic analysis have shown that several classically used exosomes markers, like MHC, flotillin, and HSP70 proteins, are similarly present in all EVs [101]. Nevertheless, a few new specific markers for different subtypes of exosomes are proposed, which are tetraspanins CD9/CD63/CD81, TSG101, and syntenin-1 for endosome-derived exosomes, thus providing guidelines to define subtypes of exosomes precisely for future functional studies.

Two areas for improvement of cancer treatment are targeting and effectiveness. Exosomes schlep efficient targets homing to tumor sites. There is abundant source of exosomes in the culture supernatant of DCs and tumor cells. CTLs and CAR-T cells possess mighty expansion ability, ensuring the outlay of exosomes to be satisfied. With the remarkable effects in anti-tumor immune response mentioned above, exosomes could substitute or boost other strategies of cell-based immunotherapy or be used as a maintenance vaccine in the future. Exosomes derived from different sources hold diverse applications in cancer therapy. In addition to the detailed discussed above, there are exosomes derived from other sources, such as mesenchymal stem cells-derived exosomes, HEK293T cells-derived exosomes, macrophage-derived exosomes, cerebral endothelial cell-derived exosomes, reticulocyte-derived exosomes and so on. Thus, it is now vital to understand exosomes derived from different sources themselves with the aim of choosing the appropriate exosomes for cancer therapy.

However, these issues should not interfere with the diffusion of such clinically important therapy for cancer. The potential development of exosomes for efficient therapeutic strategies in cancer depends on further progression.

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## Disclosure of conflict of interest

None.

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