

Review

Exosomes for Immunoregulation and Therapeutic Intervention in Cancer

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Abstract

Exosomes, as a subset of extracellular vesicles, function as a mode of intercellular communication and molecular transfer, and facilitate the direct extracellular transfer of proteins, lipids, and miRNAs/mRNAs/DNAs between cells. Cancers have adapted exosomes and related microvesicles as a pathway that can suppress the immune system and establish a fertile local and distant environment to support neoplastic growth, invasion, and metastasis; these tumor-derived exosomes affect immunoregulation mechanisms, including immune activation and immune suppression. Immune cell-derived exosomes can modulate the immune response in cancer, which supports the belief that these membranous vesicles are immunotherapeutic reagents. In this review, we discuss the recent advances in the cancer immunotherapy, roles of exosomes in cancer, immunoregulation of tumor-derived exosomes, and immunomodulation by immune cell-derived exosomes. The topics covered here highlight novel insights into the development of efficient exosome-based cancer vaccines for cancer therapeutic intervention.

Key words: Exosomes; cancer; immunoregulation; therapeutic intervention

Introduction

In mammals, the immune system is an important part of the body; it can be classified as innate or adaptive according to its origin. The adaptive immune system, which is also called the acquired immune system, includes humoral immunity and cell-mediated immunity [1,2]. In humans, B cells and T cells are the main kinds of cells involved in acquired immunity [3]. Leukocytes (including phagocytes, mast cells, and natural killer [NK] cells) are the key step of the innate immune system [1]. Dendritic cells (DCs), an important kind of phagocyte that is located in the skin, lungs, and intestines, are the link between the innate and acquired immune systems by presenting antigens to T cells [4]. Natural killer cells play key roles in clearance of malignant and virally infected cells and regulation of adaptive immune responses in human [5].

On the other hand, cancer is a major cause of

death all over the world; there are more than 100 different types of cancer [6]. Environmental and lifestyle factors are the most common risk factors for cancer, accounting for more than 90% of cases [7,8]. For example, 90% of lung cancers are caused by tobacco smoking, and 10% of cancers in males and 3% in females are due to alcohol consumption [9]. Cancer can also be induced by internal factors; while most are not inherited, some are caused by mutated genes [10]. Thus, it is very important that the immune system eliminates viral infections and inflammation to protect the host from cancer and clears away cancer cells [11]. $CD4^+$ and $CD8^+$ T lymphocytes are considered tumor immune cells and have the most important role in immune surveillance [12]. MHC molecules on the surfaces of tumor cells act as the antigen that is recognized by T cells, allowing NK cells to kill tumor cells [13,14].

Cancer Immunotherapy

The main goal of cancer immunotherapy is to specifically inhibit the malignant activity of cancer cells and leave healthy cells unaffected; it has been used to treat many diseases by activating or suppressing the immune system [15-19]. Scientists first considered the use of immunotherapy for allergies [20] and found that it was the most useful method for the treatment of asthma [21, 22]. Better immune targets and combination strategies can harness the immune system by supporting elements of the T cell anti-tumor response [23], making immunotherapy a useful treatment for all kinds of diseases. Immunotherapy can also be used for cancer, such as breast cancer [24], colon cancer [25], brain tumors [26], glioblastoma [27], lung carcinoma [28], ovarian cancer [29], and prostate cancer [30]. Three exciting targets for immunotherapy, *TNF- α* -converting enzyme, cathepsin S, and urokinase plasminogen activator, have been investigated; rituximab (one of the most commonly used monoclonal antibodies) and trastuzumab have already been used as cancer therapies [31]. As cancer immunotherapy continues to benefit from novel approaches, such as *anti-PD1* and *anti-CTLA4* antibodies, *IL-2*, and *IFN α* , there will be an increasing need to develop cancer vaccines to guide the immune system specifically toward tumor-associated antigens [32]. *IL-2* and *IFN α* have been approved for the treatment of several different tumors by the FDA. *IL-2* can not only mediate T cell response and cell differentiation [33-35] but also leads to remission in about 10% patients with metastatic melanoma [36]. *IFN* can protect the host from infection and tumor invasion [37-39]. Furthermore, Paret found that *CXorf61* was a target of immunotherapy in breast cancer [24].

Exosomes in Cancer

Exosomes are a subset of extracellular vesicles, 30 to 100 nm in diameter, that function as a mode of intercellular communication and molecular transfer and facilitate the direct extracellular transfer of proteins, lipids, and miRNAs/mRNAs/DNAs between cells. They can be isolated and purified from cell culture supernatants, which are released by many types of cells, such as immune cells and tumor cells [40-42]; they have different biological effects, depending on the type of the original cell, and play an important role in immunoregulation [43,44]. It is possible that exosomes are generated, with early endosome formation, in the endocytic pathway [45] and are released to the extracellular matrix by fusing the multi-vesicular bodies with the plasma membrane

[46,47]. Because of the different machinery of exosome biogenesis, the kinds of exosomes may be decided by the kinds of multi-vesicular bodies [48]. Exosomes naturally carry RNA and proteins and have specific characteristics with the protein content, including *Tsg101*, *Alix*, *Gag*, *Rab*, *Annexins*, *Flotilin*, *CD63*, *CD81*, and *Hsp90* [49-52], that can not only transfer messages intercellularly [53,54] but can also modulate the immune response [55-58], which indicates that exosomes can be used for the development of future exosome-based cancer vaccines.

Exosomes can carry various proteins, mRNAs, and miRNAs that result in different actions on the immune system. For example, the exosomes from B cells, combined with *MHC-II*, can directly stimulate *CD4⁺* T cells [59]; the exosomes from DCs can bind toll-like-receptor ligands and activate bystander DCs [60]; the exosomes from the chicken biliary tract can enhance *CD4⁺* T cell proliferation and inhibit avian leukosis virus replication [61]; the exosomes from leukemic cells can induce an anti-leukemic cytotoxic T cell immune response [62]; and the exosomes from rheumatoid arthritis patients have a higher amount of *TNF* than do those from individuals without rheumatoid arthritis [63]. Bhatnagar et al also found that exosomes promoted intercellular communication by activating the immune response and suggested that exosomes containing intracellular pathogens mycobacterium tuberculosis, BCG and salmonella typhimurium are an important mechanism of immune surveillance [64]. Exosomes have a role in mediating autoimmune diseases and inflammation, can induce immune tolerance, and protect against allergic sensitization, which can be used to develop therapy for allergic diseases [65]. On the other hand, Tumor-derived (TD) exosomes have become a new kind of cancer biomarker because they contain proteins, RNAs, and miRNAs and can transfer these to other cells for intercellular communication [66]. The biomarkers in exosomes of ovarian cancer, acute myeloid leukemia, melanoma, and pancreatic adenocarcinoma may be used in cancer diagnoses [67-70]. *PCA-3* and *TMPRSS2* can be easily detected in these exosomes and can be better biomarkers for prostate cancer [71]. *HSP60* is a protein carried by exosomes and can be a biomarker of large bowel cancer in humans [72]. Exosomal *miR-19a* is a biomarker for colorectal cancer [73]. All of these findings will speed up the development of new approaches to cancer diagnosis and treatment.

Immunoregulation of Tumor-Derived Exosomes

Exosomes from tumor cells are essential in tumor migration and metastasis and play an

important role in angiogenesis as a vital pathway [74-76]. Tumor-derived (TD) exosomes can suppress the function of NK cells and T cells to affect immunity in cancer, increase tumor growth by interacting with NK cells to decrease perforin secretion, decrease *cyclin D3* levels, inhibit *Jak-3* [77-79], and promote T regulator cell expansion, leading to the immune escape of tumor cells and inhibition of NK cell cytotoxicity, which is mediated by *NKG2D* and *TGF- β* [80-82]. TD exosomes are associated with the pro-tumorigenic phenotype; they facilitate immunosuppression and promote the tumorigenesis by inhibiting the immune response [83]. Recently, one study of the immune response of TD exosomes demonstrated that the exosomes triggered *NF- κ B* activation in macrophages, leading to the increased secretion of pro-inflammatory cytokines, such as *IL-6*, *TNF- α* , and *GCSF* [84]. The exosomes produced by tumor cells increased the phosphorylation of *STAT3* and *IL-6* in DCs, decreasing the cells' activity by inhibiting the differentiation of *CD14⁺* monocytes to mature antigen-presenting cells (APCs) [85]. Tumor-derived exosomes can affect immunity by decreasing the amount and activity of APCs and increasing the number of myeloid-derived suppressor cells (MDSCs), which is correlated with tumor progression, affects patient survival, and inhibits NK cells and T lymphocytes [86-89]. *FasL*-positive exosomes can promote immune escape and induce apoptosis in Kurkat and lymphoid cells [90-92]. When TD exosomes are co-cultured with priming T cells, the expression levels of *CD3* and *JAK3* are reduced and *Fas/FasL* promotes cell apoptosis [93]. This increase in suppressive immune cells is one of several mechanisms in immunity that are inhibited by exosomes; other mechanisms include decreases in cell proliferation, NK and T cell cytotoxicity, and the number and function of APCs [94,95]. Exosomes carrying *TGF- β 1* can decrease the expression of *NKG2D* receptor and reduce the activation of NK and *CD8⁺* T cells [96,97]. Immature DC exosomes display immunosuppressive activity in autoimmune diseases [98].

Although TD exosomes are predominantly immunosuppressive, they can also enhance immunostimulation. The findings of studies investigating the immunoregulation of exosomes in cancer are summarized in Table 1. TD exosomes with APCs can stimulate T cell responses [99,100]. TD exosomes can also enhance anti-tumor immunity through a gene transfer of cytokines, tumor antigens, or heat shock proteins [101-104]. Interestingly, the TD exosomes in bronchoalveolar lavage can induce epithelial cells to produce *IL-8* and activate T cell responses [105]. Moreover, the NK cells co-cultured

with TD exosomes can be induced to release granzyme B and initiate pancreatic cancer cell apoptosis [106].

Immunomodulation in Cancer by Immune Cell-Derived Exosomes

Exosomes derived from immune cells (such as APCs, dendritic cells, and NK cells) may play a crucial role in the immunomodulation of cancer. First, exosomes with antigen peptides can induce anti-tumor *CD8⁺* T cell responses in murine mastocytoma P815 and mammary carcinoma TS/A cells, causing the regression of an established tumor [107]. Raposo and his colleagues were the first to find that exosomes plus *MHC-II* can stimulate *CD4* T cells [108]. DC-derived (DCD) exosomes can inhibit tumor cell growth by injecting the exosomes into mice with tumors and observing a special anti-tumor immune response [109]. DCD exosomes activated immune effector cells, such as *BmDC*-derived exosomes with *NKG2D*, which promoted NK cell activation and proliferation and led to tumor suppression [110]. DCD exosomes carry many of the immune function-associated molecules of DCs and induce antigen-specific responses against tumor cells, which may be used as next-generation cancer immunotherapy [111,112]. High levels of *NKG2D* on NK cell-derived exosomes can be involved in NK cell-mediated surveillance of the primary tumor [113,114]. NK cell-derived exosomes enclose perforin and granzyme B to mediate anti-tumor activity both in vitro and vivo [115].

Cancer Therapeutic Intervention by Exosomes

Recently, DC-derived (DCD) exosomes have received attention in cancer immunotherapy because they play key roles in modulating the immune response. Several investigators have demonstrated that these exosomes can be used as cancer vaccines. They have been investigated in the pre-clinical setting because of their ability to stimulate an immune response and eradicate established murine tumors [116-118]. Breast tumor cells co-cultured with DCD exosomes have an increased ability to activate T cells for a more effective response, which suggests that DCD exosomes are another tool in cancer immunotherapy [119]. The anti-tumor efficacy of DCD exosomes was confirmed in melanoma patients [120]. DC immunotherapy by injecting a single dose of DCs loaded with tumor exosomes derived from non-immunogenic tumors, was also found to improve the survival of tumor-bearing mice [121].

The *Rab27a*- and *CD40L*- exosomes in tumors

elicited more potent anti-tumor immune effects, which indicated that these exosomes would be useful vaccines for lung cancer immunotherapy [122,123]. A comparison test showed that DNA vaccines that encode EV-associated antigens are promising immunotherapy tools in cancer and other diseases [119] and that *Exo/SEB* significantly stimulated apoptosis and raised the expression rates of *Bax*, *Bak*, and *Fas* [124]. Näslund found that the inclusion of B-cell epitopes in anti-cancer vaccines was crucial for the success of this immunotherapeutic intervention [125], which demonstrated that exosomes are novel and effective vaccines for treating various cancers and may provide novel insights into the development of efficient exosome-based cancer vaccines. DCD exosomes derived from genetically manipulated DCs,

engineered to promote anticancer immunogenicity, which may lead to key advances in cancer therapy [126].

In summary, exosomes can affect the immune system in different ways, depending on the original and tumor microenvironments. No matter how exosomes inhibit the immune response or promote tumor progress, a greater understanding of immunotherapy is needed, and exosomes are likely to become the most effective vaccines for cancer. Further understanding of exosome biology, especially of the molecular mechanisms involved in tumor- and immune cell-derived exosomes as cancer vaccines, is likely to provide significant insights into immunorecognition and therapeutic intervention.

Table 1. Findings in studies investigating the immunoregulation of exosomes in cancers

Function in tumor growth	Origin	Cancer cells/immune cells	Targeted molecules	Functional pathways	References		
Promoting	Tumor cells	Colorectal cancer cells	Fas, TNF	Suppressive immune cells	90		
		Melanoma cells	Fas/FasL	Apoptosis of T cells	91, 82		
		Nasopharyngeal carcinoma cells	Galectin-9/Tim-3	Induce apoptosis of T cells	92		
		Solid tumor/AML cells	JAK3, Fas/FasL	Suppress T cell and NK cells	93		
		Breast cancer cells	NF-kappa B	Decrease T cell number	84		
		Mammary carcinoma cells	Perforin, cyclin D3, JAK3	Suppress NK cell function	77		
		Ovarian tumor cells	FasL	T cell apoptosis, suppress T cell receptor	79		
		Acute myeloid leukemia cells	TGF- β 1	Suppress NK cell function	80		
		Mesothelioma cells	NKG2D	Suppress T cell and NK cell function	96		
		Squamous cell carcinoma cells	Fas/FasL	Promoting T regulatory cell expansion	82		
		Melanoma cells		Promote T regulatory cell expansion	82		
		Murine mammary tumor cells	IL-6	DC differentiation	86		
		Mammary adenocarcinoma cells	IL-6, VEGF, PGE2, TGF- β	Accumulation of myeloid-derived suppressor cells	88		
		Colon carcinoma CT26 cells, lymphoma EL4 cells, embryo NIH/3T3 fibroblasts, lung adenocarcinoma H23 cells, mammary adenocarcinoma cells	Hsp72, TLR2, STAT3	Activation and enhancement of MDSCs' suppressive ability	75		
		Ovarian cancer cells	ATF2, MTA1, ROCK1/2	Angiogenesis in ovarian serous cancer	76		
		Squamous carcinoma A431 cells, MDA-MB-231 breast adenocarcinoma cells, A549 NSCL cells, H1299 NSCL cells	Hx or Reox	Regulation of tumor metastasis and angiogenesis	98		
		Pancreatic adenocarcinoma cells	Tetraspanin D6.1A/CO-029	Induce systemic angiogenesis	99		
		Inhibiting	Tumor cells	Non-small cell lung cancer cells	MAGE peptides	T cell responses and NK cell lytic activity increased	101
				Fibrosarcoma cells	C1C2-fused antigen	Induction of a more potent antigen-specific anti-tumor immune response in vivo	103
				Murine lymphoma E.G7-OVA cells	SEA-TM	Promote induction of specific anti-tumor immune response	104
Murine colon carcinoma cells, murine melanoma cells	HSP70			Improve immunostimulatory activities	106		
Human pancreas cells, human colon cells	HSP70			Stimulate migratory and cytolytic activity of NK cells	108		
APCs	Autologous monocyte derived DCs			MAGE3 peptides, HLA-A2, HLA-BC	T cell responses	102	
	Spleen-derived murine D1 DCs			HSC73, MHCII, Mac-1 integrin, CD9, MFG-E8	Elicit potent T cell-dependent immune responses	110	
	DCs			NKG2D, IL-15R α	Promote NK cell activation and proliferation	115	
	DCs			MHCII	Elicit T cell-dependent immune responses	113	
	NK cells			FasL, CD56, perforin	Deliver cytotoxic molecules	114	

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Competing Interests

The authors have declared that no competing interest exists.

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