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REVIEW

Exosome-mediated immune regulation and its clinical application

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ABSTRACT

Immune system is a precise mechanism for maintenance of homeostasis by lymphocyte-mediated elimination of extracellular and intercellular pathogens, and abnormal cells in cytokine-, chemokine-, antibody-, and cytotoxic granule-dependent manners. Extracellular vesicles, *e.g*. exosomes, released from multivesicular endosome in immune cells have been known to be a part of the immune system. Exosomes released by antigen-presenting cells (APCs) such as macrophages and dendritic cells (DCs) regulate natural killer (NK) cells, $CD8^+$ T cells (Cytotoxic T lymphocytes [CTLs]), and $CD4^+$ T cells (Th cells) including Th1, Th2, and regulatory T (Treg) cells. In the anti-tumor immune system, NK cells and CTLs are mainly involved in the elimination of tumor cells by direct interaction. Recently, we clarified that tumor-infiltrating $CD8^+$ T cells prevent tumor invasion and metastasis by exosome-mediated destruction of tumor stroma consist of mesenchymal stem cells (MSCs) and cancer-associated fibroblasts (CAFs). In this review article, we describe the role of exosomes in controlling immune system and its clinical application.

Keywords: *$CD8^+$ T cell; exosome; extracellular vesicle; mesenchymal cell; tumor metastasis*

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Conventional immune system

NK cells and macrophages are representative innate immune cells that act as a central player at an early stage of pathological conditions such as infection and cancer. In addition, extrathymically differentiated T cells expressing γδ T cell receptor (TCR), and NK T cells expressing NK makers and invariant αβ TCR to α-galactosylceramide (αGalCer) in the context of CD1d are also classified as innate immune cells. On the other hand, intrathymic-differentiated T cells bore αβ TCR can expand vigorously by priming with specific antigen peptide/major histocompatibility complex (MHC) on dendritic cells (DCs), exhibit effector/memory phenotype, and participate in the elimination of abnormal cells, so termed as acquired immune cells[1]. Acquired immunity is composed by $CD4^+$ helper T (Th1 and Th2) cells, $CD4^+$ $Foxp3^+$ regulatory T (Treg) cells, and $CD8^+$ cytotoxic T lymphocytes (CTLs). Interferon (IFN)-γ-expressing Th1 cells support CTL induction in antigenic stimulation by DCs. Interleukin (IL)-4 secreting Th2 cells promote B cell differentiation into antibody-producing plasma cells and tumorigenesis of epithelial cells, and inhibit inflammation. $CD4^+$ $Foxp3^+$ Treg cells suppress antigen-specific T and B cell responses. In addition to Treg cells, myeloid-derived suppressor cells (MDSCs) strongly reduce T and NK cell activites and promote tumor infiltration of Treg cells[2]. Chemokines from macrophages and DCs attract innate immune cells and acquired immune cells in tumor lesion. It has been gradually clarified that exosomes released from these immunocompetent cells play a part of complicated immune responses[3].

Exosomes from T cells and NK cells

T cells strongly release exosomes with activation[4]. Treg cell exosomes have been studied to some extent, all of which are reports regarding immunosuppressive function. CD73 on Treg cells converts extracellular ATP to immunosuppressive adenosine (ADO), and inhibits T cell and NK cell activities. Treg cell exosomes also express CD73 and seem to participate in the immunosuppression[5,6]. Treg cell exosomes inhibit strongly Th1 cell

activity in an exosomal micro (mi) RNA-denpendent manner[7]. Transforming growth factor (TGF)-β and suppressive miRNAs in breast milk exosomes are retained relatively stable against temperature, pH, and freeze-thaw, and they maintain Treg cells, and prevent the onset of modern diseases such as atopic dermatitis by reduction of IgE production of B cells[8,9]. Suppressive role of Treg cell exosomes may be applicable in tolerance induction during organ transplantation[10].

$CD8^+$ T cells proliferate predominantly when spleen cells of mouse and human peripheral blood lymphocytes are cultured by stimulation with both CD3 and CD28. It has been reported that exosomes from primary culture of T cells preferential activate naive $CD8^+$ T cells, but not naive $CD4^+$ T cells[11]. $CD8^+$ T cells express FasL capable of apoptosis of Fas^+ tumor cells. However, FasL on $CD8^+$ T cell exosomes seem to promote invasion and metastasis of tumor cells, but not tumor cell killing, by matrix metalloproteinase (MMP)-9-medaited degradation of extracellular matrix proteins via Fas/FasL signaling pathway[12]. Conversely, since FasL was not found on $CD8^+$ T cell exosomes in our study, we examined the action of murine $CD8^+$ T cell exosomes in tumors in comparison with exosomes from other lymphocyte populations, tumor cells, or human T cells. Surprisingly, $CD8^+$ T cell exosomes exhibit cytotoxicity against tumor stromal cells such as MSCs and CAFs, but not tumor cells. In addition, $CD8^+$ T cell exosome-mediated destruction of mesenchymal stroma associated with the reduction of tumor invasion and metastasis (Figure 1)[13].

Studies of NK cell exosomes have not progressed because of lack of explosive growth potential compared to T cells and difficulty of cultivation in a single population. NK cell exosomes express FasL, and seem to be able to induce apoptosis of Fas+ tumor cells. It has been reported that cytotoxic substances, perforin and granzyme B, are abundantly shown in the lumen of NK cell exosomes[14].

Relationship among B cells, macrophage, and exosomes

B cells and macrophages have been known as antigen-presenting cells. Major histocompatibility complex (MHC) class II molecules express on exosome membrane released from both cells[15]. B cell exosomes entering lymph node are rapidly taken up and decomposed by subcapsular macrophages. Interaction CD169 (Siglec-1) expressed on subcapsular macrophages with sialic acid on B cell exosomes is important in this setting[16]. It has been reported that exosomes from chronic B leukemia cells have a potential to differentiate vascular endothelial cells and MSCs into smooth muscle actin+ CAFs.

When exosomes are administered systemically, most of them are engulfed by hepatic macrophages and digested in the lysosome. Scavenger receptors

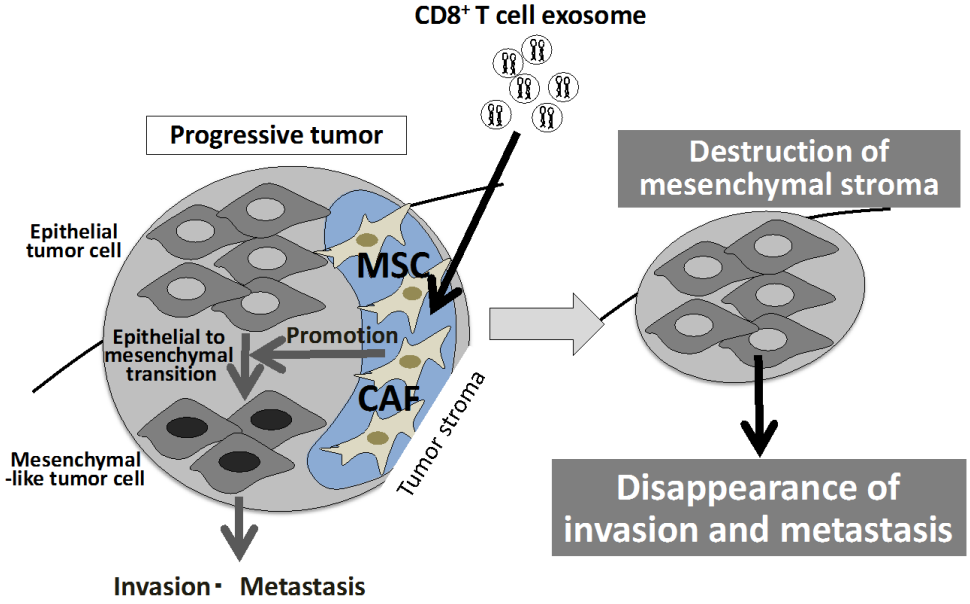

Figure 1. Prevention of tumor progression by CD8+ T cell exosomes. $CD8^+$ T cell exosomes deplete mesenchymal tumor stromal cells such as MSCs and CAFs. This exosome-mediated destruction of tumor stroma associates with reduction of tumor invasion and metastasis.

such as SR-A (Scavenger receptor class A) on hepatic macrophages seem to be a ligand for phosphatidylserine-derived negative surface charge on exosomes[17,18]. In liver metastasis of pancreatic cancer, pancreatic cancer cell-released exosomes participate in the formation of pre-metastasis niche by promotion of TGF-β and fibronectin production of hepatic stellate cells after kupffer cell activation[19], showing close relationship between exosomes and liver macrophages. Exosomes released from tumor-associated macrophages seem to promote differentiation of monocytes into macrophages in an exosomal miRNA-dependent manner[20].

DC exosomes and immune regulation

DCs reside in lymph nodes, skin and mucosal tissues in immature form. By capturing viral antigens or tumor proteins, immature DCs activate with enhanced expression of MHC class II molecules, migrate into draining lymph nodes, and present antigen peptides to specific T cells[21]. Immature DCs release exosomes more vigorously than mature DCs[22,23]. Immature DC exosomes exhibit a potent role in Treg cell activation[23,24], suggesting maintenance of homeostasis by suppressing autoimmune reactions and excessive inflammations. Whereas, mature DCs release exosomes to facilitate tumoricidal immune reactions.

Hence DC exosomes pulsed with tumor antigen peptides can induce cytotoxic T lymphocyte (CTL) responses in B cell dependent manner (unknown mechanism) *in vivo*, clinical application of DC exosomes have already begun (Figure 2)[25–27]. However, DC exosomes seem to have high NK cell activating capacity rather than CTL induction[25,26,28]. DC exosomes pulsed with α-galactosylceramide (α-GalCer) and tumor antigen peptides can activate invariant (i) NK T cells and γδ T cells concomitant with specific CTLs, resulting elicitation a strong antitumor immune responses[29]. MHC class I molecule on the exosome membrane exists as dimer at the end of the lumen region, and may have different conformation from that of the monomer[30]. Furthermore, exosomes derived from DCs from MHC class I knockout mice have T cell inducing and NK cell activating abilities comparable to those of DC exosomes from normal mice[31], suggesting MHC class I-independent mechanism of antigen presentation by DC exosomes (Figure 2).

Tumor cell exosomes and immune regulation

Immune modulatory effect of tumor cell exosomes was most developed. Tumor cell-derived exosomes promote activation and accumulation of Treg cells[32–34]. Likewise, tumor cell exosomes enhance production of prostaglandin E2, IL-6, and TGF-β of MDSCs, resulting formation of

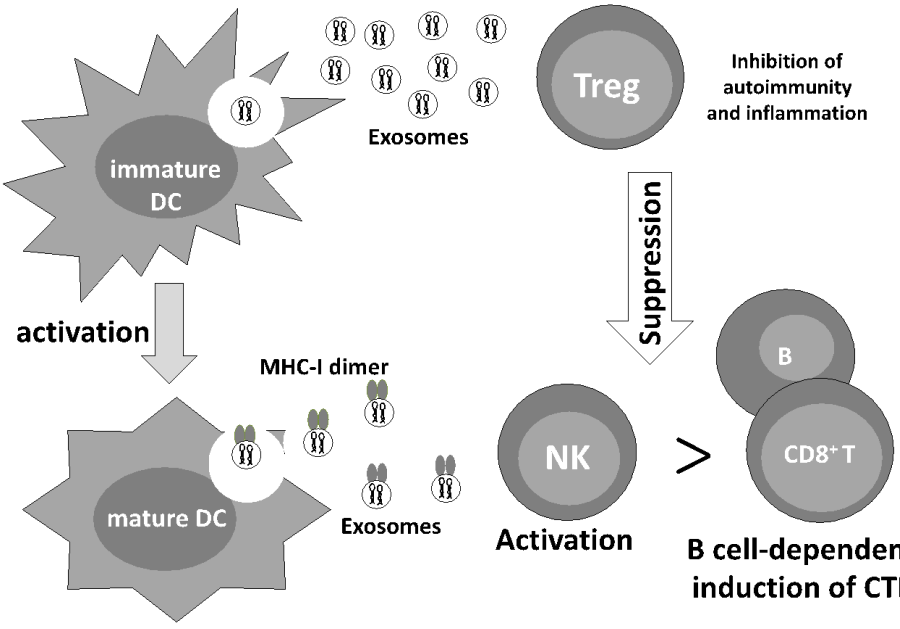

Figure 2. DC exosome-mediated activation of immune cells. Immature DCs and mature DCs release exosomes to induce Treg cells, and NK cells and CTLs, respectively. MHC class I molecules on exosomes from mature DCs express as a dimer.

strong immunosuppressive environment in tumor lesions[35,36]. NK cells, γδ T cells, and a part of CTLs recognize tumor surface MHC class I molecule-like UL16-binding protein (ULBP) [3] and MHC class I polypeptide-related sequence A (MIC-A) by interaction with NKG2D, and can lyse tumor cells. However, tumor cells released ULBP- and MIC-A-bearing exosomes, and block cytotoxicity[37,38]. Interestingly, it has been reported that tumor cell exosome-engulfed DCs produce immune competent exosomes for effective induction of anti-tumor immunity[39,40]. This seems to be related to the Type-I IFN secretion mediated by cGAS (cyclic GMP-AMP synthase)/STING (Stimulation of *IFN* gene) pathway in DCs by exosomal DNAs[41].

Tumor cells are always under hypoxic conditions and temperature stress, and are also exposed to drug stress during treatment of anticancer agents. Under these circumstances, it has been known that tumor cells release exosomes more aggressively than normal condition, and exhibit immune modulatory effects. In low oxigen, tumor cells released TGF-β-bearing exosomes, and promote and inhibit Treg cell activity and NK cell cytotoxicity, respectively[42]. Conversely, tumor cell exosomes released by high temperature stress or anticancer drug stress embed HSP (Heat shock protein)-70 and CCL (CC chemokine) -2, -4, -5, and -20 capable of promoting migration and activation of T cells, NK cells, and DCs[43,44].

Concluding remarks

In immune system, exosomes seem to inherit the function of parent cells, implying exosomes from $CD8^+$ T cells, macrophages, and B cells for treatment of tumors in addition to the already used DC exosomes. However, proteins expressed or embedded in exosomes may exhibit different function from those in the parent cells, as shown in MHC class I dimer on exosome membrane and MHC class I-independent activation of NK and CTLs by DC exosomes. Solving various issues including molecular structure should be important to elucidate the biological significance and clinical treatment of immune cell-derived exosomes.

Conflict of interest

The author declares no potential conflict of interest with respect to the research, authorship, and/or publication of his article.

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