Chapter 7
Exosomes Function in Tumor Immune Microenvironment

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Abstract Immune cells and mesenchymal stem/stromal cells are the major cellular components in tumor microenvironment that actively migrate to tumor sites by sensing "signals" released from tumor cells. Together with other stromal cells, they form the soil for malignant cell progression. In the crosstalk between tumor cells and its surrounded microenvironment, exosomes exert multiple functions in shaping tumor immune responses. In tumor cells, their exosomes can lead to pro-tumor immune responses, whereas in immune cells, their derived exosomes can operate on tumor cells and regulate their ability to growth, metastasis, even reaction to chemotherapy. Employing exosomes as vehicles for the delivery products to initiate anti-tumor immune responses has striking therapeutic effects on tumor progression. Thus, exosomes are potential therapeutic targets in tumor-related clinical conditions. Here we discuss the role of exosomes in regulating tumor immune microenvironment and future indications for the clinical application of exosomes.

Keywords Exosome · Innate immune responses · Adaptive immune responses · Tumor microenvironment · Mesenchymal stem/stromal cells

Tumor immune microenvironment is one of the hallmarks of tumor growth, progression and therapeutics, always characterized as tumor-promoting inflammation and invalid immune surveillance for tumor cells [1]. The potential link between inflammation and tumors was first discovered by Rudolf Virchow in nineteenth century, when he observed the presence of leukocytes in tumors. In last decades, detailed analysis on the immune cells in neoplastic lesion clearly demonstrated that distinct cell types of the immune system, including T lymphocytes, B lymphocytes,

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K. L. Mettinger et al. (eds.), *Exosomes, Stem Cells and MicroRNA*, Advances in Experimental Medicine and Biology 1056, https://doi.org/10.1007/978-3-319-74470-4_7

macrophages, myeloid derived suppressor cells, mast cells, dendritic cells and neutrophils, were involved in tumorigenesis and progression [2, 3].

These distinct types of immune cells control tumor progression by functioning pro-tumor immunity or anti-tumor immune responses [4, 5]. For example, by employing mice genetically engineered to be deficient for certain subtype of immune cells or blocking their infiltration in tumor, $CD8^+$ T cells, nature killer cells, $CD4^+$ helper T (Th) cells were found to contribute significantly for immune surveillance. Avoiding immune destruction by these immune cells promoted tumor progression. Also, macrophages, myeloid derived suppressor cells, and neutrophils were found to be indispensable in constructing the pro-tumor immune microenvironments and dictating tumorigenesis and progression. Consistent infiltrations of these cells tightly relate to wound healing and chronic inflammation. Indeed, chronic inflammation, such as obesity induced inflammation, environmental exposure associated inflammation, damaged cells and senescence cells-induced inflammation can build up the pro-tumor inflammatory environment and enhance the risk for tumor. Meanwhile, pro-tumor inflammatory environment can be induced by malignancy cells. Investigations have demonstrated that some oncogenes mutation in stromal cells, like *myc*, *ras* and *p53* family member, can help to construct tumor immune microenvironments through recruitment of immune cells, production of various cytokines and chemokines, as well as inhibition of anti-tumor immune responses. Tumor promoting inflammation and anti-tumor immunity coexist during tumorigenesis and progression, while their balance in tumor was controlled by microenvironmental conditions. Likewise, the same type of immune cells can exert anti-tumor immunity in one tumor and tumor-promoting inflammation in another, relying on their cytokine profiles and functions in shaping tumor progression.

The formation of tumor immune microenvironment is shaped by the communication of diverse immune cells, more importantly, controlled by tumor cells and their surrounding stroma cells, including mesenchymal stem/stromal cells (MSCs), endothelial cells, and fibroblasts [2]. These tumor cells and tumor stromal cells talk with immune cells by means of direct contact or cytokine and chemokine production in an autocrine and paracrine manner.

Besides cytokines and chemokines, exosomes released by tumor cells and tumor stromal cells is found to be pivotal in shaping tumor immune microenvironment (Fig. 7.1) [6–9]. Exosomes, as one of the most imperative extracellular vesicles and microvesicles, generate inside multivesicular bodies, or can be formed and released by budding from plasma membrane [10]. Exosome contains plenty of DNA, mRNAs, miRNAs, as well as enzymes that are known to exert an assortment of functions to shape tumor immune microenvironment and control tumor progression. The measurement of exosomes in body fluid can indicate the risk of tumorigenesis and the prognosis of the established tumors or predict the therapeutic effect in various kinds of cancers, including gastric cancer, lung cancer and prostate cancer [11–13]. Notably, exosome-derived from tumor immune cells can act on tumor cells for their growth and metastasis. In this chapter, we will decipher the role of exosomes in mediating the crosstalk between tumor cells and tumor immune microenvironment.

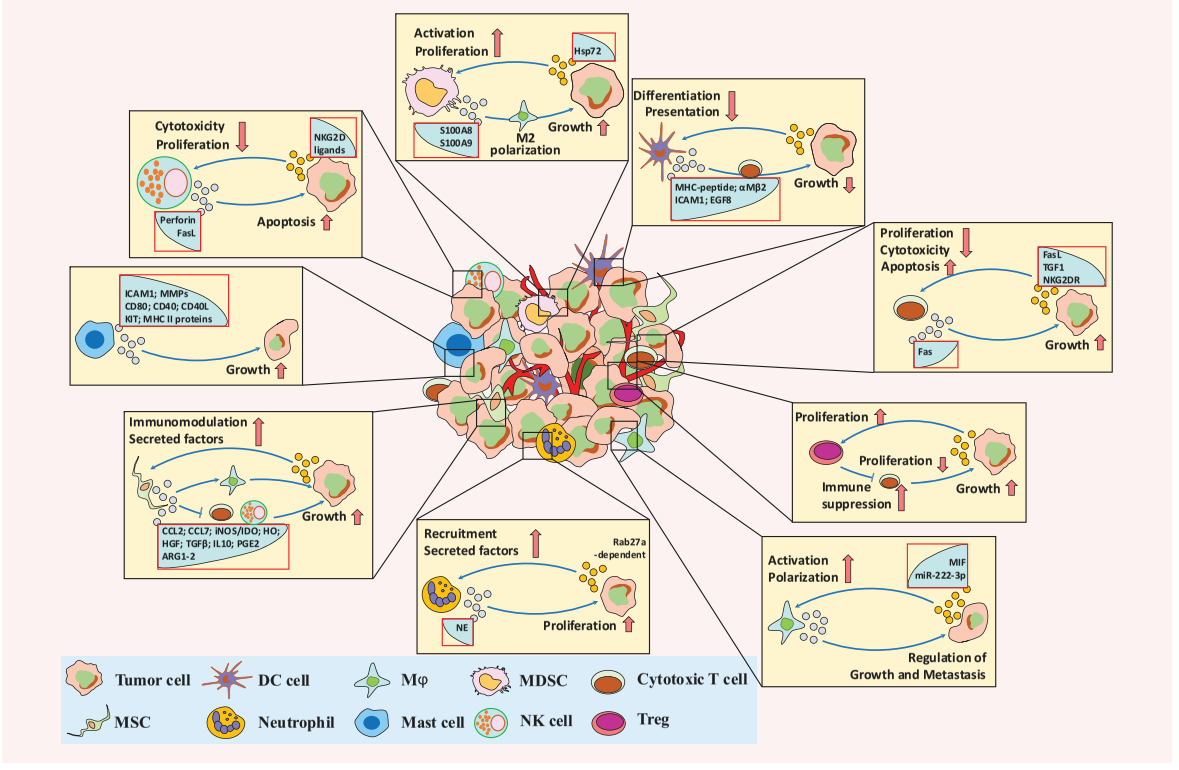

Fig. 7.1 Exosomes function in tumor immune microenvironment. In the tumor microenvironment, exosomes are released by various cell types, including tumor cells, stromal cells and immune cells. Tumor-derived exosomes participate in the recruitment, activation, differentiation, and proliferation of multiple immune cells and stromal cells. Meanwhile, exosomes produced by immune cells and tumor stromal cells regulate tumor progression and metastasis through modulating tumor microenvironment. Exosomes derived from NK cells and DCs are shown to promote tumor cell apoptosis directly or through inducing cytotoxic T cells, however, exosomes from mast cells, neutrophils, macrophages, MDSCs and T cells are shown to exert pro-tumor effects. MSCs serve as an essential member in tumor microenvironment, could also produce exosomes and promote tumor growth through blocking cytotoxicity responses of T cells and NK cells and inducing macrophages recruitment

7.1 Tumor Cell-Derived Exosomes Shape Innate Immune Responses in Tumor Microenvironment

7.1.1 Nature Killer Cells (NK cells)

NK cells are well-known in exerting immune surveillance and anti-tumor immunity by directly killing tumor cells or producing cytotoxic cytokines [14]. Those cytotoxic cytokines, such as perforin, were identified in NK cell-derived exosomes, and exerted cytotoxic activity against tumor cells [15, 16]. The dysfunction of NK cells in tumorigenesis and progression can be trained by tumor derived-exosomes. Exosomes produced by human prostate cancer cells contain ligands for natural killer group 2D (NKG2D). These exosomes downregulated the NKG2D expression on NK cells and impaired the cytotoxicity of NK cells [17, 18]. Detailed analysis on the ligands for NKG2D in tumor derived-exosomes found that MICA, MICB and ULBP1/2 are the major components in suppressing the cytotoxicity of NK cells [19]. Additionally, tumor derived-exosomes can inhibit the cytotoxicity of NK cells through suppressing perforin production [20]. However, exosome derived from tumor cells does not always destroy the cytotoxicity of NK cells. In cancer treatment, exosomes produced by hepatocellular carcinoma cells were found to contain plenty of heat shock proteins (HSPs). Consistent with studies revealing the inhibitory effects of tumor derived exosomes on NK cells, these HSP-bearing exosomes upregulated the expression of inhibitory receptor CD94, and decreased the expression of activating receptors CD69, NKG2D and NKp44. However, those exosome derived from tumor cells with chemotherapy treatment efficiently stimulated the production of granzyme B by NK cells, hence promoted the tumoradical function of NK cells [21]. Therefore, the effects of tumor derived exosomes on NK cells are still controversial and remain to be further investigated.

7.1.2 Dendritic Cells (DCs)

DCs are critical for antigen presentation and activation of adaptive anti-tumor immune response, as well as for cytokine production and immunosuppression in tumor progression [22]. DCs can process tumor antigen and present them by bound to MHC molecules on cell surface to CD4 and CD8 T cells, leading to the activation of T cells. Cytotoxic T cell activation can also be induced by exosomes released by mature DCs which harbored MHC-peptide complexes, thereby inhibiting tumor growth and eliminating established tumors [23–25]. Additionally, other membrane and immune-associated molecules were found in exosomes derived from DCs, including integrin α and β-chains (αMβ2), Ig family member ICAM-1and milk fat globule EGF factor 8, which are involved in the recruitment and activation of immune cells in the tumor microenvironment. More importantly, those membrane

associated molecules are responsible for the endocytosis of exosomes into target cells so that components in the exosomes could exert their effects efficiently [25–27]. The status of DCs in tumor microenvironment can be influenced by tumor cell-derived exosomes. In vitro experiments suggested that exosome products by TS/A mammary tumor cells could inhibit the process of DC differentiation from myeloid cells [28]. These exosomes were found to target on $CD11b^+$ myeloid precursors and induce interleukin (IL) -6 productions and its downstream signaling-STAT3, resulting in the blockade of DC differentiation. Additionally, tumor derived exosomes were link to impair the function of cytotoxic T cells through downregulating the expressions of CD11c and costimulatory receptors. Taken together, a strong relationship between tumor cells and DCs was functioned by exosomes.

7.1.3 Macrophages

Macrophages are the major cellular components of tumor immune arena. By classifying as type 1 macrophages (M1), they act anti-tumor immune responses by functioning as antigen presenting cells and producing type 1 IFN, IL-12, and nitric oxide. In contrast, type 2 macrophages (M2) are the common phenotype of tumor associated macrophages and form pro-tumor immunity. These cells always characterize with downregulated expression of MHC class II and IL-12, enhanced production of anti-inflammatory cytokines, such as IL-10, arginase, transforming growth factor β (TGFβ), as well as plenty of growth factors, and angiogenic factors [2]. The status of M2 was associated with the tumor progression and poor patient prognosis. In comparison of exosomes released by M1 and M2, study found that exosomes secreted from M1, but not M2, could enhance activity of lipid calcium phosphage nanoparticle-encapsulated Trp2 vaccine by enhancing antigen-specific cytotoxic T cell responses [29]. However, exosomes derived from M2 promoted breast cancer cell growth and invasion by transferring miR-223 [30].

The phenotype of macrophages can be fine-tuned by a wide range of stimuli and their production of mediators is specifically regulated by the signals received. In this process, exosomes released by tumor cells were verified as one of stimuli to regulate the status of macrophages. By isolating exosomes from epithelial ovarian cancer, microRNA-222-3p (miR-222-3p) was enriched and found to modulate the polarization of tumor macrophages to type 2 macrophages by targeting SOCS3 signaling pathway [31]. Moreover, studies found that exosomes derived from pancreatic ductal adenocarcinomas (PDACs) can stimulate kupffer cells and induce plenty of TGFβ production, subsequently, to form liver pre-metastatic niche by induction of fibronectin deposition and macrophage recruitment. Macrophage migration inhibitory factor (MIF) was assessed as the key component in these PDAC-derived exosomes in mediating the formation of liver inflammation and pre-metastatic niche [32].

7.1.4 Neutrophils

Neutrophils are one of the key participants in innate immune system and play both tumor-promoting and tumoricidal functions through productions of cytokines, proteases, and reactive oxygen species (ROS), as well as direct cytotoxicity and regulation of $CD8^+$ cytotoxic T cells (CTLs) responses respectively [2]. Some cytokines and mediators were found to be loaded by exosomes released by neutrophils, and promote tumor development. Neutrophil elastase (NE) was discovered in exosomes released by neutrophils and promoted the proliferation of epithelial lung cancer cells through the hydrolysis of insulin receptor substrate 1 (IRS-1) [33]. In turn, the status of neutrophils can be regulated and polarized by the stimuli in the tumor microenvironment, consequently, to shape tumor immune responses and modulate tumor progression. During the investigations on the key role of Rab27a in exosome production by breast cancer cells, exosomes were found to facilitate tumor progression by inducing systemic mobilization of neutrophils [34]. Thus, neutrophil related exosomes exert dual roles in tumor immune responses.

7.1.5 Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs have been identified in tumor microenvironment as a population of immature myeloid cells with the ability to suppress T cell activation. In molding tumor immune microenvironments, exosomes released by MDSCs, containing S100A8 and S100A9, were shown to mediate the chemotaxis of granulocytes and induce the switch of macrophages toward a type 2 macrophage phenotype [35]. Notably, MDSC accumulation in tumor microenvironment can be induced by tumor cell-derived exosomes. During this process, Hsp72 enriched in tumor-derived exosomes was demonstrated to trigger STAT3 activation in MDSCs in a TLR2/MyD88-depedent manner through autocrine production of IL-6 [36].

7.1.6 Mast Cells

The recognition on mast cell function does not limit to their responsibility for allergic reactions and removal of pathogens. The accumulation of mast cells in tumor sites accounts for the construction of tumor immune microenvironment. It has been reported that mediators released by mast cells can promote tumor growth and angiogenesis, such as matrix-degrading enzymes (MMPs), vascular endothelial growth factor (VEGF), proteases (chymase), and inhibit tumor progression, such as inflammatory cytokines [37]. In 2001, the observation that mast cells could produce exosomes was firstly reported [38]. Exosomes delivered the regulatory signals released by mast cells to T and B cells, DCs, even tumor cells. A recent study demonstrated

that mast cell line HMC-derived exosomes transferred KIT protein to lung adenocarcinoma cells, consequently, to promote tumor growth by activating SCF signaling [39]. Other components carried by exosomes released by mast cells were also related to tumor progression, such as MHC II proteins, co-stimulatory (CD86, CD40, and CD40L), adhesion-related molecules (ICAM-1), as well as matrix metalloproteinase (MMP-2, MMP-9) [40]. However, further investigations should decipher the components of exosomes released by mast cells and their detailed functions in molding tumor microenvironment and dictating tumor progression.

7.2 Exosomes Mediate the Crosstalk Between Tumor Cells and Adaptive Immune Cells

Tumor antigens processed and presented as peptide complexes with MHC class molecule by antigen presenting cells initiate T cell mediated immunity [41]. Based on their functions and cytokine productions, T cells are classified as $CD8^+$ cytotoxic T cells and $CD4^+$ Th cells, which further divide into Th1, Th2, Th17 and regulatory T cells (Tregs) [42]. These T cells enriched in the tumor microenvironment can perform both tumoricidal effects and tumor-promoting effects, relying on their functions in lysis of tumor cells and production of cytotoxic cytokines, or in construction of immunosuppressive microenvironments [2]. Accumulating evidence showed that exosome is one of major mediators for intercellular communications among adaptive immune cells, tumor cells. Exosomes can deliver many biological molecules, including proteins, lipids and nucleic acids, to modulate the function of T cell subsets.

7.2.1 Effector T Cells

Cytotoxic T cells and Th1 cells are the major warriors in T cell-mediated immune surveillance and anti-tumor immune responses. In vivo experiments demonstrated that genetically deficiency in T cells or blockade of their cytotoxic molecules can promote tumorigenesis and progression. Indeed, their high expression in tumor-bearing host was correlated with the better survival of some cancers, such as colon cancer. Insufficient T cell-mediated anti-tumor immunity always accompanies in the tumorigenesis, progression and therapeutics. The reasons for impairing T cell function can be related to tumor derived exosomes. In vitro studies showed that exosomes released by tumor cells can suppress antigen-specific $CD8^+$ T cells through inducing their apoptosis [43, 44]. Studies found that the apoptosis of T cells in tumor microenvironment can be related to the high expression of FasL on the surface of exosome derived from tumor cells [45, 46]. Additionally, tumor derived exosomes could promote T cell apoptosis through regulation of PI3K/Akt signaling

pathway [47], both intrinsic and extrinsic pathways in induction of apoptosis [48], as well as STAT activity [8]. Also, two functional receptors for T cell activation, T cell receptor and IL-2 receptor, can be negatively modulated by tumor derived exosomes, leading to the inhibition of T cell proliferation [6, 49]. By assessing the influence of tumor-derived exosomes in lymphocyte responses, membrane-associated TGFβ1 in these exosomes was found to exert the inhibition on T cell activation, as well as the promotion on the suppressive function of Tregs [50].

7.2.2 Tregs

Tregs play a tumor-promoting effect through inhibition of anti-tumor immune responses and promotion of tumor angiogenesis, while they may exert suppression of pro-tumor inflammation under certain condition. The amount of Tregs in tumor-bearing host, especially those with breast [51], gastric [52], and ovarian cancer [53, 54], is indicative of poor prognosis [55]. Therefore, elimination of the appearance of Tregs in tumor microenvironment holds the promise in enhancement anti-tumor immunity and therapeutic outcomes. Distinct from effector T cells, Tregs are resistant to apoptosis induced by tumor-derived exosomes. A close relationship between tumor-derived exosomes and Treg induction was disclosed. TGF-β and IL-10 in exosomes mediated the conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+Foxp3^+$ Tregs [56]. Those Tregs were with increased expressions of FasL, IL-10, TGF-β1, CTLA-4 [57, 58]. Similarly, after co-culture with tumor-derived exosomes, $CD4^+CD39^+$ Tregs showed higher levels of cyclooxygenase-2 (COX-2) and IL-10 [6]. These Tregs exerted their suppressive functions to limit T cell proliferation [59].

In turn, exosomes can be released by T cells with TCR signaling activation [60]. Theses exosomes led to the invasion of melanoma and lung cancer cell through secretion of MMP9, a critical enzyme in degradation of extracellular matrix component [61]. Also, exosomes released by Tregs is the mediator in construction of pro-tumor immune microenvironment. As demonstrated in B16 melanoma, exosome-derived from Tregs can inhibit T cell proliferation and IFN-γ production, as well as the cytotoxicity of $CD8^+$T cells, resulting in the destruction of anti-tumor immunity [62, 63].

7.3 Towards a Broader Understanding of Exosomes in Tumor Immune Microenvironment

Besides various types of immune cells, other cellular components in tumor microenvironment, including MSCs, fibroblasts, as well as endothelial cells, have active roles in tumor initiation, promotion, progression and metastasis. Among them, MSCs are enriched in tumor sites [64]. By sensing signals released by tumor, MSCs

were found to actively migrate to tumor sites and orchestrated the tumor immune microenvironments, together with immune cells [65]. After arriving at tumor sites, MSCs licensed by inflammatory cytokine, tumor necrosis factor α (TNFα), will change into tumor MSCs. These tumor MSCs can build up the pro-tumor immunity by facilitating the accumulation of monocytes, macrophages, and neutrophils in tumor microenvironments, with the capability to promote tumor growth and metastasis. Interestingly, tumor MSCs can endow normal MSCs with the similar potential in forming pro-tumor immunity [66]. Yet, the function of exosomes in their communication remains unclear. Detail analysis on the ménage-à-trois among tumor cells, MSCs and immune cells during the tumor growth found that tumor cell-derived exosome could educate normal MSCs with a pro-tumor phenotype. In this process, exosomes can be uptaken by MSCs and promote the enriched production of CCR2 ligands (CCL2 and CCL7), which are responsible for macrophage recruitment [67].

Multiple suppressive factors expressed by MSCs are reported to mediate their immunosuppression, including indoleamine 2, 3 dioxygenase (IDO), inducible nitric oxidase synthase (iNOS), hemeoxygenase (HO), arginase 1 and 2, hepatocyte growth factor (HGF), TGF-β, IL10 and prostaglandin E2 (PGE2) [68]. Exosomes isolated from human MSCs was also demonstrated to exert an inhibitory effect on T cell activation and IFN-γ production [69, 70]. Similar to the license function of inflammation on MSC immunosuppression, exosome-derived from MSCs with inflammatory cytokine stimulation contained multiple mediators to suppress the proliferation of T cells, B cells and NK cells, as well as the differentiation of plasma cells and antibody production [71, 72], and to induce Tregs [73]. Other stromal cells, such as cancer-associated fibroblasts and endothelial cells are critical in regulation of tumor growth, angiogenesis and metastasis. Exosomes derived from cancer-associated fibroblasts or endothelial cells can transfer the "signals" to tumor cells and promote tumor progression, yet their roles in building up tumor immune microenvironments need further investigation.

7.4 The Application of Exosomes in Tumor Immunotherapy

Not limited to pro-tumor immunity, exosomes were found to enhance anti-tumor immunity based on the diversity of their cargos, indicating their potentials in tumor treatment [74]. HSP, known to function as an endogenous signal that can increase the immunogenicity of tumors, were found in exosomes and promote the cytotoxicity of T cells and NK cells [75–78]. By employing the carrier function of exosomes, strategies were developed by modifying exosomes with high levels of tumor antigens or certain chemokines. These antigen anchored or chemokine carrying exosomes can efficiently recruit anti-tumor immune cells to the tumor sites and induce tumor-specific cytotoxicity, thereby resulting in more obvious inhibition on tumor growth [79, 80]. The optimized strategies were employed by isolating exosomes from TLR agonist activated DCs. These exosomes can induce robust activation of

tumor specific lymphocytes and promote the recruitment of cytotoxic immune cells (T cells, NK cells, and NK T cells) to the tumor site, leading to the significant suppression on tumor growth [81]. In 2008, a phase I clinical trial showed that administration of DC-derived exosomes and GM-CSF can ameliorate colorectal cancer progression through induction of tumor specific cytotoxicity by T cells [82]. Therefore, exosomes can be modified to express tumor antigens or mediators to enhance anti-tumor immunity. These armed exosomes hold the great promise in tumor treatment.

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