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Title: Immune cell-derived small extracellular vesicles in cancer treatment

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Running Title: Novel cancer immunotherapeutic approaches using immune cell-derived small extracellular vesicles that exert anti-cancer activity

Keywords: Small extracellular vesicles, Immune cells, Cancer-immune cycle, Exosomes, Cancer immunotherapy.

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ABSTRACT

Small extracellular vesicles (sEVs) secreted by most cells carry bioactive macromolecules including proteins, lipids, and nucleic acids for intercellular communication. Given that some immune cell-derived sEVs exhibit anti-cancer properties, these sEVs have received scientific attention for the development of novel anti-cancer immunotherapeutic agents. In this paper, we reviewed the latest advances concerning the biological roles of immune cell-derived sEVs for cancer therapy. sEVs derived from immune cells including dendritic cells (DCs), T cells, natural-killer (NK) cells, and macrophages are good candidates for sEV-based cancer therapy. Besides their role of cancer vaccines, DC-shed sEVs activated cytotoxic lymphocytes and killed tumor cells. sEVs isolated from NK cells and chimeric antigen receptor (CAR) T cells exhibited cytotoxicity against cancer cells. sEVs derived from $CD8^+$ T and $CD4^+$ T cells inhibited cancer-associated cells in tumor microenvironment (TME) and activated B cells, respectively. M1-macrophage-derived sEVs induced M2 to M1 repolarization and also created a pro-inflammatory environment. Hence, these sEVs, *via* mono or combination therapy, could be considered in the treatment of cancer patients in the future. In addition, sEVs derived from cytokine-stimulated immune cells or sEV engineering could improve their anti-tumor potency.

INTRODUCTION

1. Basics; biogenesis, secretion, and uptake of sEVs

Over the past decades, small extracellular vesicles (sEVs) have received considerable scientific attention due to their role in cell-cell communication and their potential in disease treatment. sEVs are in range of ~ 40 to 200 nm in diameter (1, 2) and are produced by almost all types of cells and exist in many biological fluids, such as, blood, urine, breast milk, and saliva (3, 4). Since sEVs exert diverse functions on the basis of their cell origin, it is important to understand their basic mechanism in order to interpret their function and for future application.

sEVs are generated *via* a process involving the infolding of the plasma membrane, followed by the organization of multivesicular bodies (MVBs) which contain intraluminal vesicles (ILVs). Then, MVBs fuse with the plasma membrane and the ILVs are ultimately secreted into the extracellular space as sEVs (2). Several proteins, such as Ras-related protein (Rab) GTPase, Sytenin-1, tumor susceptibility gene 101 (TSG101), apoptosis-linked gene 2-interacting protein X (ALIX), and syndecan-1, are involved in the biogenesis of sEVs (5-7). In the generation process, various cargos including proteins and nucleic acids are assembled into sEVs (8). Since the cargos and their membranes exhibit unique properties of sEVs, the similarity in the components between parent cells and their sEVs is important in the investigation of sEV function.

The secretion of sEVs follows an ordered series of events involving intracellular transport and plasma membrane fusion with MVBs. These processes undergo a specific coordination of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), Rab GTPase, integrins, tetraspanins, and cytoskeletons, which are localized on either MVBs or intracellular side of the plasma membrane. sEV release is governed by extracellular stimuli (9). For example, dendritic cells (DCs) and B cells show reinforced sEV secretion after interaction with T cells or senescence induction (10, 11). Also, mature DCs

secrete more strengthened sEVs as compared to immature DCs (11). Even if sEVs are secreted from the same type of cell, the quantity or components of sEVs differ depending on the cell status. Therefore, understanding the secretion procedure is important to characterize sEVs for biomarker and for harvest of sEVs for therapeutic application.

sEV uptake is the last stage involving transportation of components to recipient cells (5). After secretion, circulating sEVs are recruited into nearby cells in various organs and tissues *via* 4 different pathways, namely, ligand-receptor interaction, cleavage of membrane proteins that bind to target receptors, membrane fusion, and endocytic mechanisms (9). Each route needs respective membrane proteins. For example, there are highly enriched integrins on sEV surface from B cells which enhance sEV fusion with antigens (12). Also, intercellular adhesion molecule 1 (ICAM1) located on mature DC-derived sEV surface specifically interacts with the lymphocyte function-associated antigen 1 (LFA-1) receptor and thus allow sEVs to enter the antigen-presenting cells (APCs) (13). Consequently, in the aspect of specific targeting, further studies about the uptake mechanism and related biomolecules would provide more insight in engineering of sEVs for use as drug vehicles in therapeutics.

2. Promising roles of sEVs in immuno-oncology

sEVs have extensive and unique advantages in diagnosis and application compared to synthetic carriers and cell-therapy. Due to the fact that sEVs are small vesicles which originate from living cells, they possess low immunogenicity, high surface/volume ratio, and unique delivery properties (2, 14, 15). Although there are still some drawbacks, such as, low stability and yield, sEVs, with the help of engineering and isolation technique, are expected to be promising natural carries containing bioactive molecules for clinical applications (16).

In particular, recent reports focus on the use of sEVs for cancer immunotherapeutic applications (16-18), owing to the unique characteristics of tumor microenvironment (TME).

The TME typically comprises of tumor cells, immune cells, and stromal cells, such as pericytes, mesenchymal stromal cells (MSCs), and cancer-associated fibroblasts (CAFs). In TME, immune cells detect and attack tumor cells through antigen-specific and nonspecific mechanisms and simultaneously activate other immune cells by transferring neoantigens (19). On the other hand, the stroma cells promote the TME remodeling, thereby suppressing the immune cell infiltration and enhancing immune surveillance and metastasis (20). Since these dynamic reciprocal communications are conducted *via* secreted molecules as well as cell-cell interaction, understanding the relation between distant cell in TME is crucial for cancer treatment. Given their ability to mimic the function of origin cells and easily penetrate the extracellular matrix (ECM), sEVs have received scientific attention not only as cell-cell communicators but also as therapeutic drugs in the regulation of tumor progression.

Here, we summarized current knowledge regarding immune cell-derived sEVs, which are secreted from several immune cells, including DCs, T cells, natural killer (NK) cells, and macrophages for cancer treatment (Fig 1, Table 1). We also discussed some of the challenges associated with the application of immune cell-derived sEVs and future perspectives in this evolving field.

CONTENTS

DCs play a key role in launching innate and adaptive immunity as an antigen-presenting cell (21). Although the percentage of DCs is substantially low in the TME, it has been discovered that DCs are crucial for the start of acquired immunity in ‘cancer-immunity cycle’, highlighting the importance of modifying DCs function to enhance cancer immunotherapy (22, 23). Traditionally, it is known that DCs uptake and process external antigens through two pathways: either displaying on the cell surface with major histocompatibility complex (MHC) molecules

or completely digesting them. However, recent studies have discovered that absorbed antigen peptide presented by MHCs were also secreted with sEVs which can present antigens to other immune cells. Since the cancer vaccines are considered as cancer cell-derived proteins which can stimulate immune system, resulting in either treatment of existing cancer or prevention of cancer development (24, 25), it is expected that these DC-shed sEVs containing both antigen peptide and MHCs possess potential applicability as cancer vaccine. Indeed, there are several investigations revealing that sEVs from DC present cancer antigen and exhibit high immunogenicity (26-28). Moreover, based on the fact that DC-derived sEVs are more stable and amenable to manipulate than DCs (28), many researches have been explored to utilize DC-shed sEVs for cancer treatment.

In 1998, it was first revealed that MHC and T cell co-stimulatory molecules were contained in DC-secreted sEVs and were able to activate cytotoxic T lymphocytes (CTLs), thereby suppressing murine tumor progression (26). Also, sEVs shed from monocyte-derived DCs reinforced $CD4^+$ T cell survival, through interplay between T cell receptors and human leukocyte antigen (HLA) (29). Therefore, more detailed mechanism of interplay between DC-shed sEVs and immune cells contributing to reinforced immune response has been studied. To activate T cells, DC-shed sEVs directly combine with T cells or they are indirectly transferred to APCs. Especially, B cells are largely involved in DC-secreted sEV-induced T cell activation. For the polarization of T cells to the Th1 type and generation of specific T cell response, DC-shed sEVs depend on B cell activation (30). Furthermore, when DC-sEVs transfer both ICAM-1 and MHC-peptide complexes to B cells, the capability of B cell to activate T cell is enhanced (31). Another research also proved that B cells are indispensable when CTLs are activated by DC-derived sEVs (32). These studies support the importance of B cell involvement in the induction of T cell *via* DC-shed sEVs. Other studies discovered the direct interactions between DC-shed sEVs and T cells. DC-derived sEVs were taken by T cell in the spleen by C-C

chemokine receptor 7-dependant manner and enhanced the cytokine secretion (33). Also, it was revealed that LFA-1, the intermediator on T cell surface, mediated the recruitment of MHC-II-containing sEVs (34).

In addition to the contents of DC-shed sEVs, stimulators of DCs to secrete sEVs, like endotoxin, tumor-associated molecule, or cytokines, are important to enhance antitumor efficiency of sEVs. The lipopolysaccharide-induced mature DCs secrete sEVs with enriched MHC-II and ICAM-1. Consequently, sEVs can potently activate effector T cells in contrast to immature DC-shed sEVs (32). Moreover, it has been revealed that only mature DCs could secrete sEVs which would promote the interchange of MHC complexes between DCs, thereby activating $CD4^+$ T cells (35). Also, breast adenocarcinoma cell-treated DC-derived sEVs provoked more potent T cell response against tumor cells compared to sEVs from non-treated DCs (36). When DCs were stimulated by gastric tumor antigens, they secreted sEVs which stimulated T cell proliferation and improved cytotoxicity (37). Lastly, a recent *in vivo* study utilizing murine autochthonous hepatocellular carcinoma (HCC)-bearing mice model showed that treatment with sEVs derived from α-fetoprotein (AFP)-expressing DCs elicited tumor regression with enhanced anti-tumor activity and attenuated immune-suppression (38).

Similar with T cells, NK cells which recruit DC-derived sEVs exerted anti-tumor function. In melanoma cell, treatment of DC-shed sEVs to NK cell provoked proliferation and cytokine secretion *via* IL-15Rα- and natural killer group 2 member D (NKG2D)-dependent manners (39). Another kind of DC-secreted sEVs with enriched IFN-γ enhanced the efficacy of NK cell response against non-small cell lung cancer (40). Several other studies also proved that DC-derived sEV-induced NK cell activation *via* TNF receptor- (41), TLR4, and TLR1/2 ligand- (42), or HLA-BAT3-dependent manner (43). These findings highlight the potent abilities of DC-shed sEVs to regulate NK cells and offer novel application of DC-secreted sEVs in cancer therapy.

Besides affecting various immune cells, under some cancer conditions, it has been investigated that DC-derived sEVs could recognize and kill cancer cells. sEVs from DCs induced apoptosis in murine melanoma cell through TNF superfamily ligands present on their surface (41). Also, another study revealed that sEVs from hyperthermic CO_2-treated DCs showed tumor regression by elevating apoptosis and inhibiting gastric cancer cell proliferation (44). Lastly, sEVs derived from DCs also exhibited cytotoxicity against L1210 tumor cells (45).

To sum up the effects of DC-derived sEVs, they participate in reinforcing immune response under various tumor conditions. DC-secreted sEVs can directly suppress tumor progression and indirectly promote immune response against tumor. Compared to DCs, DC-shed sEVs have several advantages as therapeutical agents, such as, high stability and reduced risk of *in vivo* replication. However, more detailed researches are needed to provide a more comprehensive understanding in terms of status of DCs and interacting molecules between cancer and DC-secreted sEVs for more precise application.

2. T cell-derived sEVs

T lymphocytes are the most abundant leukocytes and participate in various immune responses, including tumor immune response. According to their phenotype, T cells can be divided into 4 groups, namely, cytotoxic T cells, helper T cells, memory T cells, and regulatory T cells. Each subtype elicits specific surface markers and produces respective effector molecules. According to vigorous studies about the relation between T cells and cancer, the role of each T cell and secreted molecules has been investigated as target of cancer therapies. Especially, the function and application of sEVs derived from T cells are getting attention with the expectation that T cell-derived sEVs would exert anti-tumor effects similar to those of their origin cells. In this review, we explained the diverse functions of sEVs from T cell according

to T cell subtypes, based on the fact that sEVs would reflect the unique function of the producer cell.

In the early stage of research on T cell-derived sEVs, the precise T cell subtype that secreted sEVs was not characterized. It was revealed that the activated human T cells produced sEVs which could stimulate resting $CD3^+$ T cell proliferation (46). Along with IL-2, T cell-shed sEVs increased $CD8^+$ T cell population which would produce cytotoxic cytokine. Therefore, it was expected that T cell-derived sEVs could exhibit anti-tumor effect. However, it was also demonstrated that human esophageal squamous cell-infiltrating T cells secreted sEVs which promoted human esophageal squamous cell carcinoma metastasis and simultaneously inhibited the proliferation of tumor (47). Recently, chimeric antigen receptor (CAR)-containing sEVs from CAR-T cells containing high level of cytotoxic molecules inhibited tumor growth (48). These studies implicate that more detailed investigations are required to disclose the function of T cell-secreted sEVs. Classifying the T cell subtype is insufficient for unveiling the precise function of T cell-shed sEVs. It was revealed that $CD4^+$ T cell-released sEVs could restrict antitumor effect of $CD8^+$ T cell against melanoma cell (49). On the other hand, a recent study found that $CD4^+$ T cell-released sEVs could promote B cell activation, proliferation, and antibody production, thereby enhancing antigen-specific humoral immune responses (50). Similar with the studies of $CD4^+$ T cell-secreted sEVs, the effects of $CD8^+$ T cell-derived sEVs on tumor are not consistent. It is known that CD8+ T cell-derived sEVs contain several cytotoxic molecules (51) and have the ability to either activate bystander T cells (52) or suppress lesional mesenchymal cells (53). However, other studies reported the protumor effect of $CD8^+$ T cell-derived sEVs. Cai Z et al. showed that activated $CD8^+$ T cell-derived sEVs promoted tumor cell invasion metastasis and exhibited little cytotoxic effect on tumor cells (54). Lastly, it was reported that sEVs derived from activated $CD8^+$ T cells inhibited

antitumor effect by decreasing the MHC in DCs and $CD8^+$ T cell activity in melanoma model (55).

The importance of T cell-secreted sEVs in cancer treatment has become apparent. They can function as mediator in cancer-immunity by regulating the activity of both immune and cancer cells. Furthermore, T cell-derived sEVs have strong ability to penetrate TME where T cells can hardly infiltrate. These findings indicate that not only T cells but also their sEVs have potential to be utilized as therapeutic agents in cancer treatment. However, the duplicity of T cell-derived sEVs is a major hurdle to the precise interpretation of sEV function and their application in tumor treatment. Therefore, more detailed molecular mechanism of T cell-shed sEVs and engineering of sEVs to reinforce the natural anti-cancer function would pave the way to improve clinical efficacy.

3. Natural killer cell-derived sEVs

NK cells are cytotoxic innate lymphocytes mediating tumor immune surveillance and clearance. NK cells kill tumor cells by secreting cytotoxic proteins, including granzyme A/B, perforin, and granulysin. In contrast to cytotoxic adaptive lymphocytes, NK cells do not require APC-mediated antigen priming. In addition, different activating and inhibitory receptors are used in NK cells as compared to $CD8^+$ T cells, supporting an increased interest for cancer treatment. However, solid tumors show remarkable challenges to the application of cell therapy because the solid tumor cells are difficult to infiltrate. Besides, dampening NK cell responses in TME is a hurdle. Unlike cells, nano-sized sEVs can easily diffuse and infiltrate tumors. Moreover, it has not been reported that NK cell-derived sEVs are influenced by immunosuppressive TME. Hence, NK cell-derived sEV therapy could overcome the limitations of NK cell therapy.

There is increasing evidence that NK cell-derived sEVs are involved in antitumor activity. These sEVs carry bioactive molecules, such as cytotoxic proteins, cytokines, and microRNAs (miRs), which exhibit tumor-killing and immunomodulatory activities. It has been reported that sEVs derived from both resting and activated NK cells exerted comparable *ex vivo* cytotoxicity against hematopoietic cancer cells and solid tumor cells but not against human peripheral blood mononuclear cell (PBMC). Both sEVs expressed similar expression levels of $CD56^{+}$, NKG2D, FasL, and lytic granules (granzyme A/B and perforins). These sEVs induce tumor cell death by Fas/FasL and lytic granule-mediated apoptosis (56). sEVs isolated from *ex vivo*-expanded NK cells contain granulysin that contribute to caspase-9/12-induced tumor apoptosis (57). sEVs released from NK-92MI cells expressed pro-inflammatory cytokine TNF-alpha, which affected melanoma cell proliferation, survival, and apoptosis (58). Besides proteins, miRs contribute to tumor inhibition. Exosomal miR-186 generated from *ex vivo*-expanded NK cells is cytotoxic to neuroblastoma. In this cancer mouse model, miR-186 prevented tumor growth and TGF beta1-dependent immune escape (59). Moreover, miR-3607-3p suppressed tumor migration and invasion by decreasing IL-26 that induced pancreatic cancer proliferation and metastasis. To improve anti-cancer effects, sEVs were isolated from IL-15-stimulated NK-92MI. These sEVs exhibited higher cytotoxicity against human cancer cell lines without affecting normal human cells (60).

Taken together, it is obvious that NK cell-derived sEVs exert cancer-specific cytotoxicity. Given that sEVs are able to cross the blood-brain barrier (BBB), NK cell-derived sEVs could be utilized in glioblastoma treatment. Moreover, sEVs overcome the limitations of NK cell therapy. Therefore, NK cell-derived sEV-based therapy could be regarded as a major cancer therapy in the future.

4. Macrophage-derived sEVs

Macrophages are mainly classified into two different polarization states, namely, pro-inflammatory M1 type and anti-inflammatory M2 type macrophages. While M2 type tumor-associated macrophages (TAMs) promote angiogenesis, tumor growth, invasion, metastasis, and drug resistance (61), M1 macrophages kill tumor cells by releasing tumor killing molecules, including, reactive oxygen species (ROS), nitric oxide synthase (iNOS), and pro-inflammatory cytokines (62). Thus, M1 macrophage-derived sEVs (M1-sEVs) could be promising sEVs for anti-cancer therapy.

Like M1 macrophages, M1-sEVs enhance anti-tumor immunity by generating pro-inflammatory environment. After exposure of macrophages, T cells, and DCs in M1-sEVs, these cells increase expression levels of pro-inflammatory cytokines. sEVs isolated from INF-gamma-stimulated macrophages can potentiate the antitumor activity of cancer vaccine by providing a pro-inflammatory microenvironment in the lymph node (63). M1-sEV-treated macrophages also enhance caspase-3-mediated tumor apoptosis. The anti-tumor effects of paclitaxel (PTX) were substantially improved when PTX was loaded into M1-sEVs (64). M1-sEVs also suppress gastric cancer (GC) by activating T cells and downregulating PD-L1 expression in cancer cells. miR-16-5p expressed in M1-sEVs mainly contributes to decrease the level of PD-L1 in GC, and increases $CD3^+$ T and INF-$gamma^+$ T cells (65). Moreover, M1-sEVs suppress hepatocellular carcinoma cell (HCC) progression by decreasing NF-kappaB expression in HCC cells.

In TME, M2 tumor-associated macrophages (TAMs) promote tumor growth and suppress antitumor immune responses by releasing angiogenic factors and anti-inflammatory cytokines (66). Thus, repolarization of M2 TAMs to M1 macrophages in TME is critical for cancer therapies. Interestingly, M1-sEVs induce the differentiation of native monocytes or M2 macrophages into activated macrophages (M1). M1-sEV treatment successfully reverts M2 to M1 macrophages in tumor tissues. Intravenous injection of M1-sEVs into tumor-bearing mice

suppressed tumor growth. Enrichment of M1 polarization-inducing miRs including miR-155, miR-125, and miR-21 in M1-sEVs addresses the capability of repolarization of M2 to M1 macrophages (67, 68).

Taken together, M1-sEVs are attractive anti-cancer therapeutics, providing pro inflammatory environment. Moreover, miRs derived from M1-sEVs play key roles in M2 to M1 repolarization and downregulation of PD-L1 in GC cells. It is expected that a variety of miRs derived from M1-sEVs would be identified as anti-cancer active molecules.

DISCUSSION

Recently, immunotherapy was emerged as an innovative cancer treatment to strengthen our immune system. Immunotherapy is classified into cellular immunotherapy, immune checkpoint inhibitors, cytokine therapy, and sEV-based immunotherapy. sEV immunotherapy has various advantages including biocompatibility, ideal bio-active molecules' carrier, BBB penetration, and their small size suitable for infiltration into solid tumors. In particular, sEV therapy could overcome the huddles of cell therapy. For example, immune cells are suppressed in TME and cannot easily infiltrate tumor tissues. On the other hand, sEVs can easily infiltrate tumor tissues without being affected by the TME. Moreover, combinational therapy of sEVs with other immunotherapies or chemotherapies potentiate efficacy. Hence, sEV-based immunotherapy is a very promising therapy.

In this review, we focused on immune cell-derived sEVs for immune cancer therapy. In contrast to cancer cell-derived sEVs, immune cell-derived sEVs exhibited anti-cancer effects. Dendritic cell-derived sEVs were developed as cancer vaccines. CAR-T cell-derived sEVs and NK cell-derived sEVs exerted cytotoxicity against cancer cells. $CD8^+$ T cell-derived sEVs inhibited tumor-associated cells including CAF and MCS. M1-sEVs provided proinflammatory environment along with M2 to M1 repolarization. However, some immune cell-derived sEVs

were not yet studied for application as sEV-based immunotherapy. For instance, reports of sEVs derived from B cells and mast cells are scarce. In case of mast cell-derived sEVs, they are uncertain for anti-cancer therapeutics because mast cells are double-edged sword in cancer therapy. While some components of mast cells induce angiogenesis and cancer shaping, their proinflammatory cytokines suppressed tumor progression (69). Therefore, further research is required on sEVs secreted from B cells, neutrophils, and mast cells to broaden sEV-based therapy.

In spites of many advantages of immune cell-derived sEVs for anti-cancer therapy, off-target effects raise concerns on clinical application. Firstly, excessive amounts of cytotoxic internal contents of sEVs lead to off-target toxicity. Although it has been no report that intrinsic sEVs derived from NK cells, DC cells, T cells and macrophages induce serious off-target toxicity, off-target effect could be observed in high does sEV treatment. Secondly, immune cell-derived sEVs could induce cytokine release syndrome (CRS) because they provide proinflammatory environment. Until now, there is no document reporting that intrinsic immune cell-derived sEVs cause CRS. However, high does may induce CRS. Thirdly, sEV contents and their effects on tissues and immune system were partially understood. In-depth studies are required such as proteomic and miR profiling along with their functions on various tissues and immune system. Collectively, we should carefully check the sEV does to avoid off-target effects for clinical application.

In the pharmaceutical industry, large-scale production and quality control are very important. For industrial application of sEVs, large scale production of immune cell-derived sEVs is required. Some cytokines and feeder cells are helpful to increase the yield of immune-derived sEVs. In case of NK cell-derived sEVs, K562 cells expressing membrane-bound IL-21 dramatically increased the yield of NK-derived sEVs by improving NK cell proliferation (70). Regarding the increase in the yield of $CD8^+$ T cell-derived sEVs, IL-12 could be utilized

for cell culture (52). sEV yield could also be improved by sEV purification method. Tangential flow filtration (TFF) is an emerging technique to concentrate sEVs with permeable membrane filtration and 'tangential flow'. This method is more time-efficient and could be scaled up, with less aggregates, as compared to ultracentrifugation. (71). Next, quality control of sEVs is required. In other words, the level of anti-cancer activity should be maintained during sEV production. Cell culture conditions should be accurately controlled to get the same quality of sEVs.

To increase the efficacy of immune-derived sEVs, sEV engineering or generation of sEVs from cytokine-stimulated cells could be considered. Firstly, CAR-T cell-derived sEVs expressed chimeric antigen receptors on their surface. These receptors enabled sEVs to specifically recognize cancer cells. In addition, co-stimulating domains were harbored in these receptors to evaluate anti-cancer activity (48). Therefore, antibody expressed on sEV surface could be utilized for cancer targeting. Moreover, expression of proper co-stimulators could augment their anti-cancer activity. Secondly, immune-derived sEVs could improve their anti-cancer activity by cytokine stimulation. For example, sEVs derived from IL-15-stimulated NK cells enhanced antitumor potency (60). In addition to NK-sEVs, other immune cell-derived sEVs could enhance anti-cancer effect by generating sEVs derived from appropriate cytokine-treated immune cells.

Recently, there were clinical trials of immune cell-derived sEVs for cancer treatment (Table 2). DC-derived sEVs with tumor antigen were treated as cancer vaccines. Phase I and II clinical trials were launched for melanoma and non-small cell lung cancer, respectively. But these clinical experiences showed limited efficacy, despite of the outstanding results in preclinical studies (40, 72, 73). sEVs derived from other immune cells including T cells, NK cells, and macrophages should be tested for clinical use of cancer immunotherapy.

Taken together, the advantages mentioned above support sEV-based therapy as a promising immunotherapy. First of all, immune cell-derived sEVs contain natural anti-cancer contents. In the future, sEV engineering or producing sEVs secreted from cytokine-stimulated immune cells could enhance antitumor potency. These sEVs could be utilized against various types of tumors especially solid and brain tumors.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FIGURE LEGENDS

Figure 1. Schematic diagram of immune cell-derived sEV function in tumor immune microenvironment.

Table 1. Summary of biomolecules and effects of immune cell-derived sEVs in cancer.

Table 2. Clinical trials of immune cell-derived sEVs for cancer treatment.

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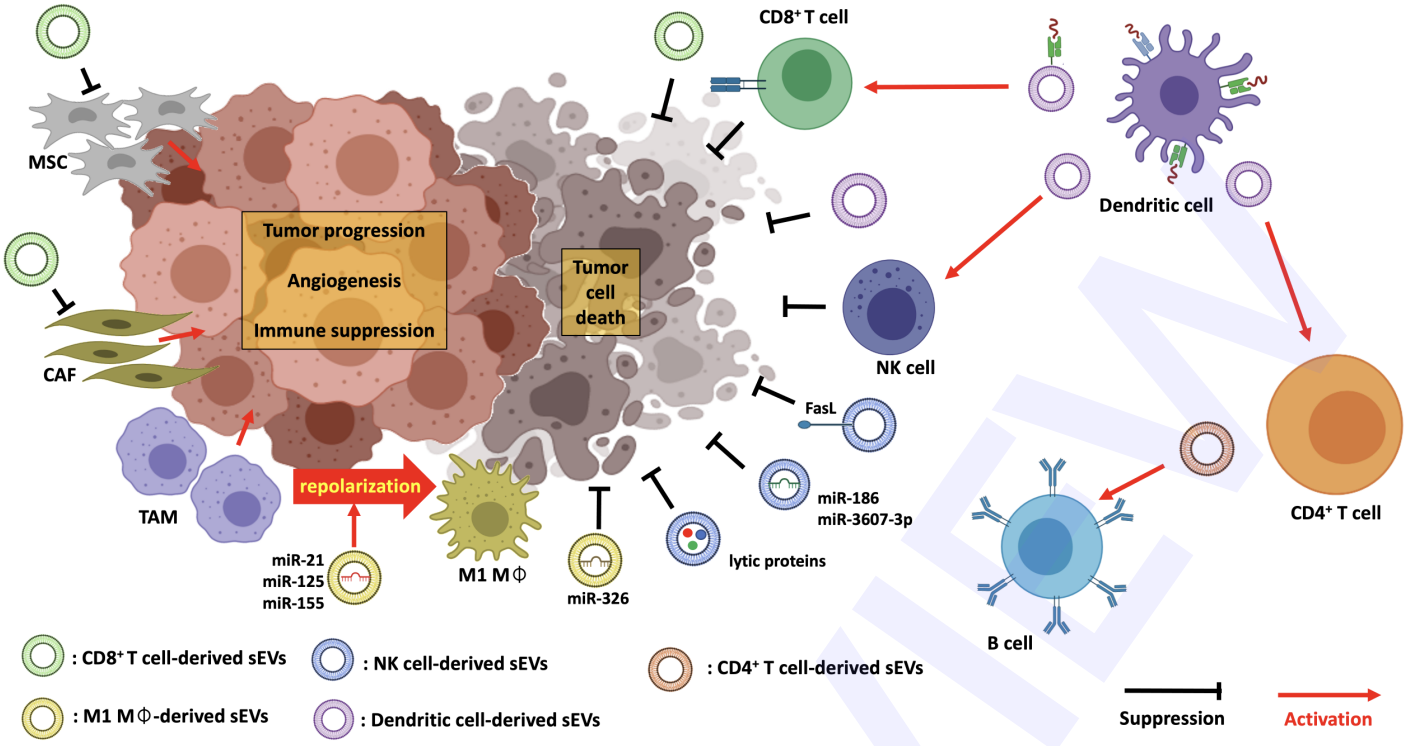

Fig. 1.

Table 1. Summary of biomolecules and effects of immune cell-derived sEVs in tumor

No.	sEV source	Functional molecules	Target cells	Response in the target cell	Ref
1	Dendritic cells	MHC-I and -II and T-cell co-stimulatory molecules	CTLs	Priming the CTL and suppressing growth of murine tumors	26
2	Dendritic cells	HLA-DR and T cell receptors	naïve $CD4^+$ T cells	Reinforcing survival of naïve $CD4^+$ T cells	29
3	Dendritic cells	MHC-peptide complexes, ICAM-1	B cells	Enhancing the ability of B cell to T cell activation	31
4	Dendritic cells	MHC-II and ICAM-1	B cells	Priming effector T cell response	32
5	Dendritic cells	CC chemokine receptor 7	T cells	Enhancing the cytokine secretion	33
6	Dendritic cells	LFA-1	T cells	Unknown	34
7	Dendritic cells	Gastric tumor antigens	T cells	Activating T-cell proliferation and enhancing effective cytotoxicity	35
8	Dendritic cells	IL-15Rα, NKG2D	Natural killer cells	Provoking NK cell proliferation and IFN-γ secretion	39
9	Dendritic cells	IFN-γ	Natural killer cells	Enhancing the efficacy of NKp30-related NK cell response	40
10	Dendritic cells	TNF receptor	Natural killer cells	NK cell activation	41
11	Dendritic cells	TLR4 and TLR1/2 ligand	Natural killer cells	NK cell activation	42
12	Dendritic cells	HLA-BAT3	Natural killer cells	NK cell activation	43
13	Dendritic cells	TNF superfamily ligands	Murine melanoma cells	Inducing caspase activation and apoptosis	41
14	Dendritic cells	Unknown	Gastric cancer cells	Suppressing gastric cancer cell proliferation and promoting apoptosis	44
15	Dendritic cells	Unknown	L1210 tumor cells	Stimulating splenic cell proliferation and enhancing cytotoxic ability	45
16	T cells	Unknown	Resting T cells	Stimulating proliferation in resting $CD3^+$ T cells	46
17	T cells	Unknown	$CD8^+$ T cells	Inducing a higher proportion of $CD8^+$ T cells	46
18	T cells	Unknown	Tumor cells	Inhibiting the proliferation of human esophageal squamous cell carcinoma	47
19	CAR-T cells	CAR proteins and cytotoxic molecules	Tumor	Inhibiting the tumor growth	48
20	$CD4^+$ T cells	Unknown	$CD8^+$ T cells	Inhibiting $CD8^+$ CTL response	49
21	$CD4^+$ T cells	Unknown	B cells	Promoting B cell activation, proliferation and antibody production	50
22	$CD8^+$ T cells	Cytotoxic molecules	Unknown	Unknown	51
23	$CD8^+$ T cells	Unknown	Bystander T cells	Activating bystander T cells	52
24	$CD8^+$ T cells	Unknown	Lesional mesenchymal cells	Attenuating the tumour invasion and metastasis	53
25	$CD8^+$ T cells	Fas	Tumor	Promoting tumor cell invasion and lung metastasis	54
26	$CD8^+$ T cells	Fas L, LFA-1	Dendritic cells and $CD8^+$ T cells	Inhibiting $CD8^+$ CTL response	55
27	Natural killer cells	CD56+, NKG2D, FasL, lytic granules	Tumor	Inducing cytotoxicity	56
28	Natural killer cells	Granulysin	Tumor	Contributing to caspase-9/12-induced tumor apoptosis	57
29	Natural killer cells	Exosomal miR-186	Neuroblastoma	Preventing tumor growth and TGFbeta1-dependent immune escape	59
30	NK-92MI cells	TNF-alpha	Melanoma	Affecting the melanoma cell proliferation, survival, and apoptosis	58
31	NK-92MI cells	Unknown	Human cancer cells	Inducing cytotoxicity	60
32	Macrophages	Unknown	Unknown	Potentiating the antitumor activity of cancer vaccine	63
33	M1 macrophages	PTX	Unknown	Enhancing the macrophage-mediated tumor apoptosis	64
34	M1 macrophages	miR-16-5p	T cells	Activating T cells	65
35	M1 macrophages	miR-16-5p	Gastric cancer cells	Downregulating PD-L1 expression in cancer cell	65
36	M1 macrophages	miR-155, miR-125, and miR-21	M2 macrophages	Repolarizing M2 to M1	67, 68

Table 2. Clinical trials of immune cell-derived sEVs for cancer treatment

No.	sEV source	Study title	Treated cancer type	Status	Ref
1	Dendritic cells (Autologous PBMCs)	Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial	Melanoma	Phase 1 (terminated)	72
2	Dendritic cells (Autologous PBMCs)	A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer	Non-small cell lung carcinoma	Phase 1 (terminated)	40
3	Dendritic cells (Autologous PBMCs)	Dendritic cell-derived exosomes as maintenance immunotherapy after first line chemotherapy in NSCLC	Non-small cell lung carcinoma	Phase 2 (terminated)	73