Emerging role of tumor-derived extracellular vesicles in T cell suppression and dysfunction in the tumor microenvironment

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ABSTRACT

Immunotherapeutic drugs including immune checkpoint blockade antibodies have been approved to treat patients in many types of cancers. However, some patients have little or no reaction to the immunotherapy drugs. The mechanisms underlying resistance to tumor immunotherapy are complicated and involve multiple aspects, including tumor-intrinsic factors, formation of immunosuppressive microenvironment, and alteration of tumor and stromal cell metabolism in the tumor microenvironment. T cell is critical and participates in every aspect of antitumor response, and T cell dysfunction is a severe barrier for effective immunotherapy for cancer. Emerging evidence indicates that extracellular vesicles (EVs) secreted by tumor is one of the major factors that can induce T cell dysfunction. Tumor-derived EVs are widely distributed in serum, tissues, and the tumor microenvironment of patients with cancer, which serve as important communication vehicles for cancer cells. In addition, tumor-derived EVs can carry a variety of immune suppressive signals driving T cell dysfunction for tumor immunity. In this review, we explore the potential mechanisms employed by tumor-derived EVs to control T cell development and effector function within the tumor microenvironment. Especially, we focus on current understanding of how tumor-derived EVs molecularly and metabolically reprogram T cell fates and functions for tumor immunity. In addition, we discuss potential translations of targeting tumor-derived EVs to reconstitute suppressive tumor microenvironment or to develop antigen-based vaccines and drug delivery systems for cancer immunotherapy.

INTRODUCTION

Communications between cancerous and healthy cells play important roles in affecting the progress of oncologic diseases. Cells can communicate with each other via a variety of mechanisms, such as using the soluble factors, directly contacting through adhesion molecules, transferring surface molecules and cytoplasmic components through nanotubules,[1] and the usage of extracellular vesicles (EVs).[2] According to the guidelines from the International Society for Extracellular Vesicles, EVs refer to a variety of cell-released, circular membrane enclosures that meet the minimal criteria in terms of their methods of isolation and characterization.[3] EVs vary in size and have different modes of biogenesis. Among them, exosomes are of endosomal origin with very small size, ranging from 30 to 150 nm in diameter.[4] Both in vitro studies using cancer cell lines and in vivo analyses of plasma, urine, and pleural effusions of patients with cancer have revealed the enhanced release of exosomes by tumor cells,[5 6] and tumor-derived exosomes are involved in many key aspects of cancer biology such as tumorigenesis,[7] drug resistance,[8] and metastasis.[9] It is important to point out that due to the lack of consensus in the scientific community on both the optimal method for the purification of exosomes and the specific markers for distinguishing different types of EVs, the term exosome used in the literature may or may not accurately describe the specific EVs being studied. In fact, the 2018 minimal information for studies of EVs (MISEV) guidelines have suggested to use the term 'EVs' instead of subtype terms.

A major obstacle for immunotherapy is tumor cell escape from immune surveillance, through inhibition of T cell function via either direct contact between tumor cells and T cells or suppressive soluble factors secreted by tumor cells. Tumor cells can use programmed death ligand-1 (PD-L1) and CD80 to directly interact with inhibitory molecules programmed death-1 (PD-1) and cytotoxic T lymphocyte antigen-4 (CTLA-4) expressed in T cells, which leads to an inhibition of T cell activation and function.[10] Increasing studies suggest that accumulation of metabolic products and metabolites such as lactate produced from tumor cells can also suppress the function and survival of T cells within the tumor microenvironment (TME).[11 12]

Emerging evidence indicates that tumor-derived EVs also play a pivotal role in facilitating tumor to escape immune surveillance.[13]

Tumor-derived EVs can modulate functions of different immune components in the TME to achieve immune suppression. For example, they can drive differentiation of monocytes towards myeloid-derived suppressor cells (MDSCs),[14] suppress effector T cell function, generate anergic-like state in natural killer T cells (NKT),[15] promote M2-like macrophage polarization,[16 17] and stimulate regulatory T cell (Treg) expansion.[18] Both MDSCs and Treg cells are well known for their abilities to suppress T cell activation, while polarization of the M2-like macrophage can promote tumor progression.[19] In contrast to immunosuppression, tumor-derived EVs also exhibit immune stimulatory properties under some circumstances. For example, tumor-derived EVs can transfer major histocompatibility complexes (MHCs) to dendritic cells (DCs), which results in the activation of T cells, leading to the inhibition of tumor progression.[20] In addition, the immunostimulatory function of tumor-derived EVs was mainly achieved either when the tumor cells were exposed to stress conditions or the components of the EVs have been modified. For example, heat-stressed tumor cells have been shown to secret EVs that help inducing the production of interleukin (IL)-6 by DCs and macrophages, leading to a switch of Treg to Th17 cells in the TME.[21] Silencing transforming growth factor beta (TGF-β) in leukemia cell-derived EVs promotes maturation and function of DCs and enhances the antigen-specific cytotoxic T lymphocyte responses.[22] Modified tumor-secreted EVs with miR-424 knocked down was also reported to increase T cell–mediated antitumor immune response and enhance the immune checkpoint blockade immunotherapy in colorectal cancer tumor models.[23] Therefore, the interplay between tumor cells and T cells as mediated by tumor-derived EVs is an emerging and exciting area. However, the molecular mechanisms responsible for the effects on T cell function and differentiation mediated by tumor-derived EVs within the TME have not been well summarized. In this review, we explore the different roles that tumor-derived EVs play in affecting T cell function and the underlying molecular mechanisms. We also discuss how immunostimulatory function of EVs can be utilized to boost the immune therapy efficacy and develop novel strategies for cancer immunotherapy.

Biogenesis of EVs and their cargo molecules

There are two major pathways for the biogenesis of EVs: vesicles are formed by outward budding from the plasma membrane, or/and vesicle formation involves the fusion of endosomal-derived multivesicular bodies (MVB) with plasma membrane and the subsequent release of intralumenal vesicle (ILVs) into the extracellular space. The relatively large-sized EVs such as microvesicles are formed by the first pathway, and exosomes are generated by the second pathway.[24] Key players for the biogenesis of EVs include the endosomal sorting complex required for transport (ESCRT) machinery and neutral sphingomyelinase (nSMAse). The ESCRT machinery is responsible for the sorting of ubiquitinated proteins into ILVs.[24] In addition, both the ESCRT machinery and the ceramide generated by the nSMAse also promote vesicle formation both at the plasma membrane and inside MVBs.[25] Besides vesicles formation machinery, members of the Rab family of small GTPases, which play well-established roles in transferring vesicles between intracellular compartments, have also been implicated in regulating the trafficking of MVB to the plasma membrane for exosome release.[26] Among the various Rab family members, Rab-27 is the best characterized. Impairing Rab27a or Rab27b can lead to an altered MVB morphology and docking to the plasma membrane.[26] Furthermore, there is evidence that the SNARE proteins such as SNAP-23 and SYX-5 are specifically required for exosome release.[27 28] Interestingly, exosome biogenesis can be enhanced by a variety of stress conditions such as hypoxia, irradiation, ethanol, or starvation.[29 30] Among these different stressors, hypoxia is of particular interest as it is a hallmark of the TME. Studies have shown that hypoxia can both enhance the production of EVs by tumor cells and alter their components.[31] As important mediators for near and long-distance intercellular communications, EVs contain a variety of cargo molecules that include lipids, proteins, nucleic acids and metabolites. All EVs share a common group of molecules that pertain to their structure and function.[32] EVs derived from different cell types have distinct components, and a certain set of proteins and RNAs are unique to each EV depending on the origins of parent cells (table 1).

The nucleic acids in the cargo of EVs, including DNAs and RNAs, have been implicated in regulating immune responses. Among different subtypes of EVs, exosomes and the effects of their components on immune cells are the most widely studied. DNAs presented in the exosomes derived from breast cancer cell E0771 have been shown to drive the activation of cGAS-STING signaling and antitumor response in mice.[33] DNAs of EVs from the oncogenic genomic DNA sequences transforming protein p21 (HRAS)-driven cancer cells can enhance the ability of circulating neutrophils to activate tissue factor procoagulant activity and IL-8, which are important factors in promoting tumor inflammation and paraneoplastic events.[34] T cells can also use its EV mitochondrial DNA as a mediator to prime DCs and enhance their responses.[35] Interestingly, DNAs present in the tumor-derived exosomes often contain a variety of clinically relevant information about tumor-specific mutations representing multiple genes such as epidermal growth factor receptor (EGFR), proto-oncogene B-Raf, RAS, isocitrate dehydrogenase 1, and human epidermal growth factor receptor 2 (HER2), which can be used in diagnosis as a promising 'liquid biopsy' material for therapy recommendations.[36] Multiple types of RNAs have been identified in EVs, including messenger RNAs (mRNAs), microRNAs (miRNAs), and other non-coding RNAs.[37] Notably, the majority of exosomal RNAs are degraded fragments with the length of less than 200 nucleotides, while some full-length RNA molecules were also found. The tumor exosomal miRNAs can regulate the immune

Table 1 Tumor-derived EVs induce alterations of immune-related molecules in T cells

Tumor types/EV sources	EV cargoes	Targeted T cell types	Changes of cytokines and immune-related molecules in T cells	T cell functional changes	References
Colorectal cancer DLD-1, WiDr cells	TGF-β1	Jurkat cell; CD4+ cells	FoxP3, LAG3, IL-10, PRF1, and GZMB ↑	EVs containing TGF-β1 inhibit T cell growth and promote Treg cell development.	183
Plasma of patients	FasL, TRAIL	Activated CD8+ T cells	NA	FasL and TRAIL in EVs induce T cell apoptosis.	78
Prostate cancer DU145 and PC3	PGE2	CD4+ T cells;	IL-2 and TNF-α ↓ Adenosine ↑	Exosomal PGE2 contributes to CD73 induction in DCs; suppress T cell response and function.	65 73 82
Melanoma MEL624; B16-F10; WM9 cells	PD-L1	CD8+ T cells	IFN-γ, IL-2, and TNF-α ↓	Exosomal PD-L1 inhibit T cell proliferation and cytotoxicity.	74
Glioma UPN933 cells; blood of patients	NA	Jurkat cell; PBMC; T cells	IL-2, IFN-γ, IL-4, IL-5, IL-17, TNF-α, GM-CSF ↓ IL-6, IL-1, G-CSF ↑	Decrease T cell activation and affect cytokine output.	184
Head and neck cancer Tu167 CGM; HN60; SCC0209	Galectin-1	CD8+ T cells	IFN-γ, CD27, CD28 ↓	Loss of CD27 and CD28 expression; suppress T cell proliferation and induce suppressor T cell phenotype.	185
PCI-13	NA	Activated CD4+ and CD8+ T cells; Treg cells	PD-L1, CTLA-4, IL-10, COX2, FOXP3, FasL ↑	Regulate expression of immune function-related genes in T cell subsets.	186
Plasma of patients	NA	CD8+ T cells	NA	CD45− exosomes induce CD39+ Treg differentiation and CD8+ T cell apoptosis.	85
Plasma of patients	CD39, CD73	CD4 + CD39+ Treg cells	5′-AMP and purines ↑	CD3− exosomes promote production of adenosine in Treg cells.	126
Oral squamous cell carcinoma Cal-27; SCC-9	HSP70	γδ T cells	IFN-γ, TNF-α, GZMB, perforin ↑	Induce γδ T cell cytotoxicity, expansion and proliferation.	100
Sera of patient	FasL	Jurkat cells and activated T cells	TCR chain ↓	Exosomal FasL induces T cell apoptosis.	77
Breast cancer MCF7	CD73, CD39	CD4+ T cells	IL-2, TNF-α ↓ Adenosine ↑	Exosomal CD73 and CD39 suppress T cell response.	65
Nasopharyngeal carcinoma TW03; C666; CNE2	miR-24–3 p;	CD4+ and CD8+ T cells	IL-1β, IL-6, IL-10 ↑ IFN-γ, IL-2, IL-17, FGF11 ↓	Exosomal miRNAs inhibit T cell proliferation and Th1 and Th17 differentiation; induce development of FOXP3+ Treg cells.	63 61
Plasma of patients, C15 and C17 cells	CCL20, Galectin-9	CD4+, CD8+, and Treg cells	TNFRSF4, SELL, ICAM1, CCR6, TNF, GZMB, TGFβ1, IL10, IL2, IL15 ↑	Exosomal CCL20 promotes Treg cell recruitment; exosomal galectin-9 induces T cell apoptosis.	81 96
Pancreatic cancer BxPC-3 cell	NA	CD8+ T cells	PERK, ATF4 and P-eIF2α ↑	Induce ER-stress and p38 dependent T cell apoptosis and cytotoxicity.	79
Ovarian cancer Ascites of patient	TGF-β1, IL-10	CD3 + CD4+ T cells	FasL, IL-10, TGF-β1, CTLA-4, GZMB and perforin ↑	Promote proliferation of Treg and convert CD4+ T cells to CD4+CD25+ Treg cells; up-regulate Treg suppressor functions.	93
Skov-3 cell line, ascites of patient	Arginase-1	CD4+ and CD8+ T cells	CD3ζ ↓	Increased arginase in EVs suppresses proliferation and immune response of CD4+ and CD8+ T cells.	64

Continued

Table 1 Continued

Tumor types/EV sources	EV cargoes	Targeted T cell types	Changes of cytokines and immune-related molecules in T cells	T cell functional changes	References
Hepatocellular carcinoma Serum of patients	14-3-3ζ	CD8^{+} T cells	PD-1, TIM-3, LAG3, CTLA-4 ↑	Exosomal 14-3-3ζ induces exhausted phenotype of T cells; impaires the functions, proliferation and activation of T cells.	95
Hepa1-6, H22, H7402 cells and HepG2 cells	SALL4/miR-146a-5p	CD3^{+} T cells	IL-2, IFN-γ, TNF-α ↓ PD-1, TIGIT, CTLA-4 ↑	Exosomal SALL4 and miRNA promote T cell exhaustion and inhibit T cell immune response.	108
Lung cancer Plasma of patients, A549, PC9, and 95D cells	circRNA-002178	CD8^{+} T cells	PD-1 ↑	circRNA in exosomes induces T cell exhaustion.	67

ATF4, activating transcription factor 4; CCR6, C-C motif chemokine receptor 6; circRNA, circular RNA; COX2, cyclooxygenase-2; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DCs, dendritic cells; EVs, extracellular vesicles; FGF11, fibroblast growth factor 11; FOXP3, forkhead box P3; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GZMB, granzyme B; ICAM1, intercellular adhesion molecule 1; IFN-γ, interferon gamma; IL, Interleukin; LAG3, lymphocyte-activation gene 3; miRNA, microRNA; NA, not available; PD-1, programmed death-1; PD-L1, programmed cell death ligand 1; P-eIF2α, phosphorylation of eukaryotic initiation factor-2α; PERK, PKR-like ER kinase; PRF1, perforin 1; SCC, squamous cell carcinoma; SELL, selectin L; TGF-β, transforming growth factor beta; TIM3, T-cell immunoglobulin and mucin-domain containing-3; TNF-α, tumor necrosis factor; TNFRSF, TNF receptor superfamily member 4; Treg, regulatory T cell.

responses by influencing gene expression and signaling pathways in recipient immune cells via the transfer of miRNAs. For example, exosomal miR-222–3p was found to downregulate suppressor of cytokine signaling 3 in monocytes and promote STAT3-mediated M2 polarization of macrophages.[38] Moreover, exosomal miRNAs can promote cancer progression by stimulating the secretion of angiogenic cytokines, including vascular endothelial growth factor, matrix metallopeptidase 2, MMP9, basic fibroblast growth factor and TGF-β.[39] Studies have also shown that tumor exosomal miRNAs, such as adenocarcinoma (PDAC)-specific miR-181–5p, miR-30a-3p, and squamous cell carcinoma (SCC)-specific miR-10b-5p may serve as diagnostic biomarkers for patients with early non-small cell lung cancer (NSCLC).[40] Besides miRNA, exosomal circular RNA-PDE8A was associated with progression and prognosis in patients with pancreatic ductal adenocarcinoma.[41]

Lipids are extremely prevalent in EVs. Exosomal lipids often mirror that of intraluminal vesicles with more cholesterol, sphingolipids, phosphatidylinositol-3-phosphate, and bis(monoacylglycerol)phosphate.[42] These lipids form lipid rafts on the exosomal membrane and mainly sustain the structure of exosomes.[43] There is an asymmetry of lipid types between the outer and inner leaflets of the membrane.[44] Specifically, sphingolipids and phosphatidylcholine are more prevalent on the outer leaflet than on the inner leaflet, while all other lipid classes are mainly on the inner leaflet.[44] It is important to note that high levels of cholesteryl ester, triacylglycerol, and cardiolipin in exosomal preparations may indicate the co-existence of lipid droplets, lipoproteins, or mitochondria within exosomes.[42] Recent studies showed that exosomes are capable of directly transporting lipids such

as cholesterol, fatty acids, and eicosanoids, from parent cells to recipient cells, which may cause the inflammation, immune or metabolic changes in certain microenvironments.[45 46] Adipocytes release lipid-filled exosomes that become a source of lipid for local macrophages and these vesicles were sufficient to induce in vitro differentiation of bone marrow precursors into adipose tissue macrophage-like cells.[45] Healthy donor serum circulating exosomes can take up free fatty acid (FFA) directly from serum via CD36 and then deliver FFA into cardiac cells.[47]

A plethora of membrane proteins have been found in EVs, including tetraspanins, flotillin, PGRL, stomatin, adhesion proteins such as L1CAM, lysosomal associated membrane proteins like LAMP2, integrins, and surface glycoproteins such as fibronectin.[48] The most found proteins are membrane transporters and proteins that mediate membrane fusions such as GTPases, annexins, and flotillin, heat shock proteins like HSP70, tetraspanins such as CD9, CD63, and CD81, multivesicular body biogenesis proteins like Alix and TSG101, and phospholipases.[49] Tumor-derived EVs often contain proteins that are important for the progression of tumors, which include immunosuppressive proteins (such as death receptor ligands FasL or TRAIL), check point receptor ligands PD-L1, and ectoenzymes engaged in the adenosine pathway (CD39 and D73).[50 51] Furthermore, enzymes that control metabolism are among the most frequently identified proteins in proteomics analysis of EVs.[52] The dynamic change of enzymes such as ATPase in tumor exosome appears to help generating an environment that benefits tumor progression.[53] In addition, tumor-derived EVs carry a unique set of tumor-associated antigens (TAAs), co-stimulatory molecules and the MHC components that could stimulate immune cells and

promote antitumor immune responses.[54] Thus, proteins carried in tumor-derived EVs could mediate either an immuno-suppressive response or immuno-stimulatory response depending on the specific contexts.

Many types of recipient cells use EV internalization as a mechanism to receive the cargos carried by the EVs. Once internalized, EVs can fuse their membranes with those of endosomes in the recipient cells, leading to the horizontal transfer of their content into the target cells.[55] The precise mechanisms targeting EV to immune cells and triggering its internalization remain largely unclear, though interactions between EV proteins and membrane receptors in the recipient cells have emerged to play important roles. For example, targeting of EVs to DCs are mediated via the interaction between EV proteins MFG-E8 and the tetraspanins CD9 and CD81 with DC proteins αvβ3 integrin and CD11a/CD54, and blocking the binding sites of CD11a using antibodies can reduce uptake of EVs into DCs.[56] Some studies claimed that since T lymphocytes are not phagocytic as macrophages, they receive information from tumor-derived EVs mainly by the interaction between their surface receptors with components in EVs.[57] One study has shown that although rat pancreatic adenocarcinoma-derived exosomes are taken up by all leukocyte subpopulations, the amount taken up by CD11b^{+} and CD11c^{+} leukocytes greatly exceed that taken up by T cells.[58] Similarly, it has been demonstrated that the ability of CD8^{+} T cells to internalize is not as good as that of other types of immune cells.[59] These studies strongly indicate that exosome internalization may not be a critical way for EVs to affect T cell responses. In support of this notion, a recent study has shown that T lymphocytes and its subsets primarily use specific surface receptors to receive information from tumor-derived EVs.[57] Specifically, dye-labeled tumor-derived exosomes were incubated with human T cells or NKT cell and B cells respectively, and it was found that T lymphocytes, unlike other mononuclear cells, do not uptake exosomes but rather respond to exosome signals delivered to the cell surface by Ca^{2+} influx.[57] It has shown that naïve CD8^{+} T cells can uptake EVs shed from antigen-presenting cells via the interactions between T cell antigen receptor, MHC class I/peptide and the intercellular adhesion molecule 1 (CD54) present on the EVs.[60] Given the important roles of EVs in regulating immune cell functions, development of more advanced and accurate methods to isolate, characterize, and manipulate EVs to elucidate the mechanisms that govern the specificity between EVs and their recipient immune cells is urgently needed for tumor immunity.

Potential mechanisms responsible for tumor-derived EVs-mediated T cell suppression

Tumor-derived EVs can suppress T cell antitumor immunity through multiple ways, including inhibiting T cell proliferation and responses, promoting Treg expansion, and inducing T cell apoptosis and exhaustion (figure 1). The molecular effects of tumor-derived EVs are achieved via different components and/or molecular processes, including delivery of DNA,[34] miRNAs,[61–63] metabolites, and amino acid degrading enzymes to T cells,[50 64 65] or direct binding with immune suppressive protein ligands on T cells[66–68] (table 1). In addition to their direct effects on T cells, tumor-derived EVs can cause T cell suppression by affecting DCs and MDSCs.

Tumor-derived EVs can suppress T cell proliferation. Exosomes derived from breast cancer cell lines have been shown to suppress T cell proliferation, and the effect was mediated via suppressive molecule TGF-β.[69] Interestingly, the production of exosomes by breast cancer cell lines can be greatly enhanced by hypoxic condition, which may contribute to therapy resistance of breast cancer.[70] Furthermore, recent evidence suggests that microRNAs from tumor-derived exosomes can also alter T cell proliferation. In a study examining the serum of patient with nasopharyngeal carcinoma (NPC), five commonly overexpressed miRNAs were found to alter T cell proliferation and differentiation via downregulation of the MAPK1 signaling pathway.[63] In addition, a high miR-24–3p level was found in the exosomes isolated from patient with NPC sera and those miR-24–3p-containing exosomes inhibit T cell proliferation via repressing fibroblast growth factor 11 (FGF11).[61] Cytokines as central important mediators in the homeostasis of lymphoid cells could promote cell survival, proliferation, and differentiation.[71 72] Studies find that mesothelioma cell exosomes strongly impair proliferative responses to IL-2 in CD4^{+} and CD8^{+} T cells in vitro, but not in Treg cells.[73] Besides these, exosomal PD-L1 secreted by mouse melanoma cells inhibits the proliferation and cytotoxicity of CD8^{+} T cells by inhibiting IL-2, interferon gamma (IFN-γ), tumor necrosis factor (TNF-α), and granzyme B in T cells in mice.[74] Additionally, PD-L1 from tumor-derived EVs suppresses T cell activation in the draining lymph node in TRAMP-C2 mice, and removal of PD-L1 from the EVs inhibits tumor growth.[75]

Tumor-derived EVs can also induce T cell apoptosis, which has been reported in EVs isolated from melanoma, colorectal cancer, pancreatic cancer, kidney adenocarcinoma tumor cells, as well as sera from patients with oral squamous cell carcinoma and NPC.[76–81] Generally, these pro-apoptotic EVs are Fas ligand positive, and they trigger apoptosis of activated T cells at least partially in a Fas ligand-dependent manner. A recent RNA sequencing study demonstrated that many of the genes linked to apoptosis and endoplasmic reticulum (ER) stress are upregulated in T cells by EVs derived from pancreatic cancer cells, meanwhile, those EVs taken up by T lymphocytes can activate p38 MAP kinase signaling, and then induce ER stress-mediated apoptosis in T cells.[79] In addition, EVs from NPC patient plasma contain galectin-9, a ligand of the membrane receptor Tim-3, and can induce apoptosis in mature Th1 lymphocytes and CD4^{+} T cells.[81]

Immunosuppressive effects on T cells by tumor-derived EVs can also be indirectly mediated by their effects on dendritic cells. For example, EVs from irradiated prostate
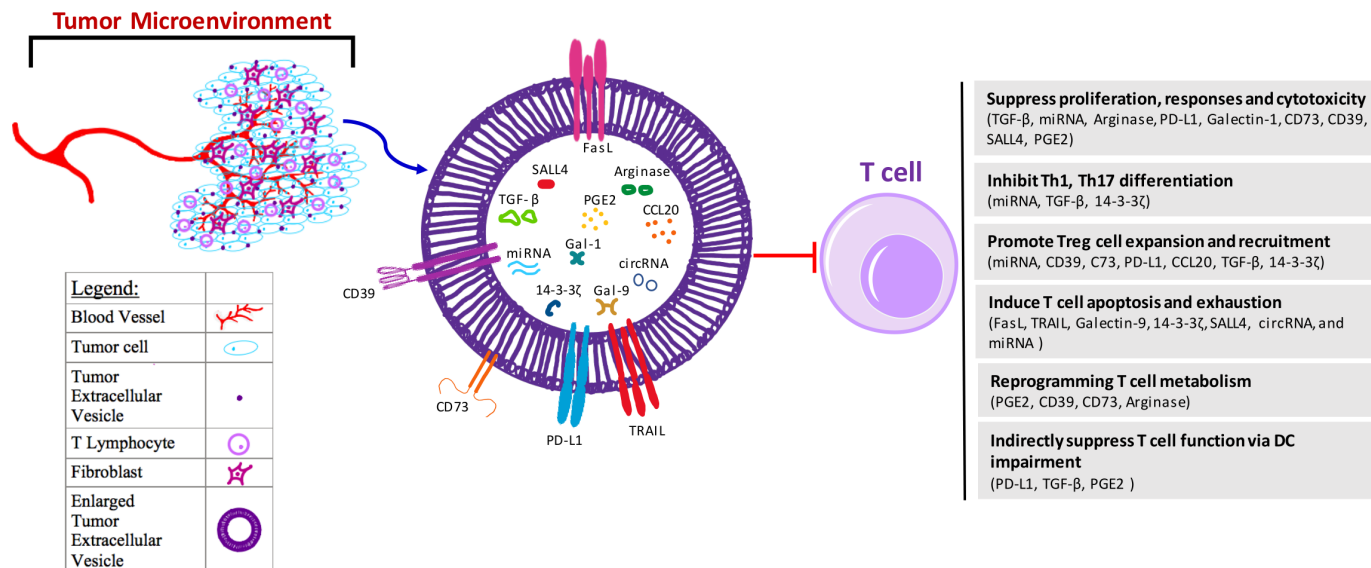
Figure 1 Mechanisms for tumor-derived EV-mediated suppression on T cells. Tumors are surrounded by different types of stromal cells within a microenvironment that the tumor closely interacts with. Within this microenvironment, there are blood vessels, fibroblasts, and T lymphocytes, as well as environmental secreted factors including exosomes derived from the tumor. These EVs further induce dysfunction of T cells in the suppressive tumor microenvironment mainly through several ways, including (1) inhibition of T cell proliferation, effector immune responses and cytotoxicity through EV components TGF-β, miRNA, arginase, PD-L1, Gal-1, CD73, CD39, SALL4 and PGE2; (2) suppression of T helper cell differentiation through EV components miRNA, TGF-β, and 14-3-3ζ; (3) expansion and recruitment of Treg cells via EV cargoes CD39, C73, PD-L1, CCL20, TGF-β, 14-3-3ζ and miRNA; (4) induction of T cell apoptosis and/or exhaustion through FasL, TRAIL, Gal-9, 14-3-3ζ, SALL4, circRNA and miRNA; (5) reprogramming of T cell metabolism through EV cargos PGE2, CD39, CD73 and arginase to increase cellular adenosine and cAMP levels, or decrease amino acid; and (6) indirect suppression of T cell function via impairing DC maturation, migration, and antigen presentation through components PD-L1, TGF-β and PGE2. DC function impairment also contributes to MDSC and Treg cells expansion, which further lead to T cell suppression. CCL20, C-C motif chemokine ligand 20; circRNAs, circular RNA; DC, dendritic cell; EV, extracellular vesicle; FasL, Fas ligand; Gal, galectin; MDSC, myeloid-derived suppressor cells; miRNAs, microRNAs; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; SALL4, spalt like transcription factor 4; TGF-β, transforming growth factor beta; Treg, regulatory T cell.

cancer impair DC antigen presentation, promoting an adenosine-mediated suppression of CD8$^+$ T cell activation.[82] Injection of EVs derived from the Lewis lung carcinoma into mice can block the maturation and migration of DCs to lymph nodes, resulting in immune suppression on T lymphocyte via a PD-L1 dependent mechanism.[83] Similar mechanisms of immunosuppression involving DCs and EVs were also observed in melanoma.[84] EVs produced by melanoma cells and accessory cells of the TME provoke immune suppression and consequently defective DC functions, which contribute to expansion of Treg cells and MDSCs as well as limitation of the cytotoxicity of T cells.[84]

These different function arrests caused by tumor-derived EVs are not mutually exclusive in the TME. In fact, they can happen simultaneously. For example, CD45$^-$ plasma-derived exosomes isolated from head and neck SCC (HNSCC) patients carry inhibitory factors that induce high levels of CD8$^+$ T cell apoptosis in advanced stage patients, producing high amounts of adenosine and promoting stage-dependent Treg differentiation.[85] Likewise, exosomes derived from multiple myeloma similarly mediate T cell suppression through both promoting proliferation of Treg cells and decreasing viability of CD4$^+$ T cells.[86]

T cell suppression mediated by tumor-derived exosomes is clearly linked to the pathogenesis and disease progression of cancers. Recent study has explored the effects of PD-L1$^+$ tumor-derived exosomes isolated from HNSCCs on immune suppression and disease progression. They identified that PD-L1$^+$ exosomes were positively associated with disease progression in patients with HNSCC and can downregulate CD69 expression of effector T cells.[87] Similarly, exosomes derived from highly metastatic murine breast cancer cells have been evaluated for their potential roles in immune suppression and promotion of metastases. Interestingly, these exosomes were taken up by CD45$^+$ bone marrow-derived cells in areas that they accumulated, leading to an accumulation of MDSCs that suppress T cells.[88 89] Additionally, tumor-derived EVs can indirectly induce T cell suppression through increasing the level of extracellular adenosine via binding adenosine A$_{2A}$ receptor in T cells.[65] The tumor-derived exosomes could inhibit NK cell cytotoxicity in addition to directly suppressing T cell proliferation, leading to a dampened anticancer immune response and promotion of cancer metastases.[88]

Tumor-derived EVs promote Treg but demote effector T cell differentiation

Upon activation, naïve T cells can differentiate into effector T helper cells (Th), including T-helper 1 (Th1), T-helper 2 (Th2), and T-helper 17 (Th17).[90] Naive CD4+ T cells can also differentiate into Treg cells.[91] The presence of abnormal quantities of Treg cells in the peripheral blood of patients with cancer is a clear indication of immune suppression.[92] In the circulation of patients with ovarian cancer, the number and activity of CD4+CD25highFoxP3+ Treg cells is higher than that in healthy donors, and a higher level of secreted exosomes was also found in the plasma of these patients.[93] EVs derived from NPC and patients with melanoma could impede the differentiation of immune-active Th1 and Th17 lymphocytes and induce differentiation of immunosuppressive Treg cells.[63 94] This conversion of conventional T cells to CD4+CD25highFoxP3+ Treg cells occurs in a TGF-β1 and IL-10 dependent manner, as the ability of tumor-derived EVs to expand Treg cells is impaired by the neutralizing antibodies specific for TGF-β1 and/or IL-10.[93] Furthermore, 14-3-3ζ, a protein that promotes the proliferation of tumor cells, is highly expressed in hepatocellular carcinoma (HCC) tissue, leading to a decrease in the percentages of effector T cells and an increase of Treg cells mediated through exosome.[95] Likewise, exosomes derived from NPC tumor cells also contain Treg-attracting chemokine C-C motif ligand 20 (CCL20), which helps to recruit conventional CD4+CD25− T cells, mediate their conversion into inhibitory CD4+CD25high Treg cells, and promote Treg cell expansion.[96] In addition to chemokines, the mutant K-RAS gene is involved in the NSCLC exosome-induced switch of naïve CD4+ T cells into a FoxP3+ phenotype in a cytokine-independent manner in a NSCLC xenograft mouse model.[97] Notably, on incubation with ovarian cancer-derived EVs, the conversed Treg showed an increased expression of FasL, IL-10, TGF-β1, CTLA-4, granzyme B and perforin, which mediate more potent suppression of responder cell proliferation.[93]

MicroRNAs present in tumor-derived EVs can act as a barrier for effective tumor immunity through initiating the differentiation of Th cells. For example, exosomes derived from the sera of patients with NPC have a high level of miR-24–3p, which can inhibit differentiation of Th1 and Th17 cells via repression of FGF11.[61] Furthermore, miR-29a-3p and miR-21–5p in exosomes derived from epithelial ovarian-associated macrophages synergistically induce the Treg/Th17 cell imbalance through directly targeting STAT3 signaling in CD4+ T cells in tumor-bearing mice.[62] Interestingly, heat-stress treatment and the production of heat shock proteins in tumor-derived EVs eliminate the imbalance of Treg/Th17 cells.[21] Exosomes derived from heat-stressed colon cancer cells contain high quantities of Hsp70, which strongly stimulates IL-6 secretion from DCs to block Treg cell development but promote differentiation of Th17 cells.[21] Blocking TGF-β1 in MC38 colon carcinoma cell-secreted EVs could significantly increase the proportion of Th1 cells and Th1-mediated responses.[98]

In addition, γδ T cells as a subtype of lymphocyte has been shown to support pancreatic oncogenesis by restraining effector T-cell activation in the TME.[99] EVs from oral SCC cells can stimulate γδ T cell expansion and function in a DC-independent manner, while hypoxic condition will attenuate this equilibrium.[100]

Tumor-derived EVs induce T cell exhaustion

T cell exhaustion is a state of T cell dysfunction with a reduced capacity to secrete cytokines and an increased expression of a panel of inhibitory molecules, including PD1, T cell immunoglobulin and mucin domain-containing protein 3 (TIM3), lymphocyte activation gene 3 protein (LAG3), CTLA-4 and T cell immunoreceptor with Ig and ITIM domains (TIGIT).[101] Exhausted T cell responses have been documented following infections by many types of virus, such as HIV, hepatitis B virus and hepatitis C virus, and have also been observed in patients with malignancies.[102] The exhausted T cells in the TME lose their effector functions to control tumor growth and display a decreased ability to produce cytokines such as IL-2, IFN-γ, and TNF-α.[103 104] Recent research has shown that tumor-infiltrating CD8+ T cells isolated from melanoma-engrafted mice have accumulated dysfunctional mitochondria, which dictate exhaustion in CD8+ T cells.[105] Furthermore, studies also focus on the relationship of lipid metabolism and exhaustion. PD-1hiCD8+ TILs from people with non-small-cell lung carcinoma contain greater lipid content than PD-1loCD8+ TILs, thus suggesting that lipid metabolism may contribute to T cell exhaustion.[106] In addition, cholesterol can induce CD8+ T cell exhaustion through an ER stress-dependent pathway in the TME.[107] Although the phenotypic and functional portraits of T cell exhaustion in the TME are well studied, the role of tumor-derived EVs in inducing T cell exhaustion has not been studied until recently. Studies have shown that miR-146a-5p in HCC-derived exosomes induces T cell exhaustion by activating anti-inflammatory M2-macrophages, while this could be stopped by blocking transcription factor SALL4.[108] In addition, it has been documented that a high expression of 14-3-3ζ in HCC-derived exosomes is correlated significantly well with an exhausted phenotype of T cells.[95] Exosomes from patient serum of lung adenocarcinoma containing circRNA-002178 could enhance PD-L1 expression via sponging miR-34 in cancer cells to induce CD8+ T cell exhaustion in vitro.[67]

Tumor-derived EVs reprogram metabolism in T cells

Naïve T cells with a quiescent state predominantly use oxidative phosphorylation to generate ATP to support immune surveillance.[109] When activated by antigen and professional antigen presenting cells, T cells must undergo rapid metabolic reprogramming and switch to aerobic glycolysis to exit quiescence and support anabolic metabolism required for proliferation and effector

function.[109 110] Specifically, activated T cells increase the expression of nutrient transporters, especially glucose transporters 1 and 3 (GLUT1 and 3),[110] and use glucose to generate ATP even in the presence of sufficient oxygen. Glycolysis provides fast energy and essential intermediates that are required for cell division and effector function.[109] Furthermore, tumor cells also increase the expression level of GLUT1 and uptake more glucose for glycolysis.[111] The enhanced utilization of glycolysis by tumor cells leads to an increased consumption of glucose and an accumulation of immunosuppressive metabolites such as lactic acid.[112] The sustained consumption of glucose by tumor cells eventually leads to a decrease of glucose levels in the TME and creates an environment with low glucose, low amino acids, and low oxygen. Effector T cells exposed to restricted glucose levels have significantly reduced anti-tumor activity.[113] In addition, elevated mechanistic target of rapamycin kinase activity and PD-1 signaling during the development of T cell exhaustion contributed to metabolic alterations including suppressed respiration, reduced glucose uptake, glycolysis and dysregulated mitochondrial energetics.[114] T cells receiving PD-1 signal were unable to engage in glycolysis but displayed an increased fatty acid oxidation (FAO) by increasing carnitine palmitoyl transferase and inducing lipolysis.[115] Notably, CTLA-4 could inhibit glycolysis in T cells without augmenting their FAO.[115] A recent study has also shown that high cholesterol content in the TME promotes CD8$^+$ T-cell exhaustion in tumor-bearing mice.[107] Interestingly, NAD precursor nicotinamide ribose could enhance the anti-tumor responses of T cells within the TME by producing effector cytokines, reducing the accumulation of depolarized mitochondria, and attenuating mitochondrial ROS (mtROS) levels in CD8$^+$ T cells.[105]

Increasing evidence suggests that tumor microenvironmental EVs could potentially rewrite metabolism in T cells in the TME[116] (figure 1). Tumor-derived EVs help creating an immunosuppressive TME by inducing apoptosis and impairing the function of effector T cells and other immune cells.[16 68 74] The effects of the tumor-derived exosomes on impacting the metabolic pathways in the recipient cells contribute significantly to a TME that is suitable for tumor cells to survive. For example, mutant K-RAS colonic cells and hepatic stellate cells release EVs containing GLUT1, which enhances glycolysis in other cells in the TME.[117 118] EVs secreted by breast cancer cell line suppress glucose uptake by lung fibroblasts and astrocytes through the downregulation of the expression level and activity of pyruvate kinase isoenzyme (PKM2) and GLUT1.[119] Low glucose availability in the TME has been shown to decrease the level of glycolytic metabolites such as phosphoenolpyruvate in T cells, weaken effector function, and render CD8$^+$ T cells less capable of preventing tumor growth.[113 120] Furthermore, limiting glucose availability or inhibiting glycolytic enzymes impairs proliferation of effector T cells and their cytokine production.[121 122]

Tumors lead to a microenvironment deficient in many nutrients, which are also required for T cell proliferation and differentiation processes. To offset the deficiency, following their activation, T cells upregulate the uptake of amino acids such as methionine and glutamine as well as enzymes for their metabolism.[123] In addition, in some T cell types, cell differentiation and development has been influenced by amino acid deficiency. An amino acid-deprived microenvironment promotes a shift in CD4$^+$ T cells toward Treg differentiation and impairs effector T cell development.[123 124] Known mechanisms that contribute to amino acid depletion include sequestration of cysteine and the production of arginase-1 (ARG1), an enzyme that speeds up the breakdown of arginine in the urea cycle. Interestingly, EVs isolated from the plasma and ascites of patients with ovarian cancer were reported to have increased arginase activity, and these ARG1$^+$ cancer EVs are able to inhibit the proliferation of CD4$^+$ and CD8$^+$ T cells, presumably by depleting arginine.[64] In addition, the increased arginase content in EVs is correlated with the decreased CD3ζ-chain levels, which is a T cell co-receptor involved in activating both the CD8$^+$ cytotoxic T cells and Th cells.[64]

One important immunosuppressive factor is adenosine, which exerts T cell immune suppressive function by binding to their receptors A_1, A_{2A}, A_{2B}, and A_3.[125] Adenosine is produced by the action of CD39 and CD73, which catalyze the conversion of ATP to AMP and the hydrolysis of AMP to adenosine, respectively. Studies have shown that tumor-derived EVs can impact adenosine production and signaling by several different ways. CD39/CD73 present on the surface of exosomes can generate extracellular adenosine through the degradation of extracellular ATP.[65] Furthermore, CD39/CD73-containing exosomes purified from diverse types of cancers display hydrolytic activity that leads to the production of adenosine from ATP in T cells, thus to inhibit T cell activation.[65 126] Furthermore, prostate cancer exosomal prostaglandin E2 induces CD73 expression in DCs thus suppressing T cell function in an adenosine-dependent manner.[82] Adenosine generated by tumor-derived EVs can directly regulate intracellular signaling in T cells. For instance, adenosine from mesothelioma exosome has been shown to elevate cAMP level in T cells through the adenosine A_{2A} receptor.[65] Interestingly, extracellular adenosine can also promote the development of Treg cells, which in turn produce more extracellular adenosine and thus form a potential positive feedback mechanism that enhances immunosuppression.[127] In addition, hydrolytic activity of exosomes leads to the production of other purine nucleosides, including inosine and hypoxanthine in Treg cells.[57 126] In support of this notion, exosomes derived from patients with HNSCC's plasma have shown to contain purine metabolites, including adenosine, inosine, hypoxanthine and xanthine, and immunosuppressive adenosine being the most prominent purine.[128] Although many progresses have been made in this field, the effects of tumor-derived EVs on T cell metabolism and their importance in anti-tumor immunity are still largely unknown. A thorough understanding of the effects of tumor-derived EVs on T

cell metabolism and function has the potential to provide new insights for improved immunotherapy for cancer.

Tumor-derived EVs and epigenetic reprogramming in T cells

In order to adapt the shifting environment, T cells dynamically modulate their transcriptional programs, which subsequently influence their differentiation and alter their function and metabolic setup.[129] The patterns of heritability of gene expression is mediated by epigenetic mechanisms that involve DNA methylation, histone post-translational modifications and non-coding RNA expression. Abnormal epigenetic patterns correlate with malfunction of effector T cells in tumors.[130 131] The epigenetic programming of T cells could be induced by changes of nutrient levels, metabolic status, and external stimuli like growth hormones and cytokines in T cells.[132] In addition, tumor-derived EVs may also epigenetically reshape the fate of T cells and contribute to T cell dysfunction in antitumor activity.

Histone acetylation is one the most extensively studied post-translational modifications and is regulated by the opposing actions of histone acetyltransferases and histone deacetylases (HDACs).[133] Studies have shown that HDACs are dysregulated in many cancers, making them a therapeutic target for the treatment of cancer.[133] Interestingly, histone deacetylase 3 (HDAC3) is required for T cell maturation,[134 135] and histone deacetylase 1 and 2 (HDAC1/2) also participate in the proper thymic development of T cells.[136] In addition, the HDAC inhibitors vorinostat, romidepsin and panobinostat have been approved for the clinical treatment of cutaneous T cell lymphoma, and belinostat is currently used for treating peripheral T cell lymphoma.[137] Besides acetylation, histone methylation also contributes to T cell dysfunction. Melanoma cells have been reported to disrupt methionine metabolism in CD8+ T cells by expressing high levels of the methionine transporter solute carrier family 43 member 2 (SLC43A2).[138] Enhanced uptake of methionine by melanoma cells led to a lowering intracellular level of methionine and the methyl donor S-adenosylmethionine in T cells, resulting in loss of dimethylation at lysine 79 of histone H3 (H3K79me2) in T cells and an impaired T cell immunity.[138] In recent years, whole-genome methylation profiling has identified a distinct methylome pattern of colorectal cancer-reactive CD8+ T cells, in which tumor-reactive markers CD39 and CD103 are specifically demethylated.[139] Latest studies demonstrated that exhausted CD8+ T cells have exhibited distinct epigenetic landscapes, including chromatin accessibility and DNA methylation patterns, from effector CD8+ T cells.[130 131]

Epigenetic changes play important roles in developing T cell dysfunctional states including exhaustion. Indeed, de novo DNA methylation by the enzyme DNMT3A could promote T cell exhaustion; interestingly, PD-1 blockade treatment can prevent the reprogramming and enhance T cell rejuvenation.[140] In addition, interrupting the mitochondrial fitness of CD8+ T cells was able to

orchestrate epigenetic reprogramming associated with exhaustion.[105] DNA-binding protein TOX and transcription factor NR4a have recently been identified as critical molecules that drive T cell exhaustion by orchestrating exhaustion-linked epigenetic and transcriptomic changes in CD8+ TILs.[141] Notably, epigenetic modification was also involved in cytokine release from T cells. Blockade of leukemia inhibitory factor led to release of the epigenetic silencing of C-X-C motif chemokine ligand 9 (CXCL9), which in turn triggers CD8+ T cell tumor infiltration and enhances tumor immunotherapy.[142]

Studies indicated that many mRNAs and proteins contained in EVs are involved in epigenetic regulation of immune cell functions in the suppressive TME, including DNA methylation, histone modification, and miRNA regulation.[143–145] Plasma exosomes containing dual methylated DNAs of CDKN2A and CDKN2B, or CDKN1B transcript contribute to diffuse large B-cell lymphoma pathogenesis.[146] As to the aspect on other immune cells, study found that irradiation of glioblastoma cell line cells promotes the tet methylcytosine dioxygenase 2-mediated demethylation of exosomal-miR-378a-3p thus inducing the decrease of granzyme B secretion by NK cells.[147] However, molecular mechanisms responsible for tumor EVs-mediated epigenetic regulation on T cells and other immune cells are still under investigation.

Targeting tumor-derived EVs is a novel strategy for tumor immunotherapy and diagnosis

Immune checkpoint blockade therapy has emerged as a promising anticancer strategy for different tumor types. Many anticancer drugs have been developed based on the immune checkpoint molecules.[148] Nevertheless, a majority of patients with cancer fail to respond to current checkpoint blockade immunotherapies. The immune suppressive function of tumor-derived EVs drives researchers to rethink the failure of current immune checkpoint blockade therapies. It has been shown that the presence of PD-L1 in tumor-derived exosomes is positively correlated with tumor activity and progression, and blocking exosome signaling in T cells with anti-PD-1 antibody effectively attenuates immune suppression mediated by the PD-L1+ exosomes.[74 75 87] Recent studies have demonstrated that directly targeting β-catenin could be an effective strategy to enhance anti-PD-1 based therapy in hepatocellular carcinoma.[149] EVs derived from ovarian tumor ascites contain phosphatidylserine (PS) which suppresses T cell activation and the signaling arrest in T cells could be stopped by a blockade of PS.[150] EVs from estrogen receptor-binding fragment-associated antigen 9 (EBAG9)-overexpressing prostate cancer cells can facilitate immune escape of tumors by inhibiting T cell cytotoxicity and regulating immune-related gene expression. This specific immune suppression could also be rescued by EBAG9 neutralizing antibody treatment.[151] In addition, other immune checkpoint molecules Tim-3 and Galectin-9 have been found to be present in large quantity in the exosomes isolated from the plasma of

the patients with non-small cell lung cancer.[152] Interestingly, the exosome-based immune checkpoint blockade strategies have now been explored to develop for cancer therapy. Exosomes engineered to contain signal regulatory protein α (SIRPα) variant can block the interaction between SIRPα and CD47, which limits the ability of macrophages, thus makes macrophages to engulf tumor cells again and promotes an intensive T cell infiltration in vivo.[153] CD47 is abundantly expressed in many tumor cells, and its interaction with SIRPα on the macrophages confers a 'don't eat me' signal to allow the tumor cells to evade the attack by macrophages. Interestingly, CD47 is also present in many EVs, which likewise protects EVs from phagocytosis by monocytes.[154] A new type of exosomes that capsule two distinct types of antibodies could simultaneously activate T cell surface CD3 and cancer cell-associated EGFR thus displaying excellent antitumor activities.[155]

In addition to enhancement of immune checkpoint blockade therapy, tumor-derived EVs could be utilized as a source of cellular antigens for development of novel tumor therapeutic vaccines.[20 156] Many molecules present in tumor-derived EVs can trigger antitumor immunity, and these include TAAs, damage-associated molecular patterns, and MHC-peptide complexes.[157–159] Immunization of mice with those tumor EVs containing TAAs and MHC-peptide complexes can enhance antigen presentation, leading to tumor-specific T cell stimulation and enhanced T cell infiltration.[157 158] It has been suggested that use of EVs as tumor antigens for the development of cancer vaccines is superior to the use of irradiated tumor cells or tumor cell lysates, which can express more concentrated antigens and lead to a better recovery in T cell proportion and is easier to store.[160 161] Furthermore, antigen-loaded exosomes can induce specific T cell responses with a Th1-type shift.[162] Exosomes loaded with immune cell ligand α-galactosylceramide (αGC) and antigen ovalbumin (OVA) can not only induce potent NK and γδ T cell innate immune responses, but also lead to synergistically amplified T cell and B cell responses.[163] In addition, treatment of tumor-bearing mice with αGC/OVA-loaded exosomes decreased tumor growth, increased antigen-specific CD8+ T cell tumor infiltration and prolonged the survival of tumor-bearing mice.[163] In fact, EV-based vaccines have recently been developed for cancer immune therapy. A heterologous human/rat HER2-specific exosome-targeted T cell vaccine has been demonstrated to stimulate CD4+ T cell responses, leading to increased induction of HER2-specific antibody and a remarkable eradication of 90% of HER2-expressed target breast cancer cells.[164] Additionally, a method for increasing the yield of EVs by cyclophosphamide treatment has been reported, which may be used to produce antigens for future design of cancer vaccines.[161] Exosome engineering approaches, such as decorating tumor peptides on the exosome surface and microfluidic technique, have also been utilized to enhance the ability of harvested exosomes in antigen presentation and T cell

activation in future vaccine design.[165 166] One attractive approach is engineering EVs to allow their specific uptake by DCs and induce antigen-specific T cell responses. This can be achieved by modifying the EV glycan surface so it can bind CD209, a DC-specific receptor that mediates antigen uptake and induction of CD4+ and CD8+ T cell activation. This approach has been successfully applied to EVs from glioblastoma and melanoma, which represent an enriched cell-free source of TAA to pulse DCs for efficient antigen delivery and initiation of antitumor immune responses.[167 168]

Tumor-derived EVs also hold great promise in cancer diagnosis, and there are some ongoing clinical trials that explore biomarkers present in these EVs for cancer diagnosis (latest information can be tracked in https://clinicaltrials.gov). EVs derived from tumors that are in pre-metastatic niches often contain pro-metastatic factors, which can be used to determine the metastatic organotropism of tumors. EVs from liquid fluids such as plasma, ascites and urine are potential reservoirs of tumor biomarkers that could be clinically useful, which enable liquid biopsies in cancer diagnosis and prognosis. In recent years, several studies have highlighted different markers contained specifically in EVs derived from different cancer subtypes. For example, EVs from pancreatic cancer contain a very promising clinical prognostic marker, macrophage migration inhibitory factor.[169] EVs from the plasma of patients with breast cancer are enriched with miR-21 and miR-1246, which can be used for the diagnosis of breast cancer or for monitoring the effectiveness of treatment.[170] In addition, ongoing clinical trials are evaluating the diagnosis marker for EVs from plasma of lung cancer (NCT04529915), and tumor-derived EVs for thyroid cancer (NCT04742608), and early prognosis marker for colorectal cancer (NCT04523389). Tumor-derived EVs may also be used to monitor the response of a patient to cancer treatment. The level of PD-L1 in the circulating EVs from patients with melanoma correlated well with the tumor response to checkpoint blockade therapy.[171] A lower level of TGF-β in EVs corresponded to an increased immune activity after chemotherapy.[159] A very recent study found a significant correlation between high levels of urokinase-type plasminogen activator receptor (uPAR) in EVs and poor clinical outcomes of immunotherapy in patients with melanoma.[172] Thus, the level of exosomal uPAR can be potentially used as a biomarker of resistance to checkpoint inhibitor immunotherapy in melanoma.

Conclusions and perspectives

Tumor-derived EVs have emerged as important mediators between malignant tumors and tumor-infiltrating immune components. It is well established that T cell function is dramatically impaired by the suppressive TME in patients with cancer. In addition, a large proportion of patients does not respond to or does not achieve durable responses to the current immunotherapies because of the dysfunction of effector T cells caused by

T cell exhaustion, anergy, and senescence.[173] Increasing evidence suggests that tumor-derived EVs are the critical factors to promote immune suppression and maintain a suppressive TME. They can suppress the function of T lymphocytes via a variety of mechanisms that include regulating T cell metabolism, affecting the differentiation of T cell subsets, and delivering immune suppressive molecular signals from the parent tumor cell to distantly situated T cells (table 1). Tumor-derived EVs have been reported to affect T cell exhaustion, however, whether they also induce other states anergy, senescence, and stemness of T cells remains largely unknown. Improved understanding of these molecular regulations will be critical for the development of effective therapeutic strategies for cancer treatment.

Although great progress has been made in understanding the effects of tumor-derived EVs on immune cells, however, one important concern in the field of tumor-derived EVs is the heterogeneous nature of these vesicles, especially those isolated from the patients. Although new assays for distinguishing cancerous from non-cancerous plasma samples have been reported,[174 175] the method for separating tumor-derived EVs and non-malignant cell-derived EVs from body fluids are not generally available. As a consequence, the total EV fractions isolated from the plasma or body fluid of patients with cancer are often referred as tumor-derived EVs. In reality, these EVs are a mix of both tumor cell-derived and non-malignant cell-derived EVs, which may not recapitulate the content, phenotypic and functional spectrum of tumor EVs in patients with cancer. These may explain the conundrum that the same tumor EVs appear to carry both suppressive and stimulatory cargoes, as these tumor EVs being investigated could be heterogeneous and stemmed from both malignant and non-malignant cells. This notion was supported by a study on EVs from the plasma of patients with melanoma.[175] Using a three-step strategy that involves immune-based capture, researchers were able to separate malignant cell-derived EVs (MTEX) and non-malignant cell-derived EVs (non-MTEX) and found that MTEX has an enrichment in immunosuppressive proteins and a paucity of immunostimulatory proteins. On the other hand, the non-MTEX has a higher ratio of stimulatory/inhibitory proteins, and this ratio varies widely in patients and negatively correlates with disease progressions.

In addition, several other challenging issues are still unknown in the field. First, the effects of individual component present in tumor-derived EVs on immune cells are unclear and worth to be studied in great details. Majority of previous studies have focused on the effects of DNA, miRNAs and immune suppressive proteins present in tumor-derived EVs on T cells, but little is known about the carbohydrates on the surface of EV, such as polysaccharides and oligosaccharides on immune cells.[176] In addition, tumor-derived EVs contain some immune suppressive metabolites, such as purines, lactic acid, fatty acids, and eicosanoids,[11 45 46 128] as well as increased levels of metabolic proteins and enzymes, such as GLUT1, PKM2, aldolase A and aldehyde dehydrogenase 3.[118 177 178] However, the precise roles of these metabolites and molecules from tumor-derived EVs in controlling T cell fate and function are still under investigation. Second, whether and how the tumor-derived EVs metabolically and epigenetically rewrite T cell differentiation and functions in tumor immunity is also not fully understood. Increasing evidence suggests that the metabolic barrier imposed on T cells by the TME is an important factor that triggers T cell suppression.[179] Furthermore, different subsets of T cells may utilize different metabolic regulation for their survival and effector function.[180] Precisely dissecting the metabolic changes in T cells induced by individual component in the tumor-derived EVs will provide critical insights for a better understanding of the failures of current immunotherapies and development of effective tumor treatments.[115] In addition to metabolic regulation in T cells, very limited information is known about how tumor EVs epigenetically regulate the factions in T cells in tumor immunity, which should be put to more efforts in the future studies. Third, novel technologies and combination strategies should be utilized to develop effective EV-based immunotherapies for future tumor treatments. Given the specific characters of EVs, nanomedicine could be as a promising form to efficiently deliver endogenous and exogenous therapeutics.[181] In addition, the applications of exosomes for combination therapy in tumors have been reported in the preclinical models. Specifically, chemotherapy drug doxorubicin and cholesterol-modified miRNA21 inhibitor are co-embedded into the lipid bilayer of healthy mice blood exosomes can be delivered to tumor cell for enhanced tumor therapy.[182] Furthermore, tumor EVs as a reliable source of tumor antigens may constitute a better immunological strategy for the development of novel tumor immunotherapeutic vaccines.

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REFERENCES

1 McCoy-Simandle K, Hanna SJ, Cox D. Exosomes and nanotubes: control of immune cell communication. *Int J Biochem Cell Biol* 2016;71:44–54.
2 Wortzel I, Dror S, Kenific CM, *et al*. Exosome-mediated metastasis: communication from a distance. *Dev Cell* 2019;49:347–60.
3 Théry C, Witwer KW, Aikawa E, *et al*. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for extracellular vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7:1535750.
4 Becker A, Thakur BK, Weiss JM, *et al*. Extracellular vesicles in cancer: cell-to-cell mediators of metastasis. *Cancer Cell* 2016;30:836–48.
5 Ludwig S, Floros T, Theodoraki M-N, *et al*. Suppression of lymphocyte functions by plasma exosomes correlates with disease activity in patients with head and neck cancer. *Clin Cancer Res* 2017;23:4843–54.
6 Andre F, Schartz NEC, Movassagh M, *et al*. Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* 2002;360:295–305.
7 Melo SA, Luecke LB, Kahlert C, *et al*. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature* 2015;523:177–82.
8 Qu L, Ding J, Chen C, *et al*. Exosome-Transmitted lncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. *Cancer Cell* 2016;29:653–68.
9 Hoshino A, Costa-Silva B, Shen T-L, *et al*. Tumour exosome integrins determine organotropic metastasis. *Nature* 2015;527:329–35.
10 Anderson KG, Stromnes IM, Greenberg PD. Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. *Cancer Cell* 2017;31:311–25.
11 Brand A, Singer K, Koehl GE, *et al*. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab* 2016;24:657–71.
12 Ho P-C, Liu P-S. Metabolic communication in tumors: a new layer of immunoregulation for immune evasion. *J Immunother Cancer* 2016;4:4.
13 Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol* 2014;14:195–208.
14 Chalmin F, Ladoire S, Mignot G, *et al*. Membrane-associated HSP72 from tumor-derived exosomes mediates STAT3-dependent immunosuppressive function of mouse and human myeloid-derived suppressor cells. *J Clin Invest* 2010;120:457–71.
15 Deng Z-B, Zhuang X, Ju S, *et al*. Exosome-like nanoparticles from intestinal mucosal cells carry prostaglandin E2 and suppress activation of liver NKT cells. *J Immunol* 2013;190:3579–89.
16 Park JE, Dutta B, Tse SW, *et al*. Hypoxia-induced tumor exosomes promote M2-like macrophage polarization of infiltrating myeloid cells and microRNA-mediated metabolic shift. *Oncogene* 2019;38:5158–73.
17 Wang X, Luo G, Zhang K, *et al*. Hypoxic tumor-derived exosomal miR-301a mediates M2 macrophage polarization via PTEN/PI3Kγ to promote pancreatic cancer metastasis. *Cancer Res* 2018;78:4586–98.
18 Ye L, Zhang Q, Cheng Y, *et al*. Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1+ regulatory B cell expansion. *J Immunother Cancer* 2018;6:145.
19 Baig MS, Roy A, Rajpoot S, *et al*. Tumor-derived exosomes in the regulation of macrophage polarization. *Inflamm Res* 2020;69:435–51.
20 Wolfers J, Lozier A, Raposo G, *et al*. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat Med* 2001;7:297–303.
21 Guo D, Chen Y, Wang S, *et al*. Exosomes from heat-stressed tumour cells inhibit tumour growth by converting regulatory T cells to Th17 cells via IL-6. *Immunology* 2018;154:132–43.
22 Huang F, Wan J, Hao S, *et al*. TGF-β1-silenced leukemia cell-derived exosomes target dendritic cells to induce potent anti-leukemic immunity in a mouse model. *Cancer Immunol Immunother* 2017;66:1321–31.
23 Zhao X, Yuan C, Wangmo D, *et al*. Tumor-Secreted extracellular vesicles regulate T-cell costimulation and can be manipulated to induce tumor-specific T-cell responses. *Gastroenterology* 2021;161:560–74.
24 Mathieu M, Martin-Jaular L, Lavieu G, *et al*. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat Cell Biol* 2019;21:9–17.
25 Trajkovic K, Hsu C, Chiantia S, *et al*. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 2008;319:1244–7.
26 Ostrowski M, Carmo NB, Krumeich S, *et al*. Rab27A and Rab27B control different steps of the exosome secretion pathway. *Nat Cell Biol* 2010;12:19–30.
27 Hyenne V, Apaydin A, Rodriguez D, *et al*. RAL-1 controls multivesicular body biogenesis and exosome secretion. *J Cell Biol* 2015;211:27–37.
28 Sedgwick AE, D'Souza-Schorey C. The biology of extracellular microvesicles. *Traffic* 2018;19:319–27.
29 Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. *Cell Mol Life Sci* 2018;75:193–208.
30 Lamichhane TN, Leung CA, Douti LY, *et al*. Ethanol induces enhanced vascularization bioactivity of endothelial cell-derived extracellular vesicles via regulation of microRNAs and long non-coding RNAs. *Sci Rep* 2017;7:13794.
31 King HW, Michael MZ, Gleadle JM. Hypoxic enhancement of exosome release by breast cancer cells. *BMC Cancer* 2012;12:421.
32 Keerthikumar S, Chisanga D, Ariyaratne D, *et al*. ExoCarta: a web-based compendium of exosomal cargo. *J Mol Biol* 2016;428:688–92.
33 Kitai Y, Kawasaki T, Sueyoshi T, *et al*. DNA-containing exosomes derived from cancer cells treated with topotecan activate a STING-dependent pathway and reinforce antitumor immunity. *J Immunol* 2017;198:1649–59.
34 Chennakrishnaiah S, Meehan B, D'Asti E, *et al*. Leukocytes as a reservoir of circulating oncogenic DNA and regulatory targets of tumor-derived extracellular vesicles. *J Thromb Haemost* 2018;16:1800–13.
35 Torralba D, Baixauli F, Villarroya-Beltri C, *et al*. Priming of dendritic cells by DNA-containing extracellular vesicles from activated T cells through antigen-driven contacts. *Nat Commun* 2018;9:2658.
36 Sharma A, Johnson A. Exosome DNA: critical regulator of tumor immunity and a diagnostic biomarker. *J Cell Physiol* 2020;235:1921–32.
37 Sato-Kuwabara Y, Melo SA, Soares FA, *et al*. The fusion of two worlds: non-coding RNAs and extracellular vesicles-diagnostic and therapeutic implications (Review). *Int J Oncol* 2015;46:17–27.
38 Ying X, Wu Q, Wu X, *et al*. Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages. *Oncotarget* 2016;7:43076–87.
39 Zhou Y, Ren H, Dai B, *et al*. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. *J Exp Clin Cancer Res* 2018;37:324.
40 Jin X, Chen Y, Chen H, *et al*. Evaluation of tumor-derived exosomal miRNA as potential diagnostic biomarkers for early-stage non-small cell lung cancer using next-generation sequencing. *Clin Cancer Res* 2017;23:5311–9.
41 Li Z, Yanfang W, Li J, *et al*. Tumor-released exosomal circular RNA PDE8A promotes invasive growth via the miR-338/MACC1/MET pathway in pancreatic cancer. *Cancer Lett* 2018;432:237–50.
42 Skotland T, Hessvik NP, Sandvig K, *et al*. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J Lipid Res* 2019;60:9–18.
43 Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* 2010;73:1907–20.
44 Skotland T, Sandvig K, Llorente A. Lipids in exosomes: current knowledge and the way forward. *Prog Lipid Res* 2017;66:30–41.
45 Flaherty SE, Grijalva A, Xu X, *et al*. A lipase-independent pathway of lipid release and immune modulation by adipocytes. *Science* 2019;363:989–93.
46 Wang W, Zhu N, Yan T, *et al*. The crosstalk: exosomes and lipid metabolism. *Cell Commun Signal* 2020;18:119.
47 Garcia NA, González-King H, Grueso E, *et al*. Circulating exosomes deliver free fatty acids from the bloodstream to cardiac cells: possible role of CD36. *PLoS One* 2019;14:e0217546.
48 Meldolesi J. Exosomes and Ectosomes in intercellular communication. *Curr Biol* 2018;28:R435–44.
49 Mashouri L, Yousefi H, Aref AR, *et al*. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer* 2019;18:75.
50 Schuler PJ, Saze Z, Hong C-S, *et al*. Human CD4+ CD39+ regulatory T cells produce adenosine upon co-expression of surface CD73 or contact with CD73+ exosomes or CD73+ cells. *Clin Exp Immunol* 2014;177:531–43.
51 Whiteside TL. Exosomes and tumor-mediated immune suppression. *J Clin Invest* 2016;126:1216–23.
52 Göran Ronquist K. Extracellular vesicles and energy metabolism. *Clin Chim Acta* 2019;488:116–21.

53 Ronquist KG, Sanchez C, Dubois L, *et al*. Energy-requiring uptake of prostasomes and PC3 cell-derived exosomes into non-malignant and malignant cells. *J Extracell Vesicles* 2016;5:29877.

54 Whiteside TL. The effect of tumor-derived exosomes on immune regulation and cancer immunotherapy. *Future Oncol* 2017;13:2583–92.

55 Chen J, Hu C, Pan P. Extracellular vesicle microRNA transfer in lung diseases. *Front Physiol* 2017;8:1028.

56 Morelli AE, Larregina AT, Shufesky WJ, *et al*. Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood* 2004;104:3257–66.

57 Muller L, Simms P, Hong C-S, *et al*. Human tumor-derived exosomes (TEX) regulate Treg functions via cell surface signaling rather than uptake mechanisms. *Oncoimmunology* 2017;6:e1261243.

58 Zech D, Rana S, Büchler MW, *et al*. Tumor-exosomes and leukocyte activation: an ambivalent crosstalk. *Cell Commun Signal* 2012;10:37.

59 Vignard V, Labbé M, Marec N, *et al*. Micrornas in tumor exosomes drive immune escape in melanoma. *Cancer Immunol Res* 2020;8:255–67.

60 Hwang I, Shen X, Sprent J. Direct stimulation of naive T cells by membrane vesicles from antigen-presenting cells: distinct roles for CD54 and B7 molecules. *Proc Natl Acad Sci U S A* 2003;100:6670–5.

61 Ye S-B, Zhang H, Cai T-T, *et al*. Exosomal miR-24-3p impedes T-cell function by targeting FGF11 and serves as a potential prognostic biomarker for nasopharyngeal carcinoma. *J Pathol* 2016;240:329–40.

62 Zhou J, Li X, Wu X, *et al*. Exosomes released from tumor-associated macrophages transfer miRNAs that induce a Treg/Th17 cell imbalance in epithelial ovarian cancer. *Cancer Immunol Res* 2018;6:1578–92.

63 Ye S-B, Li Z-L, Luo D-H, *et al*. Tumor-derived exosomes promote tumor progression and T-cell dysfunction through the regulation of enriched exosomal microRNAs in human nasopharyngeal carcinoma. *Oncotarget* 2014;5:5439–52.

64 Czystowska-Kuzmicz M, Sosnowska A, Nowis D, *et al*. Small extracellular vesicles containing arginase-1 suppress T-cell responses and promote tumor growth in ovarian carcinoma. *Nat Commun* 2019;10:3000.

65 Clayton A, Al-Taei S, Webber J, *et al*. Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production. *J Immunol* 2011;187:676–83.

66 Daassi D, Mahoney KM, Freeman GJ. The importance of exosomal PDL1 in tumour immune evasion. *Nat Rev Immunol* 2020;20:209–15.

67 Wang J, Zhao X, Wang Y, *et al*. circRNA-002178 act as a ceRNA to promote PDL1/PD1 expression in lung adenocarcinoma. *Cell Death Dis* 2020;11:32.

68 Abusamra AJ, Zhong Z, Zheng X, *et al*. Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis. *Blood Cells Mol Dis* 2005;35:169–73.

69 Rong L, Li R, Li S, *et al*. Immunosuppression of breast cancer cells mediated by transforming growth factor-β in exosomes from cancer cells. *Oncol Lett* 2016;11:500–4.

70 Muz B, de la Puente P, Azab F, *et al*. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia* 2015;3:83–92.

71 Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nat Rev Immunol* 2018;18:648–59.

72 Berraondo P, Sanmamed MF, Ochoa MC, *et al*. Cytokines in clinical cancer immunotherapy. *Br J Cancer* 2019;120:6–15.

73 Clayton A, Mitchell JP, Court J, *et al*. Human tumor-derived exosomes selectively impair lymphocyte responses to interleukin-2. *Cancer Res* 2007;67:7458–66.

74 Chen G, Huang AC, Zhang W, *et al*. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* 2018;560:382–6.

75 Poggio M, Hu T, Pai C-C, *et al*. Suppression of exosomal PD-L1 induces systemic anti-tumor immunity and memory. *Cell* 2019;177:414–27.

76 Andreola G, Rivoltini L, Castelli C, *et al*. Induction of lymphocyte apoptosis by tumor cell secretion of FasL-bearing microvesicles. *J Exp Med* 2002;195:1303–16.

77 Kim JW, Wieckowski E, Taylor DD, *et al*. Fas ligand-positive membranous vesicles isolated from sera of patients with oral cancer induce apoptosis of activated T lymphocytes. *Clin Cancer Res* 2005;11:1010–20.

78 Huber V, Fais S, Iero M, *et al*. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. *Gastroenterology* 2005;128:1796–804.

79 Shen T, Huang Z, Shi C, *et al*. Pancreatic cancer-derived exosomes induce apoptosis of T lymphocytes through the p38 MAPK-mediated endoplasmic reticulum stress. *Faseb J* 2020;34:8442–58.

80 Yang L, Wu X, Wang D, *et al*. Renal carcinoma cell-derived exosomes induce human immortalized line of Jurkat T lymphocyte apoptosis in vitro. *Urol Int* 2013;91:363–9.

81 Klibi J, Niki T, Riedel A, *et al*. Blood diffusion and Th1-suppressive effects of galectin-9-containing exosomes released by Epstein-Barr virus-infected nasopharyngeal carcinoma cells. *Blood* 2009;113:1957–66.

82 Salimu J, Webber J, Gurney M, *et al*. Dominant immunosuppression of dendritic cell function by prostate-cancer-derived exosomes. *J Extracell Vesicles* 2017;6:1368823.

83 Ning Y, Shen K, Wu Q, *et al*. Tumor exosomes block dendritic cells maturation to decrease the T cell immune response. *Immunol Lett* 2018;199:36–43.

84 Tucci M, Mannavola F, Passarelli A, *et al*. Exosomes in melanoma: a role in tumor progression, metastasis and impaired immune system activity. *Oncotarget* 2018;9:20826–37.

85 Beccard IJ, Hofmann L, Schroeder JC, *et al*. Immune suppressive effects of plasma-derived exosome populations in head and neck cancer. *Cancers* 2020;12 doi:10.3390/cancers12071997

86 Shao Q, Deng L, Liu H, *et al*. Involvement of MM cell-derived exosomes in T lymphocytes immune responses. *Oncol Lett* 2020;20:31.

87 Theodoraki M-N, Yerneni SS, Hoffmann TK, *et al*. Clinical Significance of PD-L1⁺ Exosomes in Plasma of Head and Neck Cancer Patients. *Clin Cancer Res* 2018;24:896–905.

88 Wen SW, Sceneay J, Lima LG, *et al*. The biodistribution and immune suppressive effects of breast cancer-derived exosomes. *Cancer Res* 2016;76:6816–27.

89 Chen J, Ye Y, Liu P, *et al*. Suppression of T cells by myeloid-derived suppressor cells in cancer. *Hum Immunol* 2017;78:113–9.

90 Luckheeram RV, Zhou R, Verma AD, *et al*. CD4⁺T cells: differentiation and functions. *Clin Dev Immunol* 2012;2012:925135.

91 Owen DL, Sjaastad LE, Farrar MA. Regulatory T cell development in the thymus. *J Immunol* 2019;203:2031–41.

92 Lau K-M, Cheng SH, Lo KW, *et al*. Increase in circulating Foxp3+CD4+CD25(high) regulatory T cells in nasopharyngeal carcinoma patients. *Br J Cancer* 2007;96:617–22.

93 Szajnik M, Czystowska M, Szczepanski MJ, *et al*. Tumor-derived microvesicles induce, expand and up-regulate biological activities of human regulatory T cells (Treg). *PLoS One* 2010;5:e11469.

94 Wieckowski EU, Visus C, Szajnik M, *et al*. Tumor-derived microvesicles promote regulatory T cell expansion and induce apoptosis in tumor-reactive activated CD8+ T lymphocytes. *J Immunol* 2009;183:3720–30.

95 Wang X, Shen H, Zhangyuan G, *et al*. 14-3-3ζ delivered by hepatocellular carcinoma-derived exosomes impaired anti-tumor function of tumor-infiltrating T lymphocytes. *Cell Death Dis* 2018;9:159.

96 Mrizak D, Martin N, Barjon C, *et al*. Effect of nasopharyngeal carcinoma-derived exosomes on human regulatory T cells. *J Natl Cancer Inst* 2015;107:363.

97 Kalvala A, Wallet P, Yang L, *et al*. Phenotypic switching of naïve T cells to immune-suppressive Treg-Like cells by mutant KRAS. *J Clin Med* 2019;8:1726. doi:10.3390/jcm8101726

98 Rossowska J, Anger N, Wegierek K, *et al*. Antitumor potential of extracellular vesicles released by genetically modified murine colon carcinoma cells with overexpression of interleukin-12 and shRNA for TGF-β1. *Front Immunol* 2019;10:211.

99 Daley D, Zambirinis CP, Seifert L, *et al*. γδ T Cells Support Pancreatic Oncogenesis by Restraining αβ T Cell Activation. *Cell* 2016;166:1485–99.

100 Li L, Cao B, Liang X, *et al*. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral γδ T cell equilibrium via tumor-derived exosomes. *Oncogene* 2019;38:2830–43.

101 McLane LM, Abdel-Hakeem MS, Wherry EJ. Cd8 T cell exhaustion during chronic viral infection and cancer. *Annu Rev Immunol* 2019;37:457–95.

102 JS Y, Cox MA, Zajac AJ. T-Cell exhaustion: characteristics, causes and conversion. *Immunology* 2010;129:474–81.

103 Baitsch L, Baumgaertner P, Devêvre E, *et al*. Exhaustion of tumor-specific CD8+ T cells in metastases from melanoma patients. *J Clin Invest* 2011;121:2350–60.

104 Wherry EJ. T cell exhaustion. *Nat Immunol* 2011;12:492–9.

105 Yu Y-R, Imrichova H, Wang H, *et al*. Disturbed mitochondrial dynamics in CD8⁺ TILs reinforce T cell exhaustion. *Nat Immunol* 2020;21:1540–51.

106 Thommen DS, Koelzer VH, Herzig P, *et al*. A transcriptionally and functionally distinct PD-1+ CD8+ T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade. *Nat Med* 2018;24:994–1004.

107 Ma X, Bi E, Lu Y, *et al*. Cholesterol induces CD8+ T cell exhaustion in the tumor microenvironment. *Cell Metab* 2019;30:143–56.

108 Yin J, Han Q, Xu D, *et al*. SALL4-mediated upregulation of exosomal miR-146a-5p drives T-cell exhaustion by M2 tumor-associated macrophages in HCC. *Oncoimmunology* 2019;8:1601479.

109 Magalhaes I, Yogev O, Mattsson J, *et al*. The metabolic profile of tumor and virally infected cells shapes their microenvironment counteracting T cell immunity. *Front Immunol* 2019;10:2309.

110 Hukelmann JL, Anderson KE, Sinclair LV, *et al*. The cytotoxic T cell proteome and its shaping by the kinase mTOR. *Nat Immunol* 2016;17:104–12.

111 Carvalho KC, Cunha IW, Rocha RM, *et al*. Glut1 expression in malignant tumors and its use as an immunodiagnostic marker. *Clinics* 2011;66:965–72.

112 Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009;324:1029–33.

113 Chang C-H, Qiu J, O'Sullivan D, *et al*. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* 2015;162:1229–41.

114 Bengsch B, Johnson AL, Kurachi M, *et al*. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8 + T cell exhaustion. *Immunity* 2016;45:358–73.

115 Patsoukis N, Bardhan K, Chatterjee P, *et al*. Pd-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nat Commun* 2015;6:6692.

116 Hu C, Chen M, Jiang R, *et al*. Exosome-related tumor microenvironment. *J Cancer* 2018;9:3084–92.

117 Zhang Q, Jeppesen DK, Higginbotham JN, *et al*. Mutant KRAS exosomes Alter the Metabolic State of Recipient Colonic Epithelial Cells. *Cell Mol Gastroenterol Hepatol* 2018;5:627–9.

118 Wan L, Xia T, Du Y, *et al*. Exosomes from activated hepatic stellate cells contain GLUT1 and PKM2: a role for exosomes in metabolic switch of liver nonparenchymal cells. *FASEB J* 2019;33:8530–42.

119 Fong MY, Zhou W, Liu L, *et al*. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nat Cell Biol* 2015;17:183–94.

120 Ho P-C, Bihuniak JD, Macintyre AN, *et al*. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* 2015;162:1217–28.

121 Angiari S, Sutton C, Runtsch MC. Regulation of T cell activation and pathogenicity by dimeric pyruvate kinase M2 (PKM2). *J Immunol* 2019;202.

122 Macintyre AN, Gerriets VA, Nichols AG, *et al*. The glucose transporter GLUT1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* 2014;20:61–72.

123 Klysz D, Tai X, Robert PA, *et al*. Glutamine-dependent α-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal* 2015;8:ra97.

124 Sinclair LV, Howden AJ, Brenes A, *et al*. Antigen receptor control of methionine metabolism in T cells. *Elife* 2019;8:1. doi:10.7554/eLife.44210

125 Burnstock G, Di Virgilio F. Purinergic signalling and cancer. *Purinergic Signal* 2013;9:491–540.

126 Theodoraki M-N, Hoffmann TK, Jackson EK, *et al*. Exosomes in HNSCC plasma as surrogate markers of tumour progression and immune competence. *Clin Exp Immunol* 2018;194:67–78.

127 Ohta A, Sitkovsky M. Extracellular adenosine-mediated modulation of regulatory T cells. *Front Immunol* 2014;5:304.

128 Ludwig N, Gillespie DG, Reichert TE, *et al*. Purine metabolites in tumor-derived exosomes may facilitate immune escape of head and neck squamous cell carcinoma. *Cancers* 2020;12 doi:10.3390/cancers12061602

129 Best JA, Blair DA, Knell J, *et al*. Transcriptional insights into the CD8(+) T cell response to infection and memory T cell formation. *Nat Immunol* 2013;14:404–12.

130 Pauken KE, Sammons MA, Odorizzi PM, *et al*. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. *Science* 2016;354:1160–5.

131 Philip M, Fairchild L, Sun L, *et al*. Chromatin states define tumour-specific T cell dysfunction and reprogramming. *Nature* 2017;545:452–6.

132 Lu C, Thompson CB. Metabolic regulation of epigenetics. *Cell Metab* 2012;16:9–17.

133 Li Y, Seto E. Hdacs and HDAC inhibitors in cancer development and therapy. *Cold Spring Harb Perspect Med* 2016;6:1. doi:10.1101/cshperspect.a026831

134 Hsu F-C, Belmonte PJ, Constans MM, *et al*. Histone deacetylase 3 is required for T cell maturation. *J Immunol* 2015;195:1578–90.

135 Ellmeier W, Seiser C. Histone deacetylase function in CD4⁺ T cells. *Nat Rev Immunol* 2018;18:617–34.

136 Dovey OM, Foster CT, Conte N, *et al*. Histone deacetylase 1 and 2 are essential for normal T-cell development and genomic stability in mice. *Blood* 2013;121:1335–44.

137 Suraweera A, O'Byrne KJ, Richard DJ. Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. *Front Oncol* 2018;8:92.

138 Bian Y, Li W, Kremer DM, *et al*. Cancer SLC43A2 alters T cell methionine metabolism and histone methylation. *Nature* 2020;585:277–82.

139 Yang R, Cheng S, Luo N, *et al*. Distinct epigenetic features of tumor-reactive CD8+ T cells in colorectal cancer patients revealed by genome-wide DNA methylation analysis. *Genome Biol* 2019;21:2.

140 Ghoneim HE, Fan Y, Moustaki A, *et al*. De novo epigenetic programs inhibit PD-1 Blockade-Mediated T cell rejuvenation. *Cell* 2017;170:142–57.

141 Seo H, Chen J, González-Avalos E, *et al*. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8⁺ T cell exhaustion. *Proc Natl Acad Sci U S A* 2019;116:12410–5.

142 Pascual-García M, Bonfill-Teixidor E, Planas-Rigol E, *et al*. LIF regulates CXCL9 in tumor-associated macrophages and prevents CD8⁺ T cell tumor-infiltration impairing anti-PD1 therapy. *Nat Commun* 2019;10:2416.

143 Melnik BC, John SM, Carrera-Bastos P, *et al*. MicroRNA-21-Enriched exosomes as epigenetic regulators in melanomagenesis and melanoma progression: the impact of Western lifestyle factors. *Cancers* 2020;12 doi:10.3390/cancers12082111

144 Zang X, Gu J, Zhang J, *et al*. Exosome-transmitted lncRNA UFC1 promotes non-small-cell lung cancer progression by EZH2-mediated epigenetic silencing of PTEN expression. *Cell Death Dis* 2020;11:215.

145 Sharma A. Bioinformatic analysis revealing association of exosomal mRNAs and proteins in epigenetic inheritance. *J Theor Biol* 2014;357:143–9.

146 Barıs IC, Hacıoglu S, Turk NS, *et al*. Expression and DNA methylation profiles of EZH2-target genes in plasma exosomes and matched primary tumor tissues of the patients with diffuse large B-cell lymphoma. *Clin Transl Oncol* 2021;23:1152–66.

147 Briand J, Garnier D, Nadaradjane A, *et al*. Radiotherapy-induced overexpression of exosomal miRNA-378a-3p in cancer cells limits natural killer cells cytotoxicity. *Epigenomics* 2020;12:397–408.

148 Friedman CF, Proverbs-Singh TA, Postow MA. Treatment of the immune-related adverse effects of immune checkpoint inhibitors: a review. *JAMA Oncol* 2016;2:1346–53.

149 Matsuda A, Ishiguro K, Yan IK, *et al*. Extracellular vesicle-based therapeutic targeting of β-catenin to modulate anticancer immune responses in hepatocellular cancer. *Hepatol Commun* 2019;3:525–41.

150 Kelleher RJ, Balu-Iyer S, Loyall J, *et al*. Extracellular vesicles present in human ovarian tumor microenvironments induce a phosphatidylserine-dependent arrest in the T-cell signaling cascade. *Cancer Immunol Res* 2015;3:1269–78.

151 Miyazaki T, Ikeda K, Sato W, *et al*. Extracellular vesicle-mediated EBAG9 transfer from cancer cells to tumor microenvironment promotes immune escape and tumor progression. *Oncogenesis* 2018;7:7.

152 Gao J, Qiu X, Li X, *et al*. Expression profiles and clinical value of plasma exosomal Tim-3 and galectin-9 in non-small cell lung cancer. *Biochem Biophys Res Commun* 2018;498:409–15.

153 Koh E, Lee EJ, Nam G-H, *et al*. Exosome-SIRPα, a CD47 blockade increases cancer cell phagocytosis. *Biomaterials* 2017;121:121–9.

154 Kamerkar S, LeBleu VS, Sugimoto H, *et al*. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature* 2017;546:498–503.

155 Cheng Q, Shi X, Han M, *et al*. Reprogramming exosomes as nanoscale controllers of cellular immunity. *J Am Chem Soc* 2018;140:16413–7.

156 Mignot G, Roux S, Thery C, *et al*. Prospects for exosomes in immunotherapy of cancer. *J Cell Mol Med* 2006;10:376–88.

157 Dionisi M, De Archangelis C, Battisti F, *et al*. Tumor-derived microvesicles enhance cross-processing ability of clinical grade dendritic cells. *Front Immunol* 2018;9:2481.

158 Lin W, Xu Y, Chen X, *et al*. Radiation-induced small extracellular vesicles as "carriages" promote tumor antigen release and trigger antitumor immunity. *Theranostics* 2020;10:4871–84.

159 Marar C, Starich B, Wirtz D. Extracellular vesicles in immunomodulation and tumor progression. *Nat Immunol* 2021;22:560–70.

160 Gu X, Erb U, Büchler MW, *et al*. Improved vaccine efficacy of tumor exosome compared to tumor lysate loaded dendritic cells in mice. *Int J Cancer* 2015;136:E74–84.

161 Cocozza F, Menay F, Tsacalian R, *et al*. Cyclophosphamide enhances the release of tumor exosomes that elicit a specific immune response in vivo in a murine T-cell lymphoma. *Vaccine* 2019;37:1565–76.

162 Qazi KR, Gehrmann U, Domange Jordö E, *et al*. Antigen-loaded exosomes alone induce Th1-type memory through a B-cell-dependent mechanism. *Blood* 2009;113:2673–83.

163 Gehrmann U, Hiltbrunner S, Georgoudaki A-M, *et al*. Synergistic induction of adaptive antitumor immunity by codelivery of antigen with α-galactosylceramide on exosomes. *Cancer Res* 2013;73:3865–76.

164 Xie Y, Wu J, Xu A, *et al*. Heterologous human/rat HER2-specific exosome-targeted T cell vaccine stimulates potent humoral and CTL responses leading to enhanced circumvention of HER2 tolerance in double transgenic HLA-A2/HER2 mice. *Vaccine* 2018;36:1414–22.

165 García-Manrique P, Matos M, Gutiérrez G, *et al*. Therapeutic biomaterials based on extracellular vesicles: classification of bio-engineering and mimetic preparation routes. *J Extracell Vesicles* 2018;7:1422676.

166 Zhao Z, McGill J, Gamero-Kubota P, *et al*. Microfluidic on-demand engineering of exosomes towards cancer immunotherapy. *Lab Chip* 2019;19:1877–86.

167 Dusoswa SA, Horrevorts SK, Ambrosini M, *et al*. Glycan modification of glioblastoma-derived extracellular vesicles enhances receptor-mediated targeting of dendritic cells. *J Extracell Vesicles* 2019;8:1648995.

168 Horrevorts SK, Stolk DA, van de Ven R, *et al*. Glycan-Modified apoptotic melanoma-derived extracellular vesicles as antigen source for anti-tumor vaccination. *Cancers* 2019;11 doi:10.3390/cancers11091266

169 Costa-Silva B, Aiello NM, Ocean AJ, *et al*. Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol* 2015;17:816–26.

170 Hannafon BN, Trigoso YD, Calloway CL, *et al*. Plasma exosome microRNAs are indicative of breast cancer. *Breast Cancer Res* 2016;18:90.

171 Cordonnier M, Nardin C, Chanteloup G, *et al*. Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J Extracell Vesicles* 2020;9:1710899.

172 Porcelli L, Guida M, De Summa S, *et al*. uPAR⁺ extracellular vesicles: a robust biomarker of resistance to checkpoint inhibitor immunotherapy in metastatic melanoma patients. *J Immunother Cancer* 2021;9.

173 Crespo J, Sun H, Welling TH, *et al*. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* 2013;25:214–21.

174 Wei P, Wu F, Kang B, *et al*. Plasma extracellular vesicles detected by single molecule array technology as a liquid biopsy for colorectal cancer. *J Extracell Vesicles* 2020;9:1809765.

175 Sharma P, Ludwig S, Muller L, *et al*. Immunoaffinity-based isolation of melanoma cell-derived exosomes from plasma of patients with melanoma. *J Extracell Vesicles* 2018;7:1435138.

176 Gerlach JQ, Griffin MD. Getting to know the extracellular vesicle glycome. *Mol Biosyst* 2016;12:1071–81.

177 Dai J, Escara-Wilke J, Keller JM, *et al*. Primary prostate cancer educates bone stroma through exosomal pyruvate kinase M2 to promote bone metastasis. *J Exp Med* 2019;216:2883–99.

178 Wang C, Xu J, Yuan D, *et al*. Exosomes carrying ALDOA and ALDH3A1 from irradiated lung cancer cells enhance migration and invasion of recipients by accelerating glycolysis. *Mol Cell Biochem* 2020;469:77–87.

179 Beckermann KE, Dudzinski SO, Rathmell JC. Dysfunctional T cell metabolism in the tumor microenvironment. *Cytokine Growth Factor Rev* 2017;35:7–14.

180 Beier UH, Angelin A, Akimova T, *et al*. Essential role of mitochondrial energy metabolism in Foxp3⁺ T-regulatory cell function and allograft survival. *Faseb J* 2015;29:2315–26.

181 Burnouf T, Agrahari V, Agrahari V. Extracellular vesicles as nanomedicine: hopes and hurdles in clinical translation. *Int J Nanomedicine* 2019;14:8847–59.

182 Zhan Q, Yi K, Qi H, *et al*. Engineering blood exosomes for tumor-targeting efficient gene/chemo combination therapy. *Theranostics* 2020;10:7889–905.

183 Yamada N, Kuranaga Y, Kumazaki M, *et al*. Colorectal cancer cell-derived extracellular vesicles induce phenotypic alteration of T cells into tumor-growth supporting cells with transforming growth factor-β1-mediated suppression. *Oncotarget* 2016;7:27033–43.

184 Hellwinkel JE, Redzic JS, Harland TA, *et al*. Glioma-derived extracellular vesicles selectively suppress immune responses. *Neuro Oncol* 2016;18:497–506.

185 Maybruck BT, Pfannenstiel LW, Diaz-Montero M, *et al*. Tumor-derived exosomes induce CD8⁺ T cell suppressors. *J Immunother Cancer* 2017;5:65.

186 Muller L, Mitsuhashi M, Simms P, *et al*. Tumor-derived exosomes regulate expression of immune function-related genes in human T cell subsets. *Sci Rep* 2016;6:20254.