



The role of exosomal PD-L1 in tumor immunotherapy [☆]

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ABSTRACT

Exosomes are bioactive lipid bilayer vesicles released by most cells to mediate intercellular signal communication. Tumor cells release exosomes transmitting signals cell-to-cell and between cells and organs, which will promote tumor angiogenesis, regulate tumor stromal response, immune response, and enhance tumor cells resistance, while exosomes-derived from immune cells in tumor microenvironment play a key role in inhibiting tumor growth and killing tumor cells. Programmed cell death protein 1 (PD-1) combined with Programmed cell death protein ligand 1 (PD-L1) can inhibit the activation of T cells, for tumor cells achieve immune escape by overexpressing PD-L1 and binding PD-1 on T cells. The use of anti-PD-1 / PD-L1 antibodies prevents their binding to a certain extent and partially restores T cell's activity. This article mainly discusses the role of exosomal PD-L1 in tumor progression and therapeutic efficacy after application of clinical antibodies, as well as the relation between different reactivity and immunity set points in cancer patients of different races, with different types and at different stages. Besides, we propose that exosomal PD-L1 may become targets for anti-PD-1 / PD-L1 antibody therapy, biomarkers for liquid biopsy, and drug carriers.

Introduction

Exosomes

The role of secretory vesicles from sheep reticulocytes maturation through recycling of transferrin and its receptor was first reported in 1983 [1]. Then extracellular vesicles (EVs), especially exosomes, have become a hot issue in cancer research. Exosomes are the smallest type of EVs, which are usually defined as the generally organelles-derived from endosomes within the diameter range of 30–100 nm [2]. The biogenesis of exosomes is more complex than that of Microvesicles, another type of EVs, known as extracellular vesicles with a size of usually larger than 200 nm and formed directly from the plasma membrane by shedding or budding. Different from the release of microbubble budding, the exosomes are released to the outside of the cell by exocytosis. Then some multivesicular bodies are dissolved through the lysosomal pathway, while others are fused to the cell membrane and released to

the outside of the cell to form exosomes. These exosomes contain various bioactive molecules that derived from parent cells, such as proteins (receptors, enzymes, transcription factors and extracellular matrix proteins), nucleic acids (DNA, microRNA, mRNA and other non-coding RNAs), and lipids which can change the function of recipient cell [3–6]. Here we only focus on the proteins of exosomes.

The isolation, purification, and enrichment of exosomes from cell culture supernatants, biological fluids, and tissues are of great importance, which advanced exosome research in biomedicine, detection of related diseases, and clinical application of immunotherapies. With their low density, small size and complex biological fluidity, exosomes have a major challenge for their isolation and purification. Based on the differences of exosomes' physical properties (density, specific gravity, size, and molecular weight), the traditional separation methods including ultracentrifugation and ultrafiltration, density gradient centrifugation, immunomagnetic bead method and polymer precipitation method, adopt different relative centrifugal forces, specific biological markers, and

Abbreviations: CLL, chronic lymphocytic leukemia; ER, endoplasmic reticulum; EVS, extracellular vesicles; DCS, dendritic cells; GBM, glioblastoma; GSCS, gbm-derived stem cells; HCC, hepatocellular carcinoma; IFN- γ , interferon- γ ; irAEs, immune-related adverse events; PD-1, Programmed cell death protein 1; PD-L1, Programmed cell death protein ligand 1; TILs, tumor-infiltrating leukocytes.

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Table 1
Separation methods of exosomes.

Methods	Advantages	Deficiencies	References
Ultracentrifugation	The gold standard for protein detection; Abundant exosomes can be got in a single batch.	Time-consuming; Equipment is expensive; Exosomes may be damaged; Low recovery; Precipitated exosomes may contain aggregated proteins	[71]
Ultrafiltration	Exosomes can be purified from a large number of conditioned media; A large number of exosomes can be obtained at one time	Time-consuming; Tedious operation steps; Strict operation requirements	[72]
Density gradient centrifugation	Enable different medias for further purification and isolation of exosomes		[73]
Immunomagnetic beads	Rapid separation of APC exosomes from cell supernatants; High isolation purity; Convenient for downstream analysis	Only exosomes with corresponding specific markers	[74]
Polymer coprecipitation	Low speed; Centrifugation for quick and easy isolation of exosomes	Heteropolymer particles may be co-separated contaminating exosomes; Lack specificity	[75]
size exclusion chromatography	High separation purity; Low protein content; Low lipid content	Separated from plasma	[72]
Microfluidic Chip	High recovery and purity; Short time-consuming	Requires sophisticated manufacturing technology and low circulation	[76–78]
Microfluidic method combined with immune separation	Specific separation, extraction, purification and targeted protein analysis simultaneously	Exosomes with corresponding specific markers only	[79]

different surface charges. Then, size-based size exclusion chromatography and microfluidic-based exosome separation methods using hydrodynamic characteristics, acoustic flow characteristics, and dielectrophoresis characteristics, have also been widely used recently. Besides, a method based on the combination of microfluidics and immune separation has appeared as well (Table 1).

Parent cells determine composition and content of exosomal proteins. Exosomal proteins can be divided into two classes: one is a common protein, almost in all exosomes, such as cytoskeletal proteins (tubulin, actin), Four-molecular tetraspanins (CD9, CD63, CD81, and CD82), lipid-associated proteins, heat shock protein family, etc.; the other is related to the cell source, such as proteins Alix Syntenin, Tsg101 and ESCRT family proteins. In addition, exosome-derived proteins are relatively specific, for example, exosome secreted by B lymphocytes has MHC-II molecules on the surface; and tumor cell-derived exosomes carry a large number of tumor antigens.

Compared with normal cells, tumor cells secrete more exosomes, and the contents also showed significant differences. Due to the protection of their vesicle structure, the exosomes provide stable conformational protein molecules to maintain their activity, and get to distant organs by body fluids' transporting, thus the membrane fusion works in such a way ensuring a more effective information exchange between cells [6–8]. Therefore, exosomes, as an important way to communicate information between tumors and their target cells, play a critical role in the occurrence and development of cancer. Tumor cell-derived exosomes are involved in the targeted metastasis of tumor organs, promoting the epithelial-mesenchymal transition of recipient cells and angiogenesis, forming pre-metastatic niche, and then inhibiting tumor cells when radiotherapy and chemotherapy. What's more, tumor-derived exosomes play an eye-catching role in immune escape of tumor cells as well, which we will talk about next.

PD-1/PD-L1 axis

It is well known that tumors can suppress immune responses, and negative regulatory pathways, or checkpoints, play key roles in tumors escaping detection of immune system [9–11]. Cytotoxic T-lymphocyte protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) are two of such checkpoints, which have drawn most attention. Here, we mainly discuss the role of the latter in tumor immune escape. PD-1 is

a type I transmembrane protein with 288 amino acid (aa), which is encoded by the *Pdcd1* gene of human. PD-1 can exert its effect when binding to ligands on the target cells, PD-L1 and PD-L2. PD-L1, as what we focus on, is a type I transmembrane protein with 290 aa, and encoded by *Cd274* gene in human [12,13].

PD-1 can be only expressed on T cells, especially on the activated T cells, not the resting T cells [14]. Besides immune cells (T cells, B cells, DCs, macrophages), PD-L1 can be expressed on many other cell types including epithelial cells, endothelial cells, mesenchymal stem cells, bone marrow-derived mast cells, and tumor cells [15]. PD-L1 binds the PD-1 receptor on the surface of T cells, then clusters with T cell receptors (TCR) and recruits SHP2 (Src homology 2 domain-containing tyrosine phosphatase 2) via two tyrosine-containing motifs of PD-1 (ITIM and ITSM), resulting in the suppression of T cell activation through TCR and co-stimulating CD28, which modulates the downstream pathways including PI3K/AKT, RAS/Raf, and phospholipase $C\gamma$ (PLC γ). SHP2 can suppress the activation, proliferation, survival of T cell and the T cell-dependent immune response by decreasing the cytokine expression [16–18].

Normally, PD-1/PD-L1 pathway can inhibit pathogenic self-reactive effector T cells through this mechanism, thereby avoid uncontrolled immune and prevent unwanted host lesions [19]. However, as mentioned above, PD-L1 can be expressed on the plasma membrane of tumor cells, thereby tumor cells can inhibit T immune cells activation through this mechanism as well. Therapeutic antibodies to PD-1 or PD-L1 can block this interaction and reactive cancer immunity, which has been proved successful in clinical practice [13,20]. Notably, apart from the setting of inflammation and a number of oncogenic lesions [9], PD-L1 on surface of tumor cells can also be upregulated after exposure to interferon- γ (IFN- γ) [21].

Exosomes play a key role in intercellular communication of tumor progression through their cargo and membrane surface protein. PD-L1 can be expressed on surface of various tumor cells, and suppress T cells activation when binding to PD-1 on the surface of T cells. As a membrane protein, whether PD-L1 is expressed on the surface of exosomes, and the relationship between them is still unclear. Recently, it is reported that tumor cell-derived exosomes are related with PD-L1 expression on macrophages [22]. In addition, evidences have suggested that PD-L1 can be directly expressed [23–27].

Tumor-derived exosomes affect PD-L1 expression in the tumor microenvironment

The complex tissue environment that tumor cells develop in is named tumor microenvironment, which is comprised of tumor cells, stromal cells, immunity cells and extracellular matrix. It is well known that Bidirectional communication between tumor cells and TME plays a critical role in tumor progression, invasion and metastasis. Increasing evidence has indicated that exosomes expressed from tumor cells can modulate or reeducate TME by transferring their cargo molecules, such as miRNAs, mRNAs, and some proteins (including PD-L1) [26–28].

In various types of TMEs, such as chronic lymphocytic leukemia (CLL), hepatocellular carcinoma (HCC), glioblastoma (GBM), monocytes and macrophages are important components of microenvironment, which can release tumor-supportive cytokines and express immunosuppressive molecules, including PD-L1 [29, 30]. In current study, Franziska Haderk et al. has proved that CLL-derived exosomes can target the PD-L1 expression levels of monocytes, which was connected with the Y RNA hY4 in the exosomes. They found noncoding Y RNA was highly enriched in CLL-derived exosomes, and both CLL-derived exosomes and exosomal Y RNA hY4 can induce monocytes PD-L1 expression, which was conducive to form a tumor-supportive microenvironment in CLL [31]. Researches showed that exosomes-derived from HCC cells could upregulate the expression of PD-L1 on macrophages [22], and the process was correlated to endoplasmic reticulum (ER). Recently, Jiatao Liu et al. found that ER-stressed HCC cells could express exosomes containing high levels of miR-23-3p, which would up-regulate PD-L1 expression on macrophages via PTEN/AKT pathway, thus suppressing T-cell functions [32]. And this effect can be suppressed by melatonin, a well-known hormone. There are evidences suggesting that melatonin play an essential role in immunoregulation and anti-inflammatory responses [33]. Liang Cheng et al. found that after treated with melatonin the expression of PD-L1 on exosomes-derived from macrophages decreased when exosomes-derived from normal HCC cells having the opposite effect, the mechanism of which was associated with the STAT3 signal pathway in macrophages [22]. However, it is still unknown whether melatonin can alter the PD-L1 levels on tumor-derived exosomes. Additionally, macrophages can be polarized to two sub-types, pro-inflammatory M1 phenotype and tumor supportive/propagative M2 phenotype [34]. In the TME of glioblastoma (GBM), exosomes from GBM-derived stem cells can induce monocytes to M2 macrophage, and upregulate the expression of PD-L1. As that in HCC, this process was correlated with STATs phosphorylation as well. Konrad Gabrusiewicz et al. has found that exosomes from GBM-derived stem cells directly transferred STAT3 into monocytes, which maybe contributed to the up-regulation of PD-L1. However, there may also exist other mechanisms [35].

Apart from in macrophages, effector T cells in HCC TME can be regulated. Previous research has shown that exosome-derived from HCC tumor cells pulsed DCs could significantly improve the tumor immune microenvironment of orthotopic HCC mice, and PD-1, not PD-L1, was also be upregulated in CD8+ T cells after this treatment [36,37]. In addition, evidence suggested that exosomes from lung cancer or breast cancer cells not only blocked DCs differentiation and induced cell apoptosis, but also induced the expression of PD-L1, which can be blocked by anti-PD-L1 [38].

Additionally, it was reported that in breast cancer, exosomes could transport PD-L1 from PD-L1-positive cancer cells to multiple cell types in TME, including PD-L1-negative cancer cells, macrophages and DCs (at least in vitro). Thus, exosomes could modulate immune surveillance as a trafficking vehicle to deliver PD-L1 from tumor cells into different cell types in TME [27].

In a word, immune cells such as monocytes and macrophages can directly express PD-L1 or secrete PD-L1-positive exosomes, and inhibit the activation of effector T cells and the secretion of cytokines when stimulated by sustained inflammation. Exosomes-derived from tumor cells

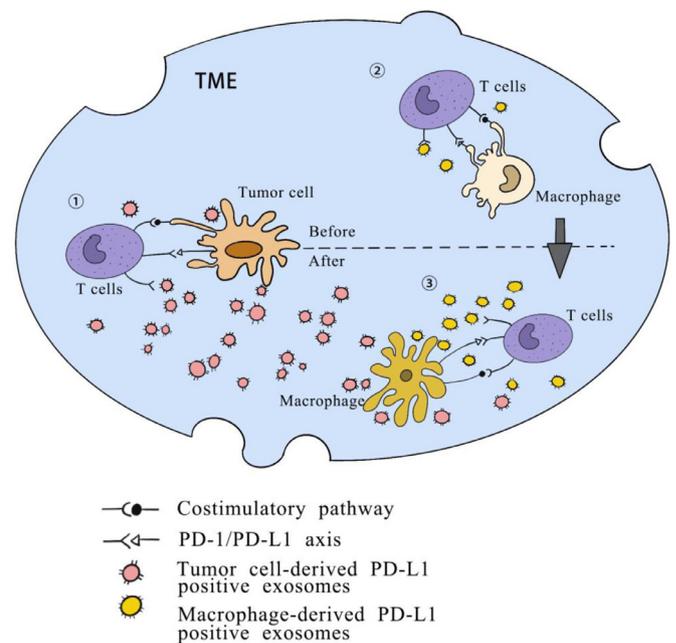


Fig. 1. Tumor cells-derived exosomal PD-L1 inhibit T cells' activation in TME.

can indirectly inhibit anti-tumor immunity by upregulating the expression of PD-L1 on various immune cells in the TME or PD-1 on effector T cells, creating a suitable microenvironment with low immunity for tumor cell growth (Fig. 1).

① In tumor microenvironment, PD-L1, a tumor cell-derived exosome, can bind to PD-1 on the surface of T cells. PD-1 can only be expressed on activated T cells. SHP2 (SRC homologous domain tyrosine phosphatase 2) is recruited through two tyrosine motifs of PD-1 (ITIM and ITSM). T cells activation is inhibited by TCR and costimulation of CD28. SHP2 can inhibit the activation, proliferation and survival of T cells and reduce the number of cells Cytokine expression inhibiting T cell-dependent immune response.

② In the absence of tumor exosomes (physiologically Limited), PD-L1 can be expressed on many other cell types including peripheral cells, endothelial cells, mesenchymal stem cells, bone marrow derived mast cells, and tumor cells;

③ Tumor cells can secrete and express exosomes containing high levels of miR-23-3p. Mir-23-3p can upregulate PD-L1 expression on macrophages through PTEN / Akt pathway, and then inhibit T cell function. PD-L1 positive exons from macrophages are up-regulated.

Tumor cells-derived exosomes expressing PD-L1

Exosomes can not only influence the PD-L1 expression of immune cells in TMEs, but also directly express PD-L1 on its plasma membrane. Recently, more and more evidences have shown that a series of tumor cell types can derive exosomes expressing PD-L1. These tumor types include breast cancer, head and neck squamous cell cancer, non-small cell lung cancer, glioblastoma and melanoma [23–27, 39].

However, if only some exosome marker proteins (such as HRS, CD63, CD81, and HSP70) and PD-L1 protein are co-precipitated at the protein level, we are not sure that PD-L1 is expressed on these exosomes. Proof of genetic level is necessary. In the exosome biogenesis, ESCRT subunit Hrs, Rab27a and nSMase2 all play important roles. nSMase2 is the key enzyme that promotes budding of intravesicular vesicles [40], Rab27a participates in the fusion of MVB to the plasma membrane [41], and Hrs, the subunit of ESCRT, mediates the recognition and sorting of exosomal cargos [42]. Mauro Poggio et al. in their research proved that not only the biogenesis and expression of exosomes could be greatly inhibited, but in the 100,000 g fraction with enriching exosomes [43], the PD-L1

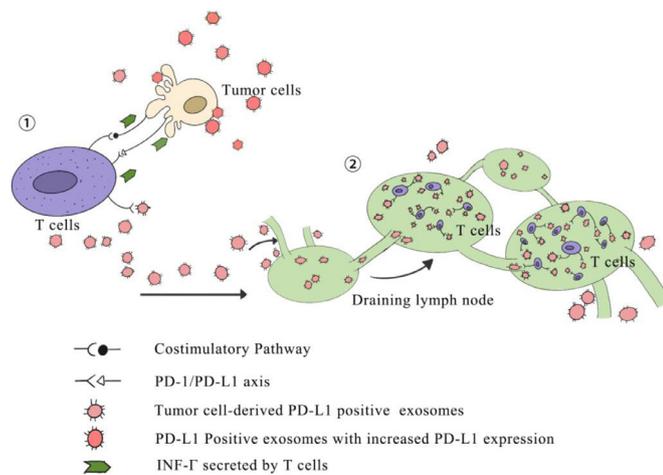


Fig. 2. Tumor-derived exosomal PD-L1 are regulated by $\text{INF-}\gamma$ and metastasized by draining lymph nodes.

protein is significantly reduced or complete absent when knocking out these genes [25]. Therefore, the expression of PD-L1 on tumor-derived exosomes is a convincing conclusion.

As a trans-membrane protein, PD-L1 on exosomes must arise from the limiting membrane of late endosomes, for it is widely known that the limiting membrane of the late endosomes arises from plasma membrane through endocytosis. However, in the process of endosome maturation, ER and Golgi directly provide material as well [44]. Mauro Poggio et al. found that exosomal PD-L1 arises from the cancer cell plasma membrane using a cell-surface biotinylation assay based on the fact that PD-L1 was glycosylated to prove exosomal PD-L1's not coming from the ER or early Golgi [25,45]. The current studies on PD-L1 mediating immunosuppression are almost based on the interaction between PD-L1 on the tumor cell surface and PD-1 on T cells. Then whether exosomal PD-L1 could function similarly as tumor cell surface PD-L1? The answer is yes. In the studies we mentioned above, exosomal PD-L1 derived from tumor cells all have the function of inhibiting the CD8 T cells activation, including proliferation, cytokine production and cytotoxicity. Notably, in glioblastoma, Franz L. Riclefs et al. suggested that this process was associated with T cell receptor relating pathway [24]. In addition, due to the unique nature of exosomes, exosomal PD-L1 obtained more features that PD-L1 on the tumor cell surface did not have. It is widely known that exosomes can be secreted into various types of body fluids, such as plasma, urine, saliva, tears, semen, etc. and reach recipient cells at a distance and deliver their contents to elicit functional response due to the structure of plasma membrane [44]. Thus, PD-L1+ exosomes could be secreted into plasma by tumor cells and function in a distance as well. In recent research, Mauro Poggio et al. reported that tumor derived exosomal PD-L1 can travel to the draining lymph node and suppress T cell activation, but with absence of exosomal PD-L1, immune competent mice not only inhibited immediate tumor growth, but also developed a robust memory toward the tumor cells [25]. (Fig. 2) This finding suggested that exosomal PD-L1 could play a role in tumor lymphatic metastasis, which has not been noticed before. In contrast, once T cells have been exposed to tumor antigens without exosomal PD-L1, T cells at the local lymph node could enable a durable systemic immune response.

Previously, studies have revealed that $\text{INF-}\gamma$ secreted from CD8+ T lymphocytes promoted PD-L1 upregulation. $\text{INF-}\gamma$ binded to the interferon gamma receptor subsequently activated JAK/STAT signaling pathway, which led to the downstream expression and activation of IRF-1, further inducing PD-L1 expression on tumor cells [21,46]. Similarly, this promotional effect of $\text{INF-}\gamma$ still worked for exosomal PD-L1. And in exosomes, this effect could be seen in the following two aspects. First,

similar with tumor cell surface PD-L1, $\text{INF-}\gamma$ can upregulate the expression of exosomal PD-L1. For example, glioblastomas can be classified into PD-L1 high and low types through their PD-L1 expression levels in the glioblastomas cells and their exosomes. Exosomes from PD-L1 high group can significantly inhibit the T cell activation, which can be partially reversed by PD-1 blockade. However, exosomes from PD-L1 low group can inhibit T cell activation as well but not via PD-1/PD-L1 pathway. After stimulated by $\text{INF-}\gamma$, this inhibition was enhanced and partially reversed by PD-1 blockade. In addition, $\text{INF-}\gamma$ has been proved not to increase the number of vesicles secreted but display increased binding to PD-1, that is, exosomal PD-L1 increased in response to $\text{INF-}\gamma$ [23,25]. (Fig. 2)

① $\text{INF-}\gamma$ secreted by T cells can promote the up-regulation of tumor-derived exosomes PD-L1; $\text{INF-}\gamma$ secreted by CD8+ T lymphocytes can promote the up-regulation of PD-L1. $\text{INF-}\gamma$ binds to $\text{INF-}\gamma$ receptor and then activates JAK / STAT signaling pathway, resulting in downstream expression and IRF-1 activation, which further induces PD-L1 expression on tumor cells. Similarly, the effect of $\text{INF-}\gamma$ on exosomes PD-L1 still exists.

② Tumor cells can directly target different effector T cells by secreting PD-L1 positive exosomes to tumor microenvironment or draining lymph nodes to inhibit anti-tumor immunity.

Tumor cells not only express PD-L1 molecules directly on the cell membrane, but also inhibit the activation of T cells to achieve immune escape. Tumor cells, especially those with high malignancy, inhibit anti-tumor immunity as well, by secreting PD-L1-positive exosomes directly to the tumor microenvironment or through draining lymph nodes to distant places, targeting different effector T cells. However, once the exosomal PD-L1 are inhibited, the body may develop sustained strong anti-tumor immunity. Blocking the production of exosomes PD-L1 or the release of exosomes may bring a new thinking to the field of immunotherapy.

Clinical application

Metastatic melanoma, lung cancer, head and neck cancer, renal cell carcinoma, urothelial carcinoma, liver cancer, gastric cancer, Hodgkin's lymphoma, Merkel cell carcinoma, large B cell lymphoma, cervical cancer, and any MSI+ tumors [47] can be treated by immunotherapies targeting the PD-1/PD-L1 (anti-PD therapy). Traditional immunotherapies aim at activating the immune system to improve the antitumor responses to kill tumor. However, anti-PD therapy recovery of T cell activity partially increases the antitumor responses. The traditional immunotherapies enhance immunity, with an increased rate of irAEs, while anti-PD therapy have less irAEs but a higher efficacy.

Immunotherapies rely on promoting the anticancer responses of immunity system. Thus, the efficacy of immunotherapies is associated with the immune profile of an individual. However, in people with overtly similar tumors, the responses of anti-PD therapy vary considerably. To evaluate the inherent immunological status of an individual, the concept of "cancer-immune set point" has been proposed, which can be defined as the equilibrium between the factors that promote or suppress anticancer immunity [20]. Therefore, the set point represents the threshold that must be surpassed for a person with anticancer to respond to immunotherapies. An array of factors contribute to this concept, including the tumor genome, T cell memory, inflamed versus non-inflamed tumours, host genetics, microbiome, environmental and other factors that influence immunity.

As described previously, tumor cells-derived exosomes play a role in anticancer immunity. We focused on several factors affecting the cancer-immune set point, which were correlated with tumor cells-derived exosomes. First, the mutational neoepitope load of a given tumor has been considered as a driver of anticancer T cell responses. In other words, the greater number of mutations in given tumor, the more probable it is, for some mutations will be immunogenic, providing targets for T cell attack [48]. To maintain a sustained anticancer response, the continued priming of native T cells may be needed, especially memory T cells, which

play a key role in this process [49]. The evidences have shown that after anti-PD-1/PD-L1 therapy, the functionality of exhausted T cells can be restored. And in mice infected with lymphocytic choriomeningitis virus, a population of short-term memory T cells selectively proliferated after PD-1 blockade [50–53]. As mentioned above, suppression of exosomal PD-L1 can induce systemic anticancer immunity. Additionally, Mauro Poggio et al. interpreted that once T cells have been exposed to tumor antigens in the absence of exosomal PD-L1, they could develop memory toward the tumor cells [25]. A study showed that exosomal PD-L1 had a more robust immunosuppressive effect than the soluble form. The exosomal PD-L1 in NSCLC patients were detected to see the applicability of immunotherapy [54].

There was a synergistic relation between exosome depletion and immunotherapy [26]. It was observed that in BALB/c 4T1 tumor mice treated with Gw4869 and α -PD-L1 mAb, the largest primary tumor burden was reduced. Compared with the single treatment, either tetracycline induced Rab27 knockdown 4T1 cell line, MC38 Rab27 / / tumor bearing mice receiving anti PD-L1 mAb, or B16-F10 melanoma mouse models treated with CD63 and PD-L1 antibodies, the therapeutic effect was significantly enhanced [23].

Therefore, exosomal PD-L1 may represent a pathway inhibiting the generation of anticancer memory T cells.

The other important factor that influences the response to anti-PD-1/PD-L1 is whether tumors harbor an inflammatory microenvironment. TME can be divided into two types according to the presence or absence of immune cells infiltration. By examining histological sections of tumor biopsies, it could distinguish three basic immune profiles that are correlated with an individual response to anti-PD-1/PD-L1 therapy [55–57]. The first profile is immune-inflamed phenotype, characterized by the presence in the tumor parenchyma of both CD4- and CD8-expressing T cells, is often accompanied by myeloid cells and monocytic cells; the immune cells are positioned in proximity to the tumor cells [57–60]. The second profile is the immune-excluded phenotype, characterized by the presence of abundant immune cells as well. However, the immune cells do not penetrate the parenchyma of these tumors but are retained in the stroma that surrounds nests of tumor cells instead [56, 57, 61]. The third profile, the immune-desert phenotype, is characterized by a paucity of T cells in either the parenchyma or the stroma of the tumor [55–57]. Although myeloid cells may be present, the general feature of this profile is the presence of a non-inflamed tumor microenvironment with few or no CD8-carrying T cells. Both the immune-excluded and immune-desert phenotype are considered as non-inflamed tumors. It is unsurprisingly that non-inflamed tumors rarely respond to anti-PD-1/PD-L1 therapy. However, a response is not assured in inflamed individuals, which indicates that immune-cell infiltration is necessary but insufficient for inducing a response.

Beyond immune-cell infiltration, the effect of anti-PD-1/PD-L1 therapy is also determined by selective expression of PD-L1 in the TME. Thus, another classification system of human cancer, termed tumor immunity in the microenvironment, based on tumor-infiltrating leukocytes (TILs) level and PD-L1 expression level in the TME, has been proposed [62–64]. Termed tumor immunity in the microenvironment, type I tumors refer to those lack of TILs in TME and PD-L1 expression, Type II those with overregulation of activated TILs, and Type III those with dysfunctional TILs activation and no expression of PD-L1. Type IV tumors express PD-L1 on the surface without infiltration of TILs. As described above, tumor cells-derived exosomes can not only regulate the PD-L1 expression of tumor cells or immune cells in TME, but exosomal PD-L1 can bind to PD-1 of T cell, playing a role in the anticancer immunity. What's more, Mauro Poggio et al. suggested that exosomal PD-L1 was resistant to anti-PD-L1 therapy, which differed from cell-surface PD-L1 [25]. Thus, apart from the PD-L1 expression on the tumor cells, the exosomes in TME should be valued as well.

Using the same strategy for all patients may be inefficient and wasteful, even increasing the irAEs rates [60,65]. Therefore it is critical to identify which immune defect is the predominant of each individual,

and which one suggests that biomarkers need to be used as predictive tools. PD-L1 is the predictive biomarker that gets most attention. In addition, the advantage of emerging biomarkers is replacing the disadvantages of traditional biomarkers, such as differences in biopsy time, location, and patients' treatment [39]. With the characteristics of non-invasive, convenient, fast and repeated tests at multiple time points, liquid biopsy has a distinctive advantage, which is beginning to emerge as the times.

However, current studies mostly focus on detecting PD-L1 of biopsies or circulating tumor cells [66–68]. Apart from circulating tumor cells and biopsies, exosomal PD-L1 can be used as predictive biomarker. Recently, Marzia Del Re et al. has reported that the response to anti-PD-1 in melanoma and NSCLC was associated with PD-L1 mRNA expression in plasma-derived exosomes, suggesting that plasma-derived exosomes can provide useful information for the response to anti-PD-1 therapy [69]. In pancreatic cancer, Alexander Lux et al. reported that plasma exosomal PD-L1 was not overexpressed in pancreatic cancer cells although it was accompanied with poorer prognosis in PDAC patients. Thus, it has no diagnostic value [70].

In short, appropriate immunotherapy can decrease the irAEs rates in the process of treatment, and the effect of immunotherapy depends on the immune setting point of TME. The setting point represents the equilibrium between the factors that promote or suppress anticancer immunity. Then PD-L1 of tumor-derived exosomes can be used as a new target for immunotherapy and as a biomarker for liquid biopsy to try to judge the applicability of patients.

Conclusion and future perspective

It's no doubt that T cell apoptosis and secretion inhibition occurs when the PD-L1 molecule expressed on exosomes, especially exosomes-derived from tumor cells, binds to PD-1 on T cells.

But it is still unknown whether it is completely consistent with PD-L1 expressed on tumor cells, and whether there is a new undiscovered site of action. If there is no such new site, why does exosomal PD-L1 and tumor PD-L1 show differences in anti-PD-1/PD-L1 treatment? If such a new site exists, can it be used as a new blocking target to improve the current efficacy of anti-PD-1/PD-L1 treatment? From what Poggio et al. found, I believe that the reason for this difference is inseparable from difference between exosomal PD-L1 and tumor PD-L1, but issues about the difference reflected in PD-L1 molecules from different sources, the different responses of the PD-1 on T cells, or the presence of a third site affecting the response of T cells, are currently unknown and provide a new direction for future research.

Exosomes can transmit the expression of PD-L1 molecules in the tumor microenvironment, which plays an important role in the construction of immunosuppressive tumor microenvironment. In addition, exosomes can act on distant target cells through body fluid circulation. Therefore, the expression of PD-L1 exosomes can inhibit T cells in distant draining lymph nodes, which plays an indispensable role in lymphatic metastasis of tumors, but further proof is needed.

PD-L1-positive exosomes inhibit the memory of anti-tumor immune T cells. They induce long-term anti-tumor immune memory and inhibit distant tumor cells through draining lymph nodes, when T cells are transiently exposed to the microenvironment without exosomal PD-L1, and develop a sustained systemic immune response. It suggests that PD-L1-positive exosomes play a strong inhibitory role in anti-tumor immunity as well. If the PD-L1-positive exosomes are cleared or blocked in vivo, creating a microenvironment without exosomal PD-L1, a long-lasting systemic anti-tumor immunity may be developed.

The above observations and prospects about the role of PD-L1 positive exosomes that derived from tumor cells all point to a same goal, blocking exosomes PD-L1 in vivo. At present, the target molecule anti-PD-1/PD-L1 treatment is PD-1 molecule on the surface of T cells or PD-L1 on the surface of tumor cells, but there is no relevant research on PD-L1 on exosomes. To achieve this goal, the difficulties not only in-

clude the unclear specificity of the exosomal PD-L1 molecule, but tumor-derived exosomes' extracting and identifying. If these problems can be overcome, blocking them by specific targets on PD-L1 positive exosomes may make new breakthroughs in tumor immunotherapy. In addition to its role in therapy, currently the most mature clinical application of exosomes is focused on liquid biopsies. At the same time, the role of PD-L1 as a biomarker has been widely recognized as well. However, the value of exosome PD-L1 as a new biomarker for liquid biopsy remains to be supported by more evidence. On the one hand, the specificity and sensitivity of the exosomal PD-L1 expression as the basis for tumor diagnosis needs further confirmation. But the relation between the high expression level of exosomal PD-L1 and poor prognosis has been sufficiently confirmed, so exosomal PD-L1 has its unique value at least in terms of tumor prognosis. On the other hand, the expression level of PD-L1 is clinically used as a marker for the evaluation of the efficacy of immunotherapy, but the efficacy of anti-PD-1/PD-L1 treatment have obvious differences even in patients with positive plasma PD-L1 expression. Exosomal PD-L1 as a new target has a more significant difference than the total PD-L1 level, so it may be more accurate for predicting the efficacy of anti-PD-1/PD-L1 treatment.

In recent years, the concept of "cancer-immune set point" has played an important role in promoting the standardization and standardization process of tumor immunotherapy. During this process, exosomal PD-L1 have significant effects for a set of elements combining cancer-immune set points, such as tumor genome, infiltration of inflammatory cells in the tumor microenvironment and T cells memory. Whether the effect of exosomal PD-L1 on anti-tumor immunotherapy can be considered as a separate factor or as an important influencing factor for the above elements, is significant for perfecting the concept of "cancer-immune set point" and promoting the application of anti-tumor immunity or immunotherapy.

In conclusion, tumor-derived PD-L1-positive exosomes have broad clinical application prospects as new targets for immunotherapy and new biomarkers for liquid biopsy. However, the research on the development and application of exosomes and PD-L1 is still in a relatively early stage. The study of exosomal PD-L1 is expected to have new developments after a new breakthrough has been made in the extraction and purification of tumor exosomes, and a new development in the mechanism of PD-1/PD-L1 blockade.

Declaration of Competing Interest

None.

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The data supporting the conclusion of this review has been included within the article.

Data deposition

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Author contribution

Jing Wang and Hao Zeng are responsible for writing and drawings; Hongwei Zhang and Yunwei Han are responsible for modification.

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