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Exosomal PD-L1: Roles in tumor progression and immunotherapy

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Abstract

The use of immune checkpoint therapies targeting programmed death-1 (PD-1) and its ligand (PD-L1) continue to show limited, durable success in clinical cases, despite widespread application. While some patients achieve complete responses and disease remission, others are completely resistant to the therapy. Recent evidence in the field suggests that tumor-derived exosomes could be responsible for mediating systemic immunosuppression that antagonizes anti-PD-1 checkpoint therapy. In this opinion article, we discuss our claim that endogenous tumor exosomal PD-L1 and tumor-derived exosome-induced PD-L1 are two of the most notable mechanisms of exosome-mediated resistance against anti-tumor immunity and we also discuss how this resistance could directly influence immune checkpoint therapy failure.

Keywords

exosomes; PD-L1; immunotherapy

Exosomes and the Cancer Connection

Exosomes are small nanoparticles, 30–150 nm in diameter, that are released via exocytosis from the surface of both normal and tumor cells. Their cargo often contains a wide assortment of RNA, DNA, proteins, miRNAs, metabolites, and other biologically active molecules [1]. They have been isolated in a variety of bodily fluids including bile, blood, breast milk, urine, cerebrospinal fluid, and saliva [2]. Given their abundance and propensity to be found in circulation, exosomes are being targeted clinically as potential biomarkers for cancer [3]. A majority of cancers have been found to secrete large quantities of exosomes which demonstrates a potential pathophysiological effect in cancer progression [4].

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Exogenous administration of tumor-derived exosomes increases primary tumor size and overall metastatic burden. Furthermore, in a variety of tumor models, deficiency in exosome secretion resulted in greater influx of activated T-cells to secondary metastatic sites and draining lymph nodes. However, only recently have we begun to appreciate the role exosomes play in conferring a systemic immunosuppressive phenotype allowing for the potentiation of primary tumor growth and metastasis. In our opinion, one of the most notable pro-tumoral exosomal effects is immune suppression mediated by expression of programmed death ligand-1 (PD-L1). Here, we provide a comprehensive overview of the roles endogenous exosomal PD-L1 and exosome-induced PD-L1 play in suppressing anti-tumor immunity and discuss the implications for current immunotherapy in cancer.

Exosomal Expression of PD-L1

One of the mechanisms by which tumor cells evade immune surveillance is via upregulating surface expression of PD-L1 which interacts with PD-1 on effector T cells shutting down their anti-tumor response. Anti-PD-1/PD-L1 monoclonal antibodies (mAb) have been developed to interfere with this process, however, the overall response is limited [5]. Accumulating evidence suggests that exosomes are a significant source of extra-tumoral PD-L1 and may be one mechanism contributing to PD-1 Ab treatment resistance. While we acknowledge that other pathogenic roles for tumor-derived exosomes exist, we will only focus on those mediated by PD-L1 in this article.

Exosomes arise from the inward budding of vesicles in the late endosome to form a multivesicular body (MVB). Immunohistochemistry staining of human breast cancer tissue revealed co-localization of exosomal marker, CD63, and PD-L1 within the MVB [6]. Fusion of the MVB with the plasma membrane releases the exosomes extracellularly. Cell surface biotinylation experiments using the colon cancer cell line, PC3, revealed that exosomal PD-L1 within the MVB originates from endocytosis of cell surface PD-L1 [7]. Confocal microscopy of non-small cell lung cancer-derived exosomes also confirmed these findings [8]. Immunoelectron microscopy and enzyme-linked immunosorbent assays also demonstrated that exosomal PD-L1 has a similar extracellular membrane topology as its cell surface counterpart [9]. Exosomes have been isolated from the supernatants of various tumor cell line cultures and human plasma through binding of their surface PD-L1 to immobilized biotinylated PD-1 antibodies [9–11].

Different tumor cell lines or types of cancer have fluctuating amounts of PD-L1 packaged into secreted exosomes. One hypothesis is that cell lines differentially load PD-L1 onto exosomes. In basal-like breast cancer cells, the endosomal sorting complexes required for transport (ESCRT) associated protein, ALIX, has been found to be directly responsible for loading PD-L1 onto exosomes from the endosomal lumen. Deficiency in ALIX in HCC1954 cells resulted in retained tumor cell surface expression of PD-L1 but decreased exosomal PD-L1 [12]. Similarly, knockdown of another ESCRT subunit, HRS, which mediates MVB formation and exosome biogenesis, in the metastatic melanoma cell line, WM9, also led to a decrease in PD-L1 expression in exosomes but retained expression in the parent cell presumably due to decreased exosome formation [9]. Elimination of exosome secretion by knocking out exosome biogenesis proteins, Rab27a and nSMase2, in PC3 cells dramatically

decreased co-expression of CD63 and PD-L1 in the sucrose fractionation [7]. Inhibition of exosome secretion using the pharmacological inhibitor GW4869 in MB231 human breast cancer cells resulted in decreased expression of exosome markers CD63 and CD81 with concomitant decreases in PD-L1 expression [6]

In addition to expression of biogenesis proteins, another characteristic of parental cells that determines exosomal PD-L1 release is metastatic capability. Metastatic melanoma cell lines were found to secrete exosomes with higher PD-L1 expression compared with primary cell lines [9]. Investigation into human WM9 melanoma xenograft-bearing nude mice demonstrated significantly increased PD-L1 expression within the exosomal compartment compared with control [9]. Exosomes extracted from the plasma of stage IV metastatic melanoma patients revealed increased exosomal PD-L1 expression compared with healthy donors [9]. Furthermore, increased exosomal PD-L1 expression level has been found to positively correlate with increasing tumor size, higher disease activity, and overall clinical stage in a variety of cancers. Interestingly, soluble PD-L1 in the plasma of head and neck squamous cell carcinoma (HNSCC) patients did not correlate with clinicopathological parameters, further suggesting exosomal PD-L1 imparts a specific phenotype in cancer [13]. It is also of note that IFN-γ stimulation which has been found to drive increased expression of PD-L1 on tumor cells was also found to increase exosomal PD-L1 expression [9, 14].

Immunosuppressive Nature of Exosomal PD-L1

The systemic immunosuppressive effects of exosomal PD-L1 can be classified into two categories based on the individual mechanism of suppression: direct endogenous exosomal PD-L1 and indirect exosome-induced PD-L1.

Direct Endogenous Exosomal PD-L1

As a secreted component from primary tumors, exosomes have been credited to possess a wide assortment of pre-metastatic niche enhancing characteristics [15]. However, thus far, the majority of these pro-tumoral characteristics have been found to be mediated by intracellular mRNA, miRNA, and protein cargo contained within the exosomes [15, 16]. Conversely, exosomal PD-L1 which is localized to the exosome surface has been associated with tumor progression in a variety of cancers including melanoma, breast cancer, HNSCC, and glioblastoma [6, 9, 13, 14]. Specifically, patients with metastatic melanoma had significantly higher levels of exosomal PD-L1 than healthy donors [9]. In breast cancer, PD-L1/exosome co-localization increased with advancing tumor grade from Grade I/I-II to Grade II-III/III [6]. PD-L1 expression on exosomes correlated with glioblastoma tumor volumes and with clinical stage and level of nodal involvement in HNSCC [13, 14]. In addition, exosome PD-L1 expression negatively correlated with postoperative survival time in pancreatic ductal adenocarcinoma patients [17].

The high level of correlation between exosomal PD-L1 expression and advanced tumor staging suggests that PD-L1 on exosomes is responsible in some capacity for augmenting tumor growth and metastasis. Previous studies in a variety of mouse models have shown that exogenous administration of tumor-derived exosomes increases metastatic potential and overall tumor burden of primary tumors [18, 19]. However, only recently has this tumor

promoting phenotype specifically been linked to exosomal PD-L1 expression. Treatment of a PD-L1 knock out (KO) 4T-1 cell line with PD-L1 expressing exosomes restored tumor growth capabilities in a dose dependent manner [6]. Similarly, exogenous administration of both PD-L1 expressing TRAMP-C2 prostate and MC38 colorectal carcinoma exosomes rescued the immunosuppression and tumor growth normally seen in wild type Rab27 deficient tumor-bearing mouse models [7]. The immunosuppression seen in the wild type TRAMP-C2 model is characterized by decreased infiltration of effector $CD8^+$ T cells and higher expression of exhaustion markers like PD-1 and TIM-3 [7]. Multiple studies have shown that in *vitro* co-culture of PD-$L1^{+/high}$ exosomes with T cells results in inhibition of T cell activation. Co-culture of PD-$L1^{high}$ but not PD-$L1^{low}$ exosomes isolated from the plasma of HNSCC patients decreased expression of the activation marker CD69 on healthy donor $CD8^+$ T cells [13]. Furthermore, the decrease in CD69 expression was rescued with the addition of α-PD-1 mAb. A similar result was seen in healthy human donor $CD4^+$ and $CD8^+$ T cells stimulated with exosomes from human glioblastoma stem-like cells [14]. Breast cancer cell line derived exosomes inhibited ERK phosphorylation and NF-κB activation in human PBMCs [6]. IL-2 secretion from PBMCs was inhibited by exosomes isolated from breast (BT-549), colon (RKO), and lung (HCC827) carcinoma cell lines [6]. Co-culture of autologous $CD8^+$ T cells from NSCLC patients with exosomes isolated from their own peripheral blood revealed that only PD-$L1^{high}$ exosomes inhibit production of pro-inflammatory cytokines such as IL-2 and IFN-γ [8].

One caveat to these studies, however, is that exosomes isolated from the plasma of human patients via immobilized antibodies are a heterogeneous mix of both tumor-derived (TEX) and non-malignant cell-derived exosomes. Therefore, it is hard to definitively conclude from these studies that the TEX alone are mediating the immunosuppressive phenotype. A recent study used immunoaffinity-based separation of human plasma exosomes to distinguish melanoma cell-derived exosomes (MTEX) from normal-cell derived exosomes (non-MTEX) [20]. They found MTEX alone were capable of downregulating expression of CD69 and inhibiting proliferation of $CD8^+$ T cells in a PD-1 dependent manner. While this data suggests tumor-derived exosomes mainly drive immunosuppression, it should be noted that non-MTEX also displayed low levels of immunosuppression and immunosuppressive ligands.

Exosomes' expression of PD-L1 on their surface allows them the ability to engage with other immune cell types not only locally within the microenvironment of their parent tumor but also systemically (Table 1). The presence and level of PD-L1 expression on tumor-derived exosomes in the peripheral blood of patients positively correlates with disease staging indicating that exosomes are an important mechanism utilized by tumors to mitigate host immune responses. A cohort of emerging evidence demonstrates in a wide variety of cancers that exosomes are able to induce an immunosuppressive phenotype through direct ligation of PD-1 resulting in inhibition of cytotoxic T cell function thus enabling tumor immune escape.

Exosomal Induction of PD-L1 on Secondary Cell Types

Exosomes have been found to modulate the immune system through an alternative, indirect mechanism by inducing PD-L1 expression on a secondary cell type. Labeling of exosomes from a variety of tumor models revealed that mainly phagocytic cells, over lymphocytes, uptake exosomes in their local microenvironment. $CD14^+$ monocytes from glioblastoma patients compared to healthy controls demonstrated increased uptake of labeled exosomes from glioblastoma stem cells [21]. Further characterization of these monocytes after treatment with glioblastoma derived exosomes revealed increased expression of MHC II, CD80, CD163, and CD206 in addition to PD-L1. Additionally, $CD14^+$ cells resected directly from glioblastoma multiform patient samples showed increased PD-L1 expression compared to these in the peripheral blood from healthy donors. The increased PD-L1 expression correlated with increased pSTAT-3, a known transcription factor in immunosuppressive M2 macrophages, and in some cases with increased phosphorylated p70S6 kinase and Erk1/2, suggesting MAPK and mTOR signaling pathways are involved in regulating the PD-L1 expression as well.

Similarly, mice receiving a single injection of labeled E0771 breast cancer derived exosomes showed the highest exosome signal in $F4/80^+CD11b^+$ macrophages in the lungs [19]. However, in this model, both subsets of αβ T cells and NK cells in the lung also demonstrated uptake signal. It is interesting to note in this experiment the exosomes trafficked predominately to the lungs over other highly perfused organs like the spleen or bone marrow. This data supports the concept that exosomes possess organotropic integrins that pre-dictate their tissue destination allowing for metastatic organotropism, the pattern of primary tumor metastasis to specific secondary tissues like breast cancer to the lung [22]. One exception to this model is the induction of PD-1 on mesenchymal stem cells from melanoma-derived exosomes [23]. The increased PD-1 expression on this cell type augmented *in vivo* tumorigenesis and tumor progression in a manner akin to exosomal stimulated $PD\text{-}L1^+$ macrophages.

One proposed mechanism by which tumor-derived exosomes drive PD-L1 expression on secondary cells is through toll-like receptors (TLRs). The non-coding RNA, Y RNA hY4, abundantly found in exosomes derived from chronic lymphocytic leukemia (CLL), has been shown to polarize monocytes towards a key CLL promoting, $PD\text{-}L1^+$ phenotype through TLR7 ligation [24]. Similarly, while the exact TLR has not been identified, miR-23a-3p in hepatocellular carcinoma-derived exosomes increased PD-L1 expression on peritoneal macrophages [25].

Overall, exosomal stimulation of macrophages/ monocytes results in polarization towards a protumorigenic phenotype, often characterized by increased PD-L1 expression and immunosuppression. However, the exact mechanisms behind the increased PD-L1 expression remains to be elucidated and is a target for future research.

Effect of Exosomal PD-L1 on Immunotherapy

The use of immune checkpoint therapies as first line treatment for a variety of cancers has become more prominent in recent years [26–28]. However, despite widespread use, the

clinical responses are still low [5, 29]. Therefore, gaining a better understanding of the mechanisms governing PD-L1 mediated immune evasion is necessary. It has been proposed that exosomal expression of PD-L1 is one mechanism by which primary immune checkpoint therapy fails [9]. Here we will review the current literature demonstrating the effect of PD-L1^{+} exosomes on immunotherapy and the associated impact on survival outcomes.

As previously described, loss of PD-L1 expression from primary tumors or depletion of exosome secretion via genetic manipulation or pharmacological inhibition decreased metastatic burden and increased overall survival in a variety of tumor-bearing mice [6–9, 30]. BALB/c 4T-1 tumor-bearing mice treated with exosome secretion inhibitor GW4869 and α-PD-L1 mAb showed the greatest decrease in primary tumor burden indicating a synergistic relationship between exosome depletion and immune checkpoint therapy [6]. Similarly, a tetracycline inducible Rab27 knockdown 4T1 cell line dramatically augmented anti-PD-L1 efficacy as compared with either monotherapy alone [6]. MC38 Rab27$^{-/-}$ tumor bearing mice receiving anti-PD-L1 mAb exhibited increased overall survival compared with either monotherapy alone [7]. A similar result was seen in a B16-F10 melanoma mouse model treated with CD63 and PD-L1 antibodies [9].

The most striking data suggesting a role for exosomes in driving PD-L1 mediated immune evasion focuses on the correlation between exosomal PD-L1 and treatment response. These studies suggest that monitoring circulating exosomal PD-L1 could be a useful way to predict tumor response to immunotherapy [9–11]. First, Chen *et al.* showed that patients who ultimately did not respond to anti-PD-1 therapy had increased levels of PD-L1^{+} circulating exosomes prior to treatment. Soluble PD-L1, microvesicular PD-L1 levels, and PD-L1 from sources other than extracellular vesicles were not statistically significant between responders and non-responders. However, if patients had low levels of exosomal PD-L1 prior to treatment and demonstrated a greater than 2.43 fold change in exosomal PD-L1 during the course of anti-PD-1 treatment, they were more likely to ultimately respond successfully to the therapy. It is thought that the increased PD-L1 expression on the exosomes is derived from increased expression on the tumor cells resulting from reinvigoration of IFN-γ producing CD8^{+} T cells. Patients lacking increased exosomal PD-L1 are presumed to be lacking an adequate reinvigoration of the IFN-γ T cell response which could explain treatment failure.

It is interesting to note that another group studying the association between exosomal PD-L1 and immunotherapy response found that an increase in circulating exosomal PD-L1 >100 pg/mL during treatment duration demonstrated a 91% positive predictive value for disease progression [10]. Similar work in HNSCC also showed patients with recurrence during treatment had increases in the TEX/total exosome ratio as well as in the number of CD3(−)PD-L1^{+} exosomes from baseline [11]. The conflicting nature of these reports highlights the importance of distinguishing TEX from non-TEX in human plasma samples and warrants further investigation into how each subset contributes to overall PD-L1 expression and disease progression.

High levels of exosomal PD-L1, either at baseline or during treatment, could antagonize anti-PD-L1 therapy by binding to the antibody itself allowing for continued exposure to

tumoral PD-L1 (Figure 1). More likely, however, is that exosomal PD-L1 is resistant to anti-PD-1 therapy and continues to exert T cell immunosuppression both directly and indirectly despite active antibody treatment. Either way, patients with the highest levels of circulating pre-treatment PD-L1$^+$ exosomes had the greatest tumor burdens, the most immunosuppression, and overall the poorest clinical outcomes [9]. Therefore, going forward it is necessary to further investigate definitively how exosomal PD-L1 adversely impacts immunotherapy and whether exosomal depletion is a viable and necessary concomitant therapy in order to generate the most efficacious immune checkpoint responses.

Concluding Remarks

Tumor-derived exosomes have been shown to promote tumor growth and metastasis in a variety of cancers. In this opinion article we have shown how one specific tumor promoting characteristic, PD-L1 expression on exosomes, imparts an immunosuppressive phenotype through three main mechanisms: direct endogenous exosomal PD-L1, indirect induced PD-L1, and PD-L1 mediated antagonism of immunocheckpoint blockade therapy (Figure 1). In general, exosomes from metastatic tumor cells display increased PD-L1 expression compared with non-metastatic cells. Furthermore, the level of PD-L1 expression on exosomes often positively correlates with severity of cancer staging and negatively with survival in a wide array of primary cancers. This finding in particular strongly supports the notion that PD-L1$^+$ exosomes specifically play an important role in mediating tumor progression.

While extensive studies have shown that exosomal PD-L1 is capable of decreasing T cell effector function in a PD-1 specific manner, the majority of these studies used exosomes from tumor cell line supernatants which is not a true mimic of the clinical picture. Exosomes isolated from the plasma of human patients are a heterogeneous mix of both tumor cell-derived and normal-cell derived exosomes. Therefore, going forward it is important to distinguish the effects of both of these subsets on mediating systemic immunosuppression. Current studies report conflicting evidence, particularly in the case of melanoma, about exosomal PD-L1 as a predictor for tumor response to immunotherapy treatment. One group reported that increased exosomal PD-L1 expression during treatment is indicative of treatment response while another suggests it demonstrates treatment failure. One hypothesis to explain these findings is differential use of heterogeneous versus tumor cell-derived exosomes.

Patients that have high levels of exosomal PD-L1 either at baseline or during treatment are often resistant to immunotherapy. One important area of future research is to identify the factors responsible for mediating the higher or lower exosomal PD-L1 levels at baseline in different patient cohorts (see Outstanding Questions). Furthermore, in patients with high exosomal PD-L1 expression prior to treatment, is exosome depletion a necessary adjunct therapy to lessen systemic immunosuppression, making patients more sensitive to anti-PD-1 therapy? Deepening our understanding of how tumor-derived exosomes influence the overall immune profile of a cancer patient is critical to optimizing current immune therapies and designing novel approaches to achieve the most robust and successful patient outcomes.

Using exosomal PD-L1 as a predictive marker for immunotherapy treatment response is a growing trend in the field. While this approach is less invasive than biopsy and could be more accurate, many questions regarding this technique still remain. Specifically, if increases in exosomal PD-L1 is indicative of treatment resistance or failure, how is exosomal PD-L1 contributing to this response? Is it through direct exosmal PD-L1 ligation of T cells or is it indirectly through secondary cell types like immunosuppressive macrophages? Providing answers to these questions can help to quickly optimize and develop exosomal PD-L1 as a predictive screening tool for use in the clinic. Having a more accurate understanding of what exosomal PD-L1 levels indicate in terms of treatment response will allow physicians to make appropriate changes in treatment strategy in a timely manner and increase the likelihood that patients achieve a more successful and durable response to immunotherapy.

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Questions

- How does endogenous exosomal PD-L1 impact on the efficacy of anti-PD-1 therapy?
- How does exosomal induced PD-L1 expression in macrophages effect anti-PD-1 therapy?
- What causes cancer patients to have higher or lower exosomal PD-L1 levels prior to the onset of immunotherapy?
- Should exosome elimination be considered for cancer patients with high exosomal PD-L1 expression prior to the onset of immunotherapy?

Trends

- Levels of exosome PD-L1 expression positively correlate with tumor staging and disease progression and negatively correlate with survival
- Tumor derived exosome PD-L1 is capable of directly binding and inhibiting T cell effector function
- Tumor derived exosomes induce increased PD-L1 expression in macrophages/monocytes
- Combination therapy of anti-PD-1 with exosome depletion decreases tumor burden and increases overall survival indicating an antagonistic role for exosome PD-L1 in immune checkpoint blockade therapy

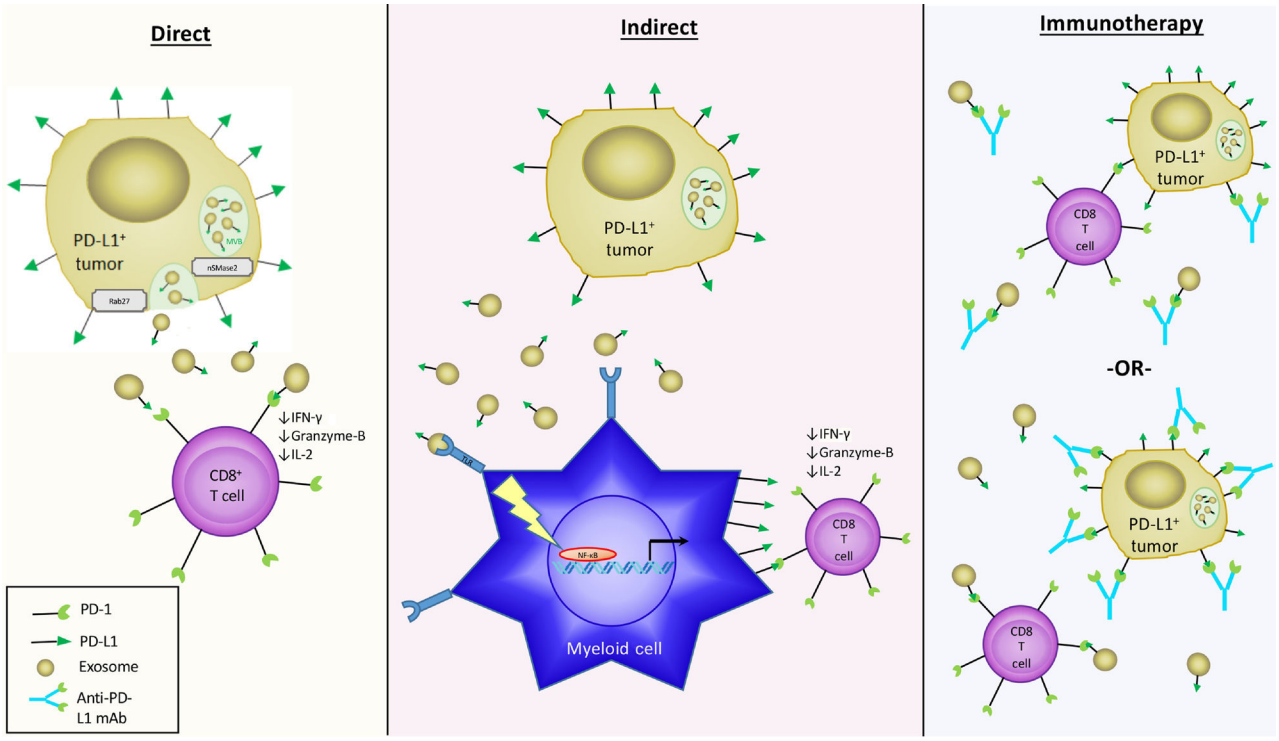

Figure 1. Mechanisms of Exosome PD-L1 Mediated Immunosuppression.

PD-L1$^+$ exosomes have been found to impart an immunosuppressive phenotype through both direct and indirect mechanisms. In general, exosomes form from the inward budding of vesicles within the multivesicular body (MVB). They are then released extracellularly through the function of Rab27 and nSMase2. In the direct mechanism (left panel), PD-L1 on the surface of tumor-derived exosomes binds directly to PD-1 on the surface of T-cells, inhibiting effector function and release of pro-inflammatory cytokines IFN-γ, IL-2, and granzyme B. In the indirect mechanism (middle panel), tumor-derived exosomes bind to a toll-like receptor (TLR) on the surface of a secondary cell type, namely myeloid cells, to induce PD-L1 expression which then binds to PD-1$^+$ T cells and inhibits effector function. Lastly, exosomal PD-L1 has been implicated as a mechanism of resistance in immunotherapy (right panel). Specifically, PD-L1$^+$ exosomes are thought to either bind to anti-PD-L1 mAb leaving tumor PD-L1 exposed or PD-L1$^+$ exosomes bind directly to PD-1 on effector T cells despite mAb treatment. In both scenarios, the blocking effect of the antibody is negated allowing for continued PD-L1 mediated immunosuppression.

Table 1.

The Effect of Exosome PD-L1 in Mouse and Human Models of Cancer

Origin of exosome	Target cells	Exosome characteristic	Effect	Refs
B16-F10 murine melanoma cell line	Purified $CD8^+$ mouse splenocytes	PD-$L1^+$	Decreased proliferation and granzyme-B production	[9]
EO771 murine breast carcinoma cell line	$CD4^+$/$CD8^+$ T cells; NK cells	PD-$L1^+$	Decreased proliferation of $CD4^+$ and $CD8^+$ T cells; decreased cytotoxic activity of NK cells against tumor cells	[19]
Lewis Lung Carcinoma-murine adenocarcinoma	AT-II cells (fresh or from MLE-12 cells)	snRNAs	Induced pro-tumor neutrophil recruiting chemokine expression	[31]
A549; H460; H1975-non-small cell lung adenocarcinoma	Jurkat Cells; NSCLC Patient $CD8^+$ T cells	PD-$L1^+$	Decreased IFN-γ production; increased apoptosis	[8]
Plasma Head and neck squamous cell carcinoma (HNSCC) patients		PD-$L1^+$	Decreased CD69 expression on human $CD8^+$ T cells; Relative exosomal PD-L1 expression associated with disease activity and clinical staging	[13]
MGC-803; SGC-7901- human gastric cancer cell line	THP-1 Cells	PD-$L1^+$	Increased IL-6, TNF-α, CCL2 secretion in an NF-κB dependent manner	[32]
MDA-MB-231 human breast cancer cell line	Human PBMC	PD-$L1^+$	Decreased T-cell mediated tumor killing; decreased ERK phosphorylation and NF-κB activation	[6]
MDA-MB-231 human breast cancer cell line	RawkB cells; THP-1 cells, BMMΦ	Palmitoylated proteins	Increased IL-6 and TNF-α secretion in a NF-κB dependent manner	[33]
Primary human Glioblastoma stem like cells (G34, G35, G44, G157)	Human PBMC	PD-$L1^+$	Decreased CD69 and CD25 expression in both $CD4^+$ and $CD8^+$ cells; decreased proliferation	[14]
MEC-1 human chronic lymphocytic leukemia cell line	J774 cells	Y RNA- hY4	Increased PD-L1 expression; increased CCL2, CCL4, and IL-6 secretion from monocytes	[24]
Glioblastoma human stem like cells (GSC20, GSC267, GSC17)	$CD14^+$ monocytes		Increased PD-L1 expression and phosphorylation of STAT3	[21]
Mel624 melanoma cell lines; WM9 melanoma cell line	Human peripheral $CD8^+$ T cells	PD-$L1^+$	Inhibited proliferation and Granzyme-B production; inhibited IL-2, IFNγ, and TNF-α secretion	[9]
H1264 human non-small cell lung carcinoma	Human peripheral $CD8^+$ T cells	PD-$L1^+$	Inhibited proliferation and Granzyme-B production	[9]
Pancreatic Ductal Adenocarcinoma Patients		PD-$L1^+$	Patients with PD-$L1^+$ exosomes had overall shorter postoperative survival time compared with PD-L1 negative patients	[17]
Tunicamycin treated HepG2 cells	mTHP-1 cells, peritoneal macrophages, and RAW264.7 cells	miR-23a-3p	Exosomal miR-23a-3p increased PD-L1 expression in macrophages via a PTEN/AKT dependent mechanism	[25]
B16F1 cell lines	Mesenchymal stem cells		Increased PD-1 expression on MSC augmenting tumorigenesis and progression	[23]
HCC1954 human breast ductal carcinoma		PD-$L1^+$	Endosomal sorting complexes required for transport (ESCRT) component ALIX was responsible for exosomal PD-L1 expression	[12]
Melanoma Patients ($CSPG4^+$ MTEX)	Human CD8+T cells	PD-L1	Decreased CD69 expression in a PD-L1 dependent manner	[20]
Melanoma Patients ($CSPG4^+$ MTEX)	Human NK cells	PD-L1	Downregulated NKG2D	[20]
SK-MEL-2	Human CD8+ T cells	PD-L1	Decreased PD-1, Ki67, and IFN-γ expression	[10]