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Invited Mini Review

Perspectives on immune checkpoint ligands: expression, regulation, and clinical implications

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In the tumor microenvironment, immune checkpoint ligands (ICLs) must be expressed in order to trigger the inhibitory signal via immune checkpoint receptors (ICRs). Although ICL expression frequently occurs in a manner intrinsic to tumor cells, extrinsic factors derived from the tumor microenvironment can fine-tune ICL expression by tumor cells or prompt non-tumor cells, including immune cells. Considering the extensive interaction between T cells and other immune cells within the tumor microenvironment, ICL expression on immune cells can be as significant as that of ICLs on tumor cells in promoting anti-tumor immune responses. Here, we introduce various regulators known to induce or suppress ICL expression in either tumor cells or immune cells, and concise mechanisms relevant to their induction. Finally, we focus on the clinical significance of understanding the mechanisms of ICLs for an optimized immunotherapy for individual cancer patients. [BMB Reports 2021; 54(8): 403-412]

INTRODUCTION

In cancer, it is the cytolytic action of cytotoxic lymphocytes that the immune system mainly elicits to restrain disease progression. However, CD8 T cells in the tumor microenvironment frequently undergo 'exhaustion', which is a distinct developmental process, because they are faced with sustained antigenic stimulation. Exhausted CD8 T cells persist but gradually lose their effector function, cytotoxicity, and proliferative capacity, leading to incompetent immunosurveillance.

Exhausted CD8 T cells typically express a panoply of inhibitory immune checkpoint receptors (ICRs), which are triggered by cognate ligands to regulate T-cell response via a downstream signaling pathway. For instance, programmed cell death 1 (PD-1, CD279), one of the notable ICRs, is expressed on exhausted CD8 T cells. Upon being bound with programmed cell death-ligand 1 (PD-L1, B7-H1, CD274), PD-1 counters either CD28 signaling or a T-cell receptor (TCR) by activating SH2 domain-containing protein tyrosine phosphatase-2 (SHP-2) and renders the T cells hypofunctional in many ways (1). Therefore, in exhausted CD8 T cells, blocking the interactions between ICRs and immune checkpoint ligands (ICLs) is a plausible strategy for de-repressing CD8 T cells. Indeed, immune checkpoint blockade therapy has demonstrated clinical efficacy against various types of tumors (2). Multiple immune checkpoint pathways other than PD-1/PD-L1 have been investigated (3), and the pathways vary from patient to patient because of the heterogeneity of the tumor microenvironment. Since most ICRs were co-expressed as part of a larger co-inhibitory gene program (4), the 'functional' inhibitory pathways are determined by the expression of ICLs. Therefore, it is essential to understand which factors contribute to the inter-patient differences in ICL expressions. In this review, we summarize various factors that modulate the expression of ICLs within the tumor microenvironment (Table 1).

PD-1 LIGANDS

The promising outcome of therapies targeting the PD-1 axis has highlighted the need to elucidate the molecular regulation of its ligands. PD-1-mediated T-cell inhibition can be attributed to both of two well-known ligands of PD-1, which are PD-L1 and programmed cell death-ligand 2 (PD-L2, B7-DC, CD273) (Figs. 1 and 2).

PD-L1

Given that antibodies against PD-L1 have shown an efficacy similar to that of the antibodies against PD-1, which can block interactions with both PD-L1 and PD-L2, PD-L1 is a more dominant ligand of PD-1 than is PD-L2. PD-L1 expression by tumor cells often occurs during malignant transformation and without ongoing immune response (5). However, apart from intrinsic factors, which give rise to constitutive expression of PD-L1 in tumor cells, extrinsic factors within the tumor micro-

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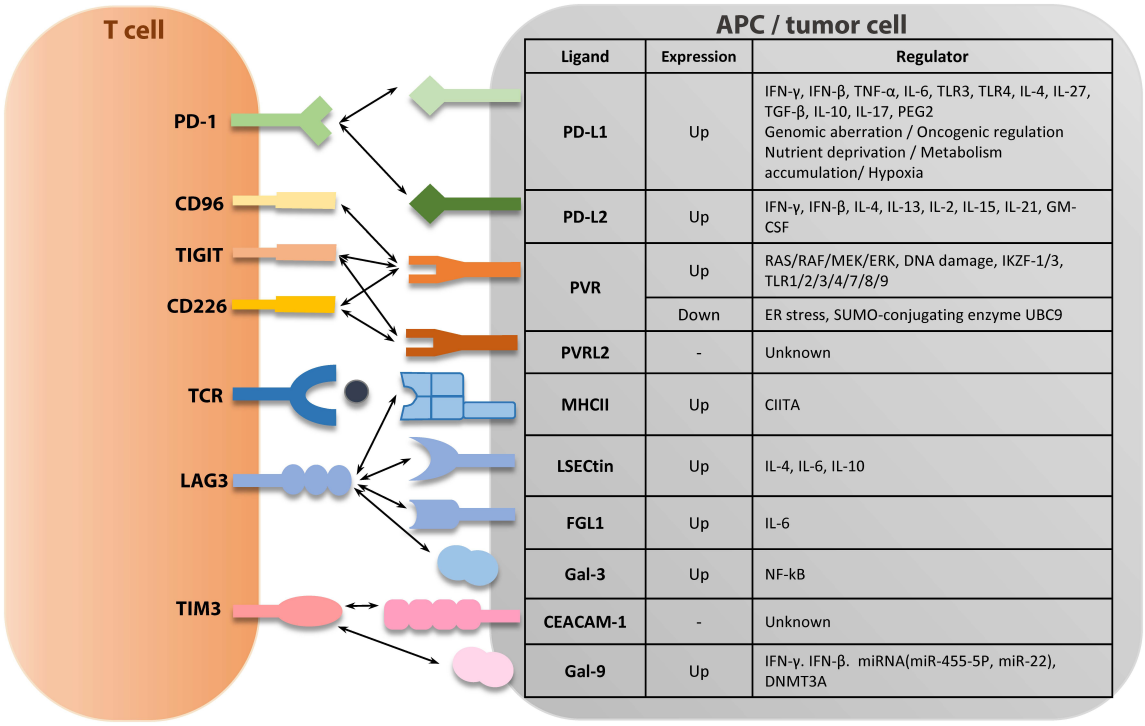
Table 1. Overview of multiple immune check point ligand regulators

Receptor	Ligand	Regulator	Expression	
			Tumor cell	Immune cell
PD-1	PD-L1	IFNγ	Multiple tumor cell (10, 11)	Dendritic cell (24, 25), neutrophil (27), monocyte/macrophage (24, 26)
		IFNβ	H melanoma (11)	Dendritic cell (28), neutrophil (27)
		TNFα	H breast cancer (12), H prostate/ colon cancer (13), H renal cell carcinoma (14)	Dendritic cell (29), monocyte/macrophage (30, 31)
		IL-6	H prostate cancer (15), H lung cancer (16)	Dendritic cell (29), monocyte/macrophage (32)
		TLR3	H neuroblastoma (21)	Dendritic cell (40)
		TLR4	H bladder cancer (20)	Dendritic cell (43), monocyte/macrophage (41, 42)
		IL-4	H renal cell carcinoma (14)	-
		IL-27	H ovarian cancer (18)	-
		TGFβ	H lung cancer (19)	-
		IL-10	-	Monocyte (33, 35, 36)
		IL-17	-	Monocyte/macrophage (33)
		IL-1β	-	Dendritic cell (29)
		PGE2	-	Monocyte/macrophage (44)
		Nutrient deprivation	Multiple tumor cell (6, 7)	-
		Metabolite accumulation	H lung cancer (8)	-
		Hypoxia	Multiple tumor cell (9)	-
		Oncogenic signaling	Multiple tumor cell (5)	-
		microRNA	Multiple tumor cell (5)	-
	PD-L2	IL-4	H esophageal adenocarcinoma (49)	Dendritic cell (51), monocyte/macrophage (42, 51, 52)
		IFNγ	H melanoma (11), H lung cancer (19), H colorectal cancer (47), H brain tumor (48)	Monocyte/macrophage (24, 36, 50)
		IFNβ	H melanoma (11)	
		IL-13	H esophageal adenocarcinoma (49)	
		IL-2/IL-15/IL-21	-	Monocyte/macrophage (54)
		GM-CSF	-	Dendritic cell/macrophage (53)
TIGIT	PVR	RAS/RAF/MEK/ERK	Fibroblast (60)	-
		DNA damage	H melanoma (63, 64)	-
		IKZF-1/3	H melanoma (65)	-
		ER stress	H hepatoma (66)	-
		SUMO-conjugating enzyme UBC9	H melanoma (67)	-
		TLR1/2/3/4/7/8/9	-	Dendritic cell/macrophage (69)
TIM3	Galectin-9	IFNγ	-	Monocytes/gMDSCs (74, 79)
		IFNβ	H leukemia/M colon cancer (73)	-
		microRNA	H chondrosarcoma (80), liver cancer (81)	-
		DNMT3A	H cervical cancer (82)	-
LAG3	MHCII	IFNγ	H osteosarcoma/H melanoma (84)	-
	Galectin-3	NF-kB	H leukemia (89)	Macrophage (90)
	LSECtin	IL-6/IL-10	H/M melanoma (86)	-
		IL-4	-	Dendritic cell (91)
	FGL-1	IL-6	H hepatocellular carcinoma (93)	-

M, mouse; H, human.

environment can contribute to PD-L1 expression. Many of the factors in the tumor vicinity, such as nutrient deprivation (6, 7), metabolite accumulation (8), and hypoxia (9), are observed in common in different types of tumors, concomitantly causing PD-L1 upregulation. Further, an inflammatory tumor microenvironment provides the assorted factors that adjust PD-L1



Ligand	Expression	Regulator
PD-L1	Up	IFN-γ, IFN-β, TNF-α, IL-6, TLR3, TLR4, IL-4, IL-27, TGF-β, IL-10, IL-17, PEG2 Genomic aberration / Oncogenic regulation Nutrient deprivation / Metabolism accumulation/ Hypoxia
PD-L2	Up	IFN-γ, IFN-β, IL-4, IL-13, IL-2, IL-15, IL-21, GM-CSF
PVR	Up	RAS/RAF/MEK/ERK, DNA damage, IKZF-1/3, TLR1/2/3/4/7/8/9
	Down	ER stress, SUMO-conjugating enzyme UBC9
PVRL2	-	Unknown
MHCII	Up	CIITA
LSECtin	Up	IL-4, IL-6, IL-10
FGL1	Up	IL-6
Gal-3	Up	NF-kB
CEACAM-1	-	Unknown
Gal-9	Up	IFN-γ. IFN-β. miRNA(miR-455-5P, miR-22), DNMT3A

Fig. 1. Multiple immune checkpoint receptor-ligand interactions between T cells and APCs or tumor cells. The regulators of each immune checkpoint ligand are indicated on the right side (Up, positive regulator; Down, negative regulator).

expression. IFN-γ, primarily secreted by effector T cells and NK cells, is the most potent inducer of PD-L1 for various tumor-cell lines (10); hence PD-L1 expression in tumor cells may reflect concurrent T-cell responses. Mechanistically, in human melanoma cell lines, IFN-γ-induced upregulation of PD-L1 is mediated by JAK1/2 – STAT1 activation and, eventually, direct binding of Interferon Regulatory Factor 1 (IRF1) to PD-L1 promoter (11). Garcia-Diaz *et al*. also clarify that IFN-α and IFN-β induce PD-L1 expression, but to a lesser extent than does IFN-γ (11). Besides IFN, other inflammatory mediators regulate PD-L1 expression as well. For example, TNF-α increases PD-L1 expression in human breast cancer cells by promoting deubiquitination mediated by COP9 signalosome complex subunit 5 (CSN5) (12). When treated alone or in combination with IL-17, TNF-α upregulates PD-L1 expression in both human prostate cancer and colon cancer cells, mediated by the Akt/NF-κB and ERK/NF-κB pathways, respectively (13). In human renal cell carcinoma, TNF-α or IL-4 increases PD-L1 expression, accompanied by NF-κB or STAT6 activation, respectively. Combined treatment of the two has additive effects (14). IL-6 has been reported to increase transcription of the PD-L1 gene by means of either JAK/STAT3 signaling in human prostate cancer cells (15) or MEK/ERK signaling in human lung cancer cells (16). IL-6 also increases PD-L1 expression at the post-transcriptional level by JAK1-mediated phosphorylation, which promotes glycosylation and stabilization of the PD-L1 protein in hepatocellular carcinoma cells (17). IL-27 can upregulate PD-L1 by means of STAT3 signaling in human ovarian cancer cell lines (18). Among anti-inflammatory cytokines, TGFβ was reported to increase PD-L1 expression in an Smad2-dependent manner in human lung cancer cells (19). Other than cytokines, stimulation of Toll-Like Receptor 4 (TLR4) by a lipopolysaccharide (LPS) induces PD-L1 expression in human bladder cancer cells via the ERK/JNK pathway (20). In human neuroblastoma cells, PD-L1 can be upregulated in response to TLR3 stimulation, with simultaneous TLR9 ligation mitigating TLR3-mediated upregulation of PD-L1 (21).

Though PD-L1 expression is often represented by a tumor proportion score, which quantifies PD-L1 expression solely from viable tumor cells, accumulating evidence supports that a combined positive score, which integrates PD-L1 expression of tumor and non-tumor cells, is a more predictive biomarker (22), implying the significance of PD-L1 expressed by non-tumor cells in PD-1 blockade. Indeed, PD-L1 expressed by tumor-infiltrating immune cells promotes immune escape via diverse mechanisms (23). PD-L1 expressed on antigen-presenting cells (APCs), including dendritic cells and macrophages, delivers inhibitory signals during crosstalk with PD-1-expressing T cells or sequesters co-stimulatory molecule, CD80, in *cis*. Also, PD-L1 from activated T cells engages with PD-1 expressed on

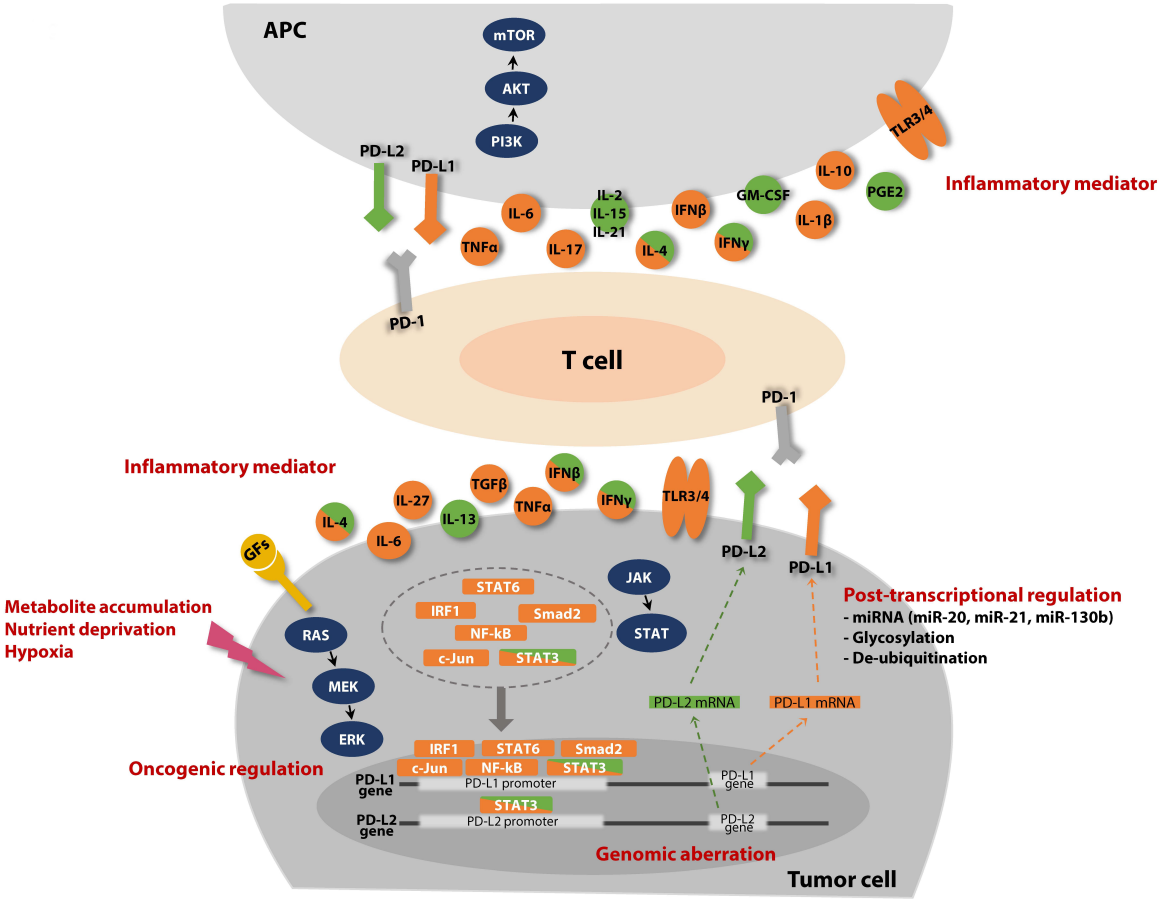


Fig. 2. Schematic overview of PD-L1 and PD-L2 positive-expression regulators in APCs (Up) or Tumor cells (Down). Molecules colored in orange or green regulate PD-L1 or PD-L2 expression, respectively. Molecules colored in orange and green regulate PD-L1 and PD-L2 expression at the same time.

other T cells or macrophages, the latter promoting M2 polarization. By the way, PD-L1 expressed on T cells can lead to an anergic state or apoptosis in activated T cells by acting as a receptor *per se*.

As in tumor cells, IFN-γ induces PD-L1 in multiple types of immune cells, including monocytes (24, 25), monocytes-derived dendritic cells (24, 26), macrophages (26), and neutrophils (27). Although marginal, IFN-β also increases PD-L1 expression in neutrophils (27) and dendritic cells (28). Among other pro-inflammatory cytokines, TNF-α, IL-1β, or IL-6 upregulates PD-L1 expressed on monocyte-derived dendritic cells (29), whereas PD-L1 expressed on monocytes/macrophages is upregulated by TNF-α (30, 31), IL-6 (32), or IL-17 (33). However, IL-17-induced upregulation of PD-L1 is indirectly mediated by several cytokines, particularly IL-10, produced by IL-17-activated monocytes. Another inflammatory cytokine, IL-12, regulates PD-L1 expressed on macrophages (34). Though IL-12 upregulates PD-L1 expression in monocyte-derived macrophages by means of increased IFN-γ production, it also can downregulate PD-L1 expression in THP-1, a human monocytic cell line, presumably by decreased IL-10 production with inability to produce IFN-γ.

The role of anti-inflammatory cytokines in regulation of PD-L1 expression has also been investigated. As briefly mentioned above, IL-10 upregulates PD-L1 expression in monocytes (33, 35, 36) and dendritic cells (37). Yet, IL-10-induced upregulation of PD-L1 is observed only in immature, monocyte-derived dendritic cells, not in LPS-matured one. The contradictory role of TGFβ in determining PD-L1 expression was observed, in that it upregulates PD-L1 expression in dendritic cells (38, 39), but downregulates it in monocytes (30). Also, TLR3 signaling induces PD-L1 expression in dendritic cells (40), and TLR4 signaling induces PD-L1 expression in monocytes (41), macrophages (42), and dendritic cells (43). Prostaglandin E2 (PGE2), which is a bioactive lipid that is closely connected with inflammation, is also involved in the induction of PD-L1 expression in myeloid cells, including macrophages and myeloid-de-

rived suppressor cells (MDSCs) (44).

PD-L2

As a second ligand for PD-1, PD-L2 also endows PD-1 with an inhibitory function, although its mechanisms are not fully understood. Given that PD-L2 binds to PD-1 with a higher affinity than that of PD-L1 (45) and that its expression has been reported in many human malignancies, it is worth speculating about how its expression is regulated within the tumor microenvironment. PD-L2 expression was initially thought to be restricted to dendritic cells or macrophages, but recent studies reveal that PD-L2 expression is less restricted than previously thought. For example, a considerable proportion of peritoneal B1 B cells constitutively express PD-L2, which positivity enriches Phosphatidylcholine-specific B1 cells, as is crucial for innate defense against invading pathogens (46).

Like PD-L1, PD-L2 is upregulated by IFN-γ treatment in tumor cells (11, 19, 47, 48). In line with how IFN-γ orchestrates PD-L2 upregulation in tumor cells, IFN-β has a similar effect via promoting STAT3 interaction with PD-L2 promoter (11). IL-4 and IL-13, T_h2-type cytokines produced during Barrett's metaplasia, have been reported to induce PD-L2 expression in esophageal adenocarcinoma (49).

In immune cells, IFN-γ and T_h2 cytokines are involved in PD-L2 expression. IFN-γ induces PD-L2 expression in human monocytes (24, 36, 50), and IL-4 induces in murine macrophages (42, 51, 52) and dendritic cells (51) in a STAT6-dependent manner. Yamazaki *et al*. also identified the granulocyte-macrophage colony stimulating factor (GM-CSF) as an inducer of PD-L2 in both macrophages and dendritic cells, but its regulatory mechanism, which encompasses a transactivation effect of PU.1/ IRF4 and histone modification by PU.1/ p300, has been demonstrated recently (53). Additionally, common γ chain cytokines, such as IL-2, IL-15, and IL-21, can induce PD-L2 expression in monocytes or macrophages (54). IL-10 also upregulates PD-L2 in monocytes (36), but downregulates PD-L2 in mature dendritic cells (24).

T-CELL IMMUNORECEPTOR WITH IG AND ITIM DOMAINS (TIGIT) LIGANDS

TIGIT is an inhibitory receptor, mainly expressed by NK cells, regulatory T cells, memory T cells, and exhausted CD8 T cells. When TIGIT was identified for the first time, it was also reported that human TIGIT can bind to three ligands, Poliovirus receptor (PVR, NECL5, CD155), PVR-related 2 (PVRL2, Nectin2, CD112), and PVR-related 3 (PVRL3, Nectin3, CD113), among which PVR has the highest affinity for TIGIT (55). PVR/ TIGIT engagement suppresses T-cell responses by phosphorylating immunoreceptor tyrosine-based inhibitory motif (ITIM) in the cytoplasmic tail of TIGIT or disturbing PVR/DNAX-associated molecule 1 (DNAM-1, CD226) engagement, which bolsters T-cell response.

PVR is an adhesion molecule frequently overexpressed in many types of solid and hematological malignancies, and its overexpression is associated with poor prognosis (56-59). Since PVR is a member of the Nectins and Nectin-like (Necl) family of molecules and is involved in various physiological processes, including cell-cell adhesion, movement, proliferation, and differentiation, its overexpression can have a pro-tumorigenic effect in a manner intrinsic to tumor cells. In parallel, the oncogenic RAS/RAF/MEK/ERK signaling pathway upregulates PVR expression by means of direct binding of activator protein-1 (AP-1) to PVR promoter in mouse fibroblasts (60), a fact that is expected to be applicable to murine tumor cells. Despite the presence of the AP-1 binding sequence within the PVR promoter/enhancer in some human tumor cell lines (61), it is unclear if the RAS/RAF/MEK/ERK signaling increases PVR expression in human tumor cells as well. However, in terms of anti-tumor immune responses, whether PVR overexpression has a pro-tumorigenic or anti-tumorigenic role remains uncertain. In regulating the anti-tumor immune response, PVR can bind to three different receptors—DNAM-1, TIGIT, and CD96—and contradictory effects can occur depending on the receptor that it binds to (62). Nevertheless, many previous studies present PVR as a stimulator of NK-cell function, emphasizing its interaction with activating receptor DNAM-1. For example, PVR can be induced as a part of an ATM/ATR-dependent DNA damage response ignited by either inherent genotoxic stress or genotoxic drug treatment in human multiple myeloma (MM). Particularly, when genotoxic stress imposed by nitric oxide and the related reactive nitrogen species induces PVR, the effect depends on transcriptional regulation by E2F1. Subsequently, induced PVR makes these tumors eliminable by means of DNAM-1 ligation *in vitro* (63, 64). Also, immuno-modulatory drugs targeting Cereblon, which breaks down transcriptional repressors of PVR, such as Ikaros family zinc finger protein-1 and -3 (IKZF1/3), also upregulate PVR expression and provoke NK-cell-mediated cytolysis *in vitro* (65). In terms of post-transcriptional regulation, when there is Endoplasmic reticulum (ER) stress, ER-associated degradation-related molecule HRD1 increases and promotes PVR degradation in human hepatoma cell lines (66). In parallel, dysregulated small ubiquitin-like modifier (SUMO) conjugation, which results from SUMO-conjugating enzyme overexpression, also facilitates PVR degradation in human MM cell lines (67). Both studies demonstrated that diminished PVR expression caused tumor cells to evade recognition and elimination by NK cells. However, given that TIGIT binds to PVR with higher affinity than that of DNAM-1 (55, 68) and that TIGIT is highly expressed on tumor-infiltrating lymphocytes, how PVR expression on tumor cells affects effector cells *in vivo* needs further investigations.

Regarding tumor-infiltrating immune cells, it was reported that tumor-associated APCs represent a higher level of PVR than do circulating APCs, although factors responsible for upregulated PVR were not addressed in the study (69). However, since NF-kB signaling increases PVR expression on APCs upon

TLR stimulation (70), an inflammatory microenvironment within tumor tissue may contribute to PVR upregulation in APCs (Fig. 1).

T-CELL IMMUNOGLOBULIN DOMAIN AND MUCIN DOMAIN 3 (TIM-3) LIGANDS

Tim-3, known as a hallmark of exhausted T cells, is one of the most commonly targeted checkpoints for immunotherapy. Four ligands have been described for Tim-3: Galectin-9 (Gal-9), Phosphatidylserine (PtdSer), High-mobility group box 1 (HMGB1), and Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). Among them, Gal-9 and CEACAM1 have been reported to attenuate TCR signaling by dissociating HLA B-associated transcript 3 (BAT3) from Tim-3 (71, 72). Specifically, in the absence of the two ligands, Tim-3 is bound to BAT3, which interacts with Lymphocyte-specific protein tyrosine kinase (Lck). Since Lck-mediated phosphorylation of the TCR complex is critical for TCR downstream signaling, dissociation of BAT3 and subsequently, Lck upon Tim-3 ligation, impairs adjacent TCR downstream signaling and ultimately leads to apoptotic cell death. Furthermore, the intracellular interaction between Tim-3 and CEACAM1 supports maturation and surface trafficking of Tim-3. Therefore, the absence of CEACAM1 within T cells leads to intracellular accumulation of Tim-3 and its inability to interact with ligands, unleashing T cells from Tim-3-mediated inhibition. On the other hand, PtdSer, which is exposed on the outer leaflet of apoptotic cells, triggers Tim-3 expressed on CD8+ dendritic cells or a subset of macrophages and induces phagocytosis; or extracellular HMGB1, a kind of alarmin, can be released into the tumor microenvironment and form a complex with free DNA. Since the formation of the complex assists internalization of DNA into dendritic cells to activate endosomal TLRs, sequestration of HMGB1 by Tim-3 can curtail the formation of the complex and the following dendritic cell activation. Until now, interaction between Tim-3 and PtdSer or HMGB1 has been known to affect T-cell function indirectly, but whether PtdSer or HMGB1 directly affects Tim-3-expressing T cells has to be evaluated.

Although expression of Gal-9 (73-75) or CEACAM1 (72, 76-78) in tumor cells and/or immune cells has been addressed, the factors implicated in their regulation have not been well explored. It has been reported that expression of Gal-9, the first ligand for Tim-3, is regulated by IFN (73, 74, 79). Unlike PD-1 ligands, IFN-β is a more robust regulator of Gal-9 expression in various cell lines than is IFN-γ. Moreover, Gal-9 has to be released extracellularly to serve as a ligand for Tim-3, and IFN-β or IFN-γ also enhances Gal-9 secretion even in some of the tumor cells in which IFN-β or IFN-γ fails to increase Gal-9 expression or macrophages that possess constitutive Gal-9 expression (73). However, the other two studies delineated IFN-γ-mediated upregulation of Gal-9 in monocytes and granulocytic MDSCs, respectively, without covering the effect of IFN-β. Also, microRNA-dependent post-transcriptional regulation (80, 81) and DNA (cytosine-5)-methyltransferase 3A (DNMT3A)-mediated epigenetic modulation (82) of Gal-9 in tumor cells have been reported (Fig. 1).

LYMPHOCYTE ACTIVATION GENE-3 (LAG-3, CD223) LIGANDS

LAG-3 inhibits the anti-tumor immune response by synergizing with PD-1; a combination of anti-PD-1 and anti-LAG-3 is undergoing clinical trials. Notwithstanding the incomplete picture of molecular mechanisms, five ligands for LAG-3 have been discovered so far: MHC II, Galectin-3 (Gal-3), Liver sinusoidal endothelial cell lectin (LSECtin), α-synuclein fibrils (α-syn), and Fibrinogen-like protein 1 (FGL1). Unlike other ligands, LAG-3 bound to α-syn is associated with intercellular delivery of pathological α-syn fibrils in the nervous system, which is irrelevant to immune response. Although it had been reported that LAG-3 can bind to MHC II with high affinity and regulate CD4 T cells by impeding interaction between MHC II and CD4, more recent studies have shown that discriminatory binding of LAG-3 to a stable MHC II/peptide complex (pMHC II) suppresses CD4 T cells by transducing inhibitory signals (83). Moreover, Maruhashi *et al*. also found that APCs that abundantly express stable pMHC II inhibit activation of CD8 T cells via LAG-3-dependent mechanism. In this regard, since MHC II transactivator (CIITA) can induce expression of MHC II accessory molecules, which are involved in pMHC II formation, as well as that of MHC II, IFN-γ-mediated upregulation of CIITA expression may increase pMHC II in various types of cells, including certain tumor cells (84). Alternatively, other ligands, Gal-3 (85), LSECtin (86), and FGL1 (87), have been reported to engage with LAG-3 to negatively regulate CD8 T cells in the tumor microenvironment. Even though Kouo *et al*. demonstrated that intratumoral CD8 T cells and stromal cells are major sources of Gal-3 (85), Gal-3 is also secreted by many types of tumor cells (88). It is reported that Gal-3 is exocytosed from stromal cells and endocytosed by tumor cells in pre-B cell lymphoma. Afterwards, internalized Gal-3 auto-activates Gal-3 transcription accompanied by NK-κB signaling in tumor cells (89). Though the study rarely provides direct evidence for causality between NF-κB and Gal-3, it is conceivable that increased Gal-3 transcription is a result of NF-κB activation, taking into account the NF-κB-mediated Gal-3 expression in other types of cells (90, 91). LSECtin or FGL1 is expressed in the liver under normal physiological conditions and is highly upregulated in some tumor cells (86). Although LSECtin is induced by IL-6 or IL-10 treatment in tumor cells, it can also be induced in human monocyte-derived dendritic cells by IL-4 treatment (92). IL-6 also increases FGL1 in human hepatoma cells (93). Future studies are required to verify the precise molecular mechanisms of these ligands and the possibility of their cooperation in LAG-3-mediated T cell inhibition (Fig. 1).

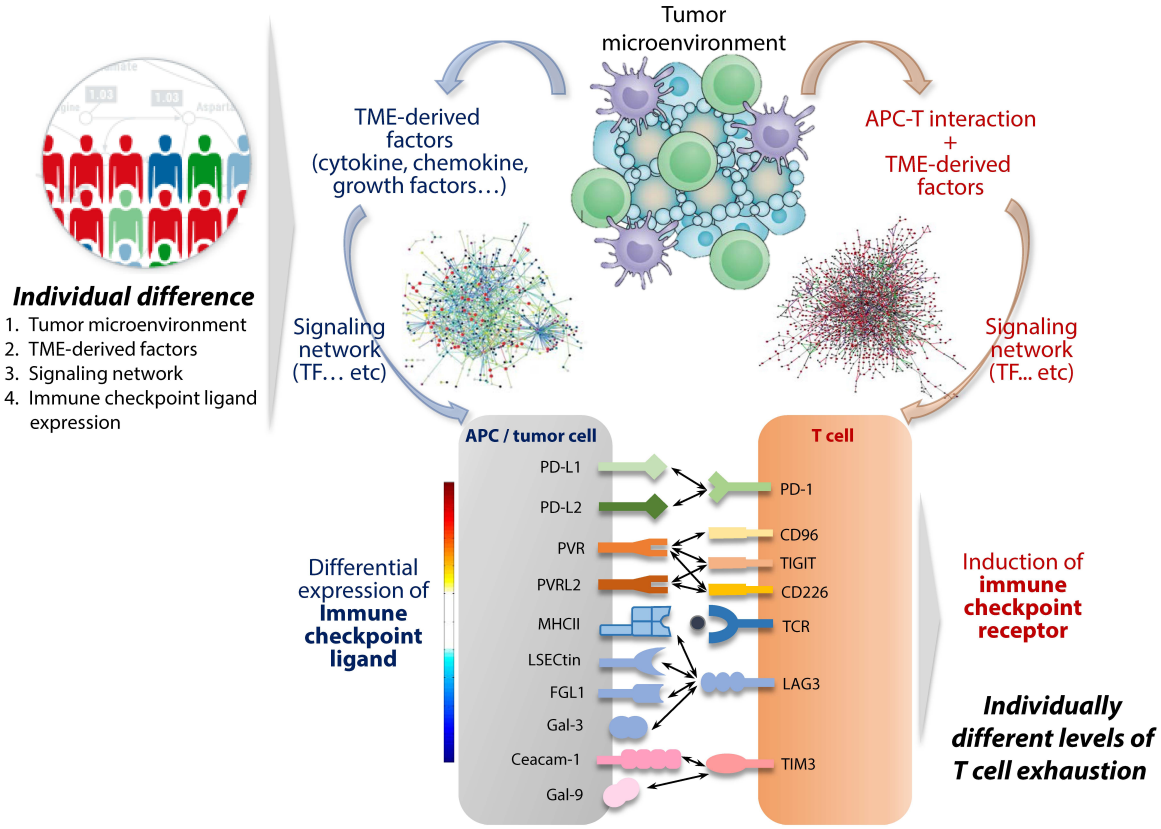


Fig. 3. ICRs are co-regulated and simultaneously expressed by common factors. In contrast, Individual difference in TME-derived factors or signaling network induce diverse patterns of ICLs. Heterogeneity in expression of ICLs can afford patient stratification for customized ICB therapy.

CONCLUSIONS

In the tumor microenvironment, tumor cells build a permissive environment for growth, executing distinct strategies. As a tumor progresses, ICRs are simultaneously induced by common factors, whereas the expression of the corresponding ICLs has overlapping but independent regulation. Further, there are diverse regulators in the tumor microenvironment, each of which may have inconsistent effects on ICL expression depending on the cellular context, creating individually different expressions of ICLs. Because ICL expression patterns could be of relevance to indicate the activity of immune checkpoint pathways, differential expression profiles of ICLs among individuals can be used to predict the treatment response of the immune checkpoint blockade. For instance, many studies have shown that PD-L1 expression is associated with superior response to PD-1 blockade (94). Our previous study demonstrated that the complementary expression patterns of PVR and PD-L1 are key determinants for PD-1 blockade (95). Another study revealed that CD276, which is an incompletely elucidated ICL, could be used as potential biomarker for PD-1 blockade (96). By querying a set of verified ICLs and identifying robust ICL-based biomarkers, it is possible to achieve a higher responder rate and tailor precision immunotherapies. (Fig. 3).

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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