Oral Oncology 51 (2015) 221–228

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Review

Intrinsic and extrinsic control of expression of the immunoregulatory molecule PD-L1 in epithelial cells and squamous cell carcinoma

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ARTICLE INFO

Article history:
Received 13 September 2014
Received in revised form 13 November 2014
Accepted 20 November 2014
Available online 12 December 2014

Keywords:
Head and neck cancer
Squamous cell carcinoma
PD-L1 (B7-H1, CD274)
Carcinogenesis
Immune regulation
Immunotherapy
Keratinocytes
Epithelial–mesenchymal transition

SUMMARY

Recent clinical results for PD-1 blockade therapy have demonstrated durable tumor control with minimal immune-related adverse effects. PD-L1 is induced in non-lymphoid tissue cells and tumor cells, in addition to tissue-recruiting immune cells, under inflammatory conditions triggered by several cytokines, especially IFN-γ, and exogenous stimuli delivered by pathogen-associated molecular patterns. Receptor-mediated signaling molecules that affect the cell cycle, proliferation, apoptosis, and survival (including NF-κB, MAPK, PI3K, mTOR, and JAK/STAT) are involved in PD-L1 induction. PD-L1 expression in tumor cells is also triggered by the signals described above, but in some instances, intrinsic cell alteration associated with carcinogenesis contributes to PD-L1 induction. The tumor suppressor genes *PTEN* and *Lkb1* and epithelial–mesenchymal transition-related molecules are also involved in the regulation of PD-L1 expression. Notably, squamous cell carcinoma of the head and neck (SCCHN) often exhibits both host immunosuppression and cytogenetic alternations of tumor cells. Precise understanding of how PD-L1 expression is controlled will allow the development of effective approaches to PD-1 blockade therapy for patients with SCCHN.

Introduction

Antigen-specific T cell responses are controlled by the balance between co-stimulatory and co-inhibitory signals [1,2]. Modulation of such co-signal pathways is beneficial when immune intervention is necessary. To date, four immunomodulatory biologicals that target co-signal pathways have been approved for clinical use: Abatacept (a fusion protein composed of the Fc region of human immunoglobulin (Ig) G1 fused to the extracellular domain of CTLA-4), Belatacept (a fusion protein composed of the Fc region of human IgG1 fused to the extracellular domain of CTLA-4, with two amino acid exchanges), Ipilimumab (a humanized anti-CTLA-4 monoclonal antibody (mAb)), and Nivolumab (a humanized anti-PD-1 mAb) (Fig. 1) [3,4]. The first two agents target CD28-CD80/CD86 co-stimulatory pathways and are used to achieve immune suppression in patients with autoimmune diseases or those who have undergone transplantation. The latter two agents target either the CTLA-4 [5,6] or PD-1 [7–9] co-inhibitory pathway and are used as immunostimulatory drugs in cancer therapy.

Under normal physiological conditions, co-inhibitory pathways play important roles in the maintenance of self-tolerance and in protection against excessive tissue damage induced by immune responses. Thus, such pathways function as immune checkpoints [10,11]. The contributions of two immune checkpoint receptors, CTLA-4 and PD-1, are quite different, because the expression of both the receptors and their ligands is controlled differentially in both time and space during progression of an immune response (Table 1) [1,2,5,6,8,12]. Differential contributions of the CTLA-4 and PD-1 co-inhibitory receptors create different expression phenotypes in mice singly deficient for either receptor, and different clinical effects are evident upon application of blockade therapy. In contrast to CTLA-4-deficient mice, which develop rapidly progressing lethal systemic lymphoproliferative disorders accompanied by infiltration of multiple organs by activated polyclonal T cells, PD-1-deficient mice exhibit slow, strain-specific, and organ-specific diseases of autoimmunity including lupus-like proliferative arthritis and glomerulonephritis (on the C57BL/6 background), and dilated cardiomyopathy (on the BALB/c background). CTLA-4 blockade (treatment with ipilimumab) in patients with advanced melanoma prolonged survival. However new types of adverse event, namely immune-related adverse events (irAE) such as colitis, hepatitis, dermatitis, endocrinopathies, and

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http://dx.doi.org/10.1016/j.oraloncology.2014.11.014
1368-8375/

neuropathies were often observed [13,14]. A recent retrospective review from 14 completed phase I-III trials of ipilimumab in patients with advanced melanoma indicated that irAE occurred in 64.2% of patients [15]. Grade 3 to 4 irAE have been reported in around 20–30% of patients, depending on clinical trials [16].

PD-1 blockade (treatment with nivolumab) in patients with treatment-refractory solid tumors gives durable and persistent tumor regression with minimal irAE [17–21]. A study of 107 patients with melanoma who initiated treatment with nivolumab between 2008 and 2012 revealed that overall survival was 16.8 months; 1- and 2-year survival rates were 62% and 43%, respectively [20]. Objective responses were observed in 31% of patients (33 of 107) and durable responses were observed for all doses tested (0.1–10 mg/Kg). The appearance of irAE was 54%, but grade 3–4 adverse events were only seen in five patients (5%). PD-1 blockade seems to benefit from less toxicity than CTLA-1 blockade. PD-L1 expression in tumor cells is associated closely with the clinical response to anti-PD-1 therapy [18,21]. However, this also implies that some patients with PD-L1-expressing tumor cells will not benefit from anti-PD-1 therapy. A recent phase 1 trial of combined therapy with ipilimumab and nivolumab in 53 patients with advanced melanoma showed an objective-responses rate was 40% and grade 3–4 adverse events in 53% of patients, values similar to those for monotherapy [22].

To predict the efficacy of and optimize anti-PD-1 therapy, alone or in combination, it is important to understand the mechanisms controlling PD-L1 expression. In this review, we focus on the regulation of PD-L1 expression in both non-lymphoid tissue cells and malignant cells with a particular focus on epithelial cells and squamous cell carcinoma (SCC), and we discuss intrinsic and extrinsic regulation of PD-L1 expression.

PD-L1 expression in epithelial cells

PD-1 interacts with two ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273), which exhibit quite different expression patterns [23]. Although PD-L1 is abundant in immune cells and parenchymal tissue cells, PD-L2 expression is very limited in dendritic cells and macrophages after activation. PD-L1 expression at the mRNA level is high in normal human organs including the heart, skeletal muscle, placenta and lungs, as well as in the heart and lungs of mice; however, PD-L1 protein expression in healthy subjects was not detectable immunohistochemically using an anti-PD-L1 mAb [8,24,25], because of the lower sensitivity of histological staining using antibodies. Interestingly, PD-L1 protein is induced in various non-lymphoid tissue cells, including epithelial, endothelial, smooth muscle cells, in response to inflammatory cytokines present at disease sites [26–32]. In the oral mucosa and skin of patients with lichen planus (a chronic inflammatory mucocutaneous disease characterized by massive T cell infiltration under the epithelium), substantial expression of PD-L1 was detected in keratinocytes (KCs) located near the basement membrane [29]. PD-L1 protein expression in primary cultured human oral KCs was upregulated upon stimulation with IFN-γ, TNF-α, or IL-1β. Of these effectors, IFN-γ induced PD-L1 expression most potently. Similarly, IFN-γ enhanced PD-L1 expression markedly in murine epidermal KC *in vitro* [33]. In a murine contact hypersensitivity (CH) model, the hapten 2, 4-dinitrofluorobenzen (DNFB) induced high levels of PD-L1 on epidermal KCs [33]. In inflamed, but not normal skin, PD-L1 is expressed by subsets of microvessels and KCs [34]. PD-L1 in dermal fibroblasts is also induced by IFN-γ stimulation [35]. PD-L1 expression in renal tubular epithelial cells (TEC) may be detected in patients with renal diseases such as interstitial nephritis, lupus nephritis, and IgA nephropathy [36]. B7-H1 expression on TEC was induced via stimulation with IL-1α, LPS, TNF-α, or anti-CD40 mAb. These results indicate that epithelial PD-L1 expression is induced by inflammatory stimuli both *in vitro* and *in vivo*.

Immune regulation by epithelial cell-associated PD-L1

Demonstration of an actual contribution of tissue-associated PD-L1 to disease requires ingenuity, because PD-L1 is easily inducible in both non-lymphoid tissue cells and tissue-infiltrating immune cells such as T cells, dendritic cells, and macrophages *in vivo*. Studies using PD-L1-deficient mice, bone marrow chimera systems, and/or PD-L1 transgenic mice revealed the contributions of pancreatic islet cell-associated PD-L1 in autoimmune diabetes [37], of graft tissue-associated PD-L1 in organ transplantation

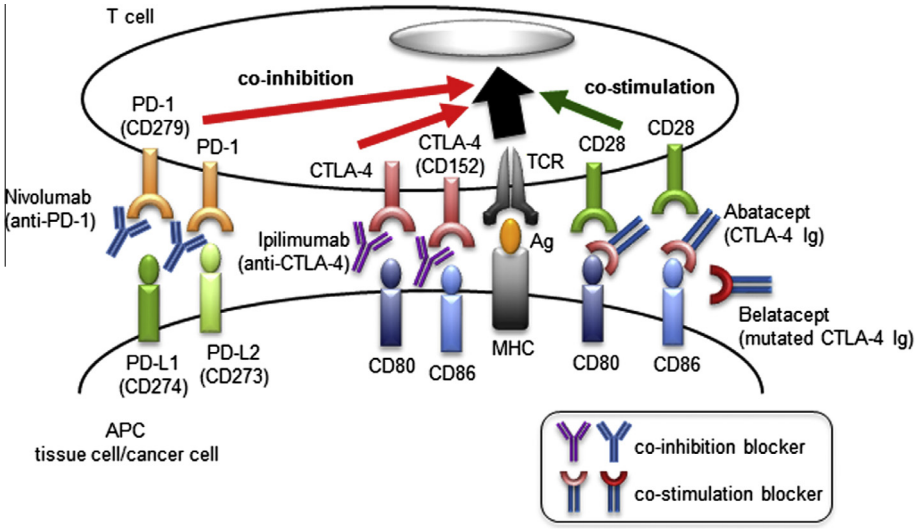

Fig. 1. Immunomodulatory biologicals targeting co-signal pathways. Antigen-specific T cell responses are controlled by the valance of co-stimulation and co-inhibition. Abatacept and belatacept block the CD28:CD80/CD86 co-signal pathway, while ipilimumab and nivolumab block the CTLA-4:CD80/CD86 and PD-1:PD-L1/PD-L1 co-inhibitory pathway, respectively. Blockade of co-stimulatory or co-inhibitory pathways efficiently inhibits or enhances T cell-mediated immune responses.

Table 1
Comparison between CTLA-4 and PD-1 immune checkpoint receptors.

Issue	CTLA-4	PD-1
Expression in T cells	Early activated T cells Natural regulatory T cells	Effector T cells Induced regulatory T cells Tumor-infiltrating CD8 + T cells
Surface expression on T cells	Transient and low	Stable and high
Expression other than T cells	None	B cells, NK cells, myeloid cells
Location of positive cells	Regional lymph nodes	Peripheral inflammatory tissues
Inhibitory roles	Inhibition of T cell activation	Inhibition of effector T cells and NK cells
Involvement in self-tolerance	High	Moderate
Phenotypes of gene knockout mice	Lethal systemic lymphoperiferative diseases with polyclonal lymphocyte activation	Non-lethal, individually and strain-dependent differences organ-specific autoimmune diseases
Ligand	CD80, CD86	PD-L2 (CD273/B7-DC), PD-L1 (CD274/B7-H1)
Ligand expression	Antigen-presenting cells (CD80, CD86) Activated T cells (CD80) Rare induction on non-lymphoid tissue cells at inflammatory sites (CD80, CD86)	Most activated lymphoid cells (PD-L1) Non-lymphoid tissue cells at inflammatory sites (PD-L1) Activated macrophages and dendritic cells (PD-L2)
Ligand expression in tumor cells	Inducible but not so often	Often inducible

[38], of host tissue-associated PD-L1 in acute GVHD [39,40], and of KC-associated PD-L1 in mucocutaneous inflammation [33,41].

*K14/PD-L1*tg mice overexpress PD-L1 in KCs [33]. In such mice, CH responses induced by abdominal skin-painting (sensitization) and subsequent ear skin-painting (challenge) are impaired markedly. Adoptive transfer of hapten-sensitized T cells into *K14/PD-L1*tg mice induces lower ear swelling. In PD-L1-overexpressing skin, the effector function of infiltrating $CD8^+$ T cells was impaired dramatically, but such impairment was abrogated by the addition of an anti-PD-L1 mAb [33]. Adoptive transfer of PD-$1^{-/-}$ OT-I $CD8^+$ T cells into mice expressing OVA in epidermal KCs (K14-OVA mice) induced severe GVHD-like disease associated with abundant expression of PD-L1 in all T cells, dendritic cells, Langerhans cells, and KCs [41]. In addition, knockdown of KC PD-L1 in K14-OVA mice enhanced activation of OT-I T cells. These results indicate a requirement for onsite regulation of KC PD-L1 expression via interactions of autoantigen-expressing KCs with autoreactive $CD8^+$ T cells. In summary, the results indicate that PD-1 is highly expressed on tissue-recruited effector $CD8^+$ T cells, and that KC-associated PD-L1 directly suppresses effector T cell generation and activation at sites of local inflammation. Such interaction between PD-1 and PD-L1 is involved in peripheral tolerance by preventing excessive local inflammatory responses.

PD-L1 expression in SCC and the prognostic implications thereof

Early reports measured PD-L1 expression in various types of solid tumor, including squamous cell carcinoma (SCC) of the lung, esophagus, and head and neck [25,42–44]; other types of carcinoma of the breast, gut, colon, pancreas, kidney, bladder, and ovary; and melanomas and glioma [25,45]. PD-L1 was expressed in 66% (16 of 24) of freshly isolated SCC of the head and neck (SCCHN) [42]. The extent of histologically determined PD-L1 expression in SCC was not uniform; PD-L1 was found on plasma membrane and/or in the cytoplasm, but with either focal or diffuse distribution [25,42,44]. The specific tumor microenvironment may greatly affect PD-L1 induction and distinct molecular mechanisms of such induction may exist. PD-L1 is also expressed at various levels in cultured SCCHN cell lines, and its expression is upregulated in response to the proinflammatory cytokines, IFN-γ, TNF-α and IL-1β [42,44]. As is true of normal tissues, IFN-γ is a key cytokine triggering de novo PD-L1 induction in tumor cells. PD-L1 blockade by a mAb efficiently augmented the effects of adaptive T cell immunotherapy in a murine model of PD-L1-transfected SCC (SCCVII) [42] and inhibited the growth of de novo induced $PD\text{-}L1^+$ SCC (NRS-1) [44]. These results suggested the potential utility of PD-L1 blockade therapy in clinical situations.

In an early clinicopathological study, the expression levels of both PD-L1 and PD-L2 were analyzed in 52 surgically resected non-small cell lung carcinoma (NSCLC) patients including those with SCC and adenocarcinoma [46]. No relationship was evident for the expression levels of PD-L1 and PD-L2 with either clinicopathological variables or postoperative survival; however, in the same specimens, significantly fewer tumor-infiltrating lymphocytes (TILs) were observed in PD-L1-positive tumor regions, and the proportions of $PD\text{-}1^+$ TILs were significantly lower in such regions. In a study of 41 patients with esophageal SCC, the mRNA and protein levels encoding PD-L1 and PD-L2 were closely correlated, as assessed immunohistologically [43]. Both $PD\text{-}L1^+$ and $PD\text{-}L2^+$ patients experienced significantly poorer prognosis than those who expressed neither form of PD-L, but no significant correlation between PD-L1 expression and TIL number was evident [43].

A recent study has found that 39 of 45 cases of oral SCC showed tumor PD-L1 expression and that the PD-L1 expression level was related to a lower density of intratumoral $CD8^+$ TILs [47]. However, the tumor-associated PD-L1 status did not affect survival. In human papillomavirus (HPV)-associated HNSCC with greater lymphocyte infiltration, PD-L1 staining in tonsilar SCC was evident on the membranes of HPV^+ tumor cells and $CD68^+$ tumor-associated macrophages, which correlated with the numbers of $CD8^+$ TILs expressing high levels of PD-1 [48]. Another study evaluating oropharyngeal SCC showed that PD-L1 was expressed in 49.2% and 34.1% of HPV mRNA-positive and -negative cases, respectively. No correlation was evident between PD-L1 status and survival [49]. A more recent study of 340 NSCLC patients, including 178 SCC patients, found that the PD-L1 mRNA and protein expression levels, as assessed via *in situ* hybridization and tissue microarray, respectively, were in agreement, and that high PD-L1 expression levels were associated with elevated TILs and better survival [50].

SCC studies that explored the correlation between tumor-associated PD-L1 status and clinicopathological features and prognosis have yielded variable results. The methods used to evaluate PD-L1 expression, the timing of biopsy, and the tissue origins differed among studies, as did the monoclonal/polyclonal antibodies and detection methods used, the definition of positive expression, staining intensities, and staining distributions. It is also possible that the capacity of PD-L1 to exert opposite effects under different circumstances contributed to the observed variability. The extent of lymphocyte infiltration and tumor immunogenicity differed both individually and at the tissue-specific level. Such factors may also have affected the results. Further work using larger

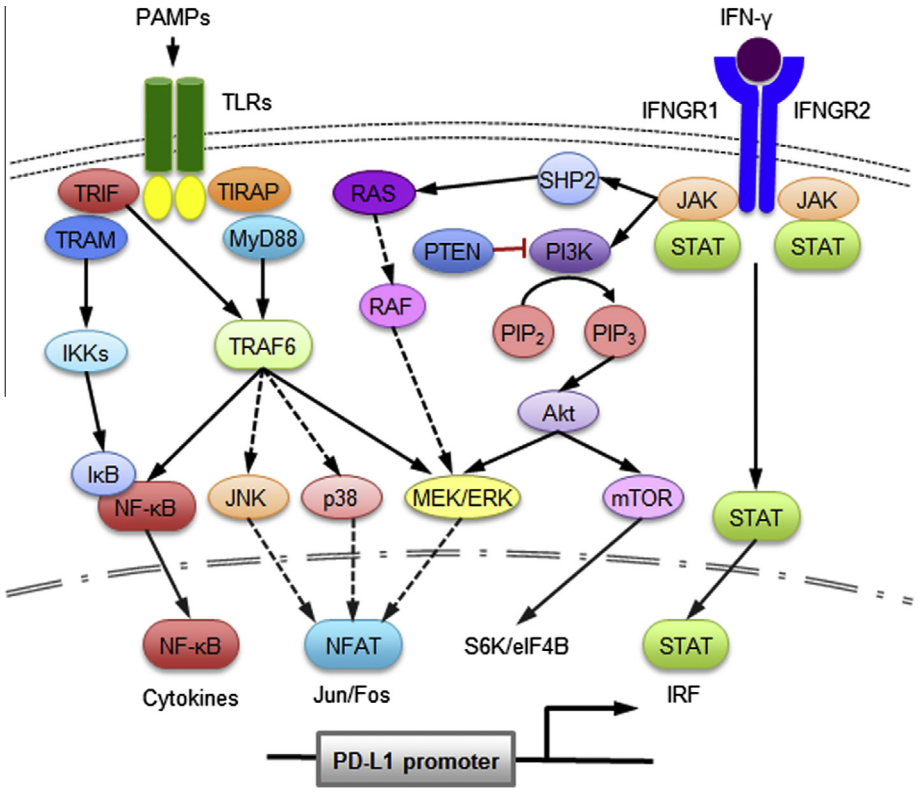

Fig. 2. Induction of PD-L1 by various signaling molecules. Signal transduction via PAMPs and IFN-γ results in phosphorylation of NF-κB, MAPK (JNK, p38 and ERK), mTOR and STAT, and downstream signaling mediated by these molecules triggers the nuclear translocation of various transcription factors. Binding of the factors, NF-κB, NFAT, and STAT/IRF to the PD-L1 promoter further induces transcription and translation of PD-L1.

cohort sizes and uniform evaluation methods will yield more definitive results.

Extrinsic control of PD-L1 expression

Up-regulation of PD-L1 in immune cells and several cancer cells is heavily dependent on TLR- or IFN-γ-mediated signaling pathways [51–53]. TLR-4 signaling in bladder cancer cells upregulated PD-L1 expression, and inhibitors of ERK or JNK attenuated such upregulation [53]. Blockade of the MEK/ERK or MyD88/TRAF6 pathway inhibited the PD-L1 expression induced by IFN-γ and TLR ligands. Signal transduction via IFN-γ/STAT1 triggered MEK/ERK phosphorylation in plasma cells from a multiple myeloma patient, and inhibition of STAT1 reduced PD-L1 expression [52]. The principal target of IFN-γ signaling, interferon regulatory factor-1 (IRF-1), was also upregulated in plasma cells exposed to IFN-γ, but the levels fell after treatment with ERK inhibitors, suggesting a major role for the MEK/ERK pathway in IFN-γ signaling [52]. In the lung cancer cell line A594, an electrophoretic mobility shift assay (EMSA), site-directed mutagenesis, and a knockdown experiment using siRNA all revealed that IRF-1 was primarily responsible for both constitutive PD-L1 expression and early induction of PD-L1 after IFN-γ stimulation. In addition, IRF1 synthesized de novo, acting through the JAK/STAT pathway was involved in late induction of PD-L1 [35]. In myelodysplastic syndrome blast cells, PD-L1 was upregulated via NF-kB activation in response to IFN-γ and TNF-α [54]. IFN-γ-stimulated dermal fibroblasts exhibited nuclear translocation of NF-κB mediated by phosphorylation of ERK1/2 and PI3K, increasing PD-L1 promoter activity and gene expression [55]. Knockdown of PKD2, a downstream target of PI3K activated by PKC, decreased PD-L1 expression in IFN-γ-stimulated human oral SCC [56]. In addition, several microRNAs (miR), single-stranded RNA molecules that repress translation and silence genes, have been shown to regulate PD-L1 induction, post-transcriptionally. miR-513 directly regulated PD-L1 mRNA and protein expression by targeting the PD-L1 3-untranslated region in human biliary epithelial cells stimulated by IFN-γ [57].

All above results suggest that extrinsic stimuli acting via TLRs or IFN-γ receptor modulate the expression and activation of various down-stream signaling molecules, such as NF-kB, MAPK, PI3K, mTOR and JAK/STAT, that affect cell cycle progression, cell proliferation, and activation or regulation of transcription factors. Such signaling molecules further regulate the nuclear translocation of transcription factors to the PD-L1 promoter (Fig. 2 and Table 2).

Intrinsic cellular control of PD-L1 expression and carcinogenesis

Apart from the data summarized above, several reports have suggested that intrinsic cellular changes associated with carcinogenesis induce PD-L1 expression (Table 2 and Fig. 3). PD-L1 expression in human breast cancer is strongly associated with proliferative Ki-67 expression and cell cycle progression that is independent of host PD-1 [58]. T cell lymphoma cells carrying the oncogenic nucleophosmin (NPM)-anaplastic lymphoma kinase (ALK), which is involved in malignant transformation, induce high levels of PD-L1 expression via STAT3 and ERK activation [59,60]. Inactivation of the tumor suppressor gene phosphatase and tensin homolog (*PTEN*) is often observed in mouse SCC [61,62]. PTEN negatively regulates the phosphatidylinositol 3-kinase (PI3K)/AKT pathway, alternations of which are also evident in human SCC, together with a reduction in/loss of PTEN. In human glioma, loss of *PTEN* has been correlated with enhanced PD-L1 expression

Table 2
Signaling molecules involved in PD-L1 induction.

Cell type	Triggering factor	Signaling molecule involved	Reference
Normal cell			
Mouse			
Keratinocyte (overexpressing PD-L1)	Carcinogenesis	Slug, Twist	23, 57
Human			
Biliary epithelial cell	IFN-γ	miR-513	48
Dermal fibroblast	IFN-γ	PI3K, ERK1/2, NF-κB	46
Cancer cell			
Mouse			
SCCHN	Carcinogenesis (Loss of PTEN)	PI3K/Akt/mTOR	61
Lung SCC	Inactivation of Lkb1 and PTEN	Kras, PI3K	65
Human			
Bladder cancer cell	TLR-4L	MEK/ERK	53
Breast cancer cell	Carcinogenesis	Ki-67	58
Colorectal cancer	miR-20b, -21, -130	PI3K	63
Glioma	Loss of PTEN	PI3K/Akt	45
Lung adenocarcinoma epithelial cell	IFN-γ	JAK/STAT/IRF-1	35
MM plasma cell	IFN-γ	STAT1, MEK/ERK	52
	TLR-2L, TLR-4L, TLR-9L	MyD88/TRAF6	
Myelodysplastic syndrome blast cell	IFN-γ, TNF-α	NF-κB	54
Oral SCC	IFN-γ	PI3K, PKC, PKD2	56
T cell lymphoma	NPM-ALK	STAT3, ERK1/2	59, 60

PTEN (phosphatase and tensin homolog); Lkb1 (liver kinase B1); TLR (Toll-like receptor); IFN (interferon); TNF (tumor necrosis factor) NPM-ALK (neucleophosmin-anaplastic lymphoma kinase); PI3K (phosphoinositide 3-kinase); ERK (extracellular signal-regulated kinase); NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells); Akt (or PKB or protein kinase B); mTOR (mammalian target of rapamycin); Kras (Kirsten rat sarcoma viral oncogene homolog); MEK (mitogen-activated protein kinase kinase); JAK (Janus kinase); STAT (signal transducer and activator for transcription); IRF (interferon regulatory factor); MyD88 (myeloid differentiation primary response gene 88); TRAF (tumor necrosis factor receptor associated factor); PKC (protein kinase C); PKD (protein kinase D).

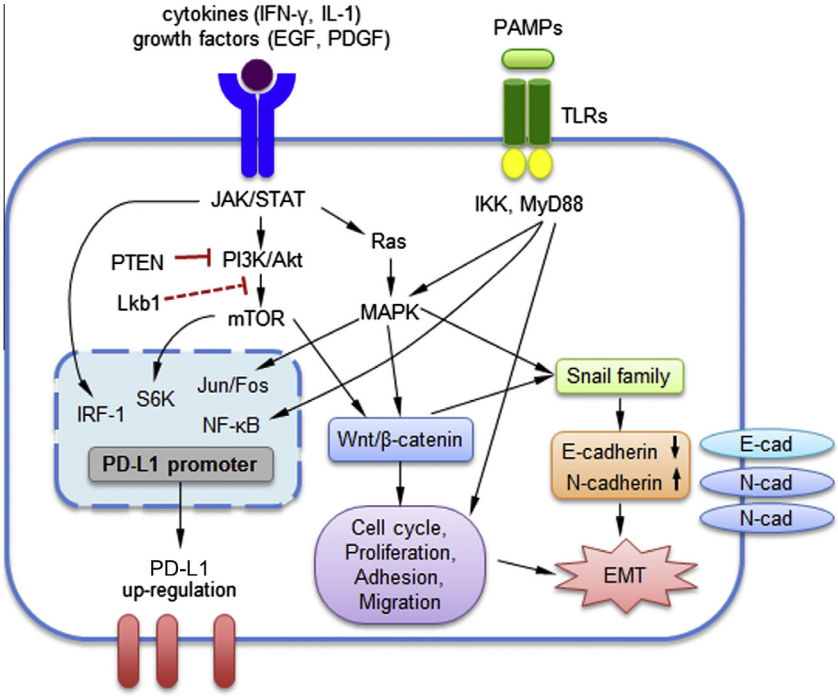

Fig. 3. Related signaling molecules for PD-L1 induction and carcinogenesis. PAMPs, growth factors, and cytokine stimulation transduces signals via MyD88/IKK/NF-κB, JAK/STAT/IRF1, PI3K/Akt/mTOR/S6K and MAPK/Jun/Fos, triggering PD-L1 upregulation. In addition, PTEN and Lkb1 negatively regulate PI3K/Akt and/or mTOR-mediated signaling, resulting in modulation of PD-L1 expression. These signaling molecules also link to cell intrinsic alternation related to carcinogenesis, such as cell cycle, cell proliferation, adhesion, migration, and EMT. PI3K/Akt and MAPK are the upstream events of Wnt/β-catenin. Snail/E-cadherin and MyD88/IKK/NF-κB pathways directly control cell cycle, proliferation, adhesion and migration.

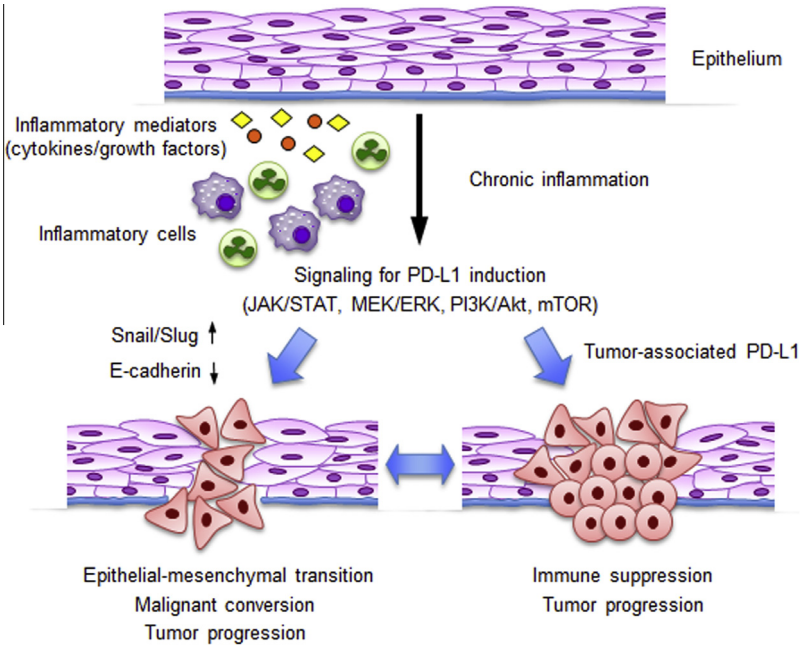

Fig. 4. A possible link between PD-L1 induction and malignant conversion. Inflammatory mediators such as cytokines and growth factors cause transient induction of PD-L1 expression in epithelial cells. However, continuous and/or repeated stimuli (chronic inflammation) may induce irreversible cell intrinsic changes that promote EMT and carcinogenesis in addition to PD-L1 induction. Tumor-associated PD-L1 regulates the host immune responses and promotes tumor progression.

[45]. In colorectal cancer, miR-20b, -21 and 130 inhibited PTEN expression, resulting in PD-L1 overexpression [63]. The KC-specific deficiency of *PTEN* induced epidermal hyperplasia and accelerated tumor formation [64]. Simultaneous activation of *Kras* and inactivation of *Lkb1* (also known as serine-threonine kinase 11) induce lung SCC formation. Although a deficiency of either *PTEN* or *Lkb1* did not drive tumor formation, simultaneous inactivation of *PTEN* and *Lkb1* resulted in the development of murine lung SCC exhibiting elevated PD-L1 expression [65]. One of the most frequently mutated oncogenes in patients with NSCLC is the epidermal growth factor receptor (*EGFR*) gene. Recent reports demonstrated that activating *EGFR* mutations were associated with increased PD-L1 expression in surgically resected NSCLC and ectopic expression of mutant *EGFR* in bronchial epithelial cells induced PD-L1 expression [66,67]. Inhibition of EGFR signaling by the EGFR tyrosine kinase inhibitor erlotinib downregulated surface expression of PD-L1 in EGFR mutation-positive NSCLCL cells, but not in the EGFR wild-type cells [67]. These results suggest a possible link between carcinogenesis and PD-L1 expression.

The epithelial–mesenchymal transition (EMT) is critical in the conversion of normal epithelial cell to tumor cell during SCC carcinogenesis. Down-regulation of E-cadherin and up-regulation of N-cadherin are closely with such a conversion. We reported that PD-L1 transgenic mice overexpressing PD-L1 in KCs (*K14/PD-L1*tg mice) [33] exhibited clearly accelerated SCC formation in a carcinogen 3-methylcholanthrene (MCA)-induced tumor model [33,68]. Prior to tumor formation, atypical cellular changes such as disturbed cell alignment and chromatin condensation were evident in basal cells of *K14/PD-L1*tg mice at an early stage of MCA injection. E-cadherin expression was down-regulated significantly in PD-L1-overexpressing KCs. Regulation of E-cadherin expression is controlled by the Snail family of transcriptional repressors [69,70]. We found that *K14/PD-L1*tg-derived SCC exhibited significantly higher levels of *Slug* (*Snai2*) and *Twist* (*Twist1*) than those of wild type mice-derived SCC. Slug and Twist may play critical roles in epithelial KC migration by repressing E-cadherin.

In KCs, ultraviolet radiation activates the ERK and p38 MAPK cascades and increases Snail and Slug expression [71]. Wnt/β-catenin pathways are also involved in the EMT process [72]. Overexpression of Akt in SCC lines caused EMT characterized by down-regulation of numerous epithelial cell-specific proteins, including E-cadherin and β-catenin [73]. Various downstream signaling cascades triggered by growth factor receptors, such as Akt/mTOR, NF-kB and MAPK, are involved in the EMT. Most EMT-related signaling molecules overlap with those involved in extrinsic induction of PD-L1, as described above (Fig. 3). Therefore, it is difficult to differentiate the precise events induced by exogenous stimuli from changes intrinsic to cells. It is conceivable that the persistent PD-L1-induced exogenous stimuli may transduce constitutive signals that activate expression of intracellular proteins required for the EMT and carcinogenesis (Fig. 4). It seems that elevated tumor-associated PD-L1 levels are caused not only by exposure to extracellular cytokines secreted by tumor bystander cells, but also by intrinsic cancerous changes linked to the EMT and carcinogenesis.

Concluding remarks

Over the past decade, appreciation of the utility of anti-PD-1 or anti-PD-L1 therapy has grown. Taube et al. commented that one of the most intriguing findings from early clinical trials evaluating PD-1 pathway blockade therapy to treat advanced solid tumors was the correlation between pretreatment tumor PD-L1 expression and treatment response [21]. Most tumors in the patients from earlier clinical trials were melanoma, NSCLC, and renal-cell cancers, that which are all rather highly immunogenic and exhibit higher levels of lymphocyte infiltration. A more recent large-scale study involving 636 primary breast carcinoma patients demonstrated that half of all breast cancer expressed PD-L1 mRNA [74,75]. Higher PD-L1 mRNA expression levels are associated closely with elevated TIL numbers and longer recurrence-free survival. However, only 16% of breast cancers showed prominent TIL infiltration, whereas 12% showed both high TIL numbers and PD-L1 expression. This implies that the majority of breast cancers (46%) exhibited PD-L1 expression but low TIL numbers. Such a patient group would benefit less from PD-1 blockade therapy alone, since

intrinsic cellular changes triggered by carcinogenesis may be responsible for PD-L1 expression. In such a group, additional treatments to modulate intracellular signaling associated with cell malignancy and/or to stimulate immune cell recruitment to tumors may be required. Although no large-scale study has been performed in SCCHN patients to evaluate the correlation between PD-L1 expression and TILs, such patients are often immunocompromised because of active immune regulation by tumor-associated macrophages, myeloid-derived suppressor cells, and regulatory T cells [76]. In addition, cytogenetic alternations are often seen in SCCHN [77]. A phase III clinical trial of nivolumab versus cetuximab, methotrexate or docetaxel (as chosen by physicians) in patients with recurrent or metastatic SCCHN (NCT02105636) presently is ongoing. Consideration of PD-L1 expression mechanisms will be important to optimize treatment approaches in SCCHN patients.

Conflict of Interest

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgement

The authors thank the members of the Azuma laboratory for their experimental data and helpful discussions. This work was supported by Grants-in-Aid for Scientific Research (No. 20249075, 23249082, 26253086) from the Japan Society for the Promotion of Science (JSPS).

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