Regulation of PD-L1: a novel role of pro-survival signalling in cancer

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Evasion of immune system is a hallmark of cancer, which enables cancer cells to escape the attack from immune cells. Cancer cells can express many immune inhibitory signalling proteins to cause immune cell dysfunction and apoptosis. One of these inhibitory molecules is programmed death-ligand-1 (PD-L1), which binds to programmed death-1 (PD-1) expressed on T-cells, B-cells, dendritic cells and natural killer T-cells to suppress anti-cancer immunity. Therefore, anti-PD-L1 and anti-PD-1 antibodies have been used for the treatment of cancer, showing promising outcomes. However, only a proportion of patients respond to the treatments. Further understanding of the regulation of PD-L1 expression could be helpful for the improvement of anti-PD-L1 and anti-PD-1 treatments. Studies have shown that PD-L1 expression is regulated by signalling pathways, transcriptional factors and epigenetic factors. In this review, we summarise the recent progress of the regulation of PD-L1 expression in cancer cells and propose a regulatory model for unified explanation. Both PI3K and MAPK pathways are involved in PD-L1 regulation but the downstream molecules that control PD-L1 and cell proliferation may differ. Transcriptional factors hypoxia-inducible factor-1α and signal transducer and activation of transcription-3 act on the promoter of PD-L1 to regulate its expression. In addition, microRNAs including miR-570, miR-513, miR-197, miR-34a and miR-200 negatively regulate PD-L1. Clinically, it could increase treatment efficacy of targeted therapy by choosing those molecules that control both PD-L1 expression and cell proliferation.

Key words: immune checkpoint, Akt, MAPK, NF-κB, STAT3, HIIF-1A

introduction

Evasion of the immune system is essential for cancer development, progression and resistance to treatment. While immunocompromised individuals are associated with increased cancer incidence and poor prognosis, it is now known that effective immune responses are involved in destruction of cancer cells by many therapeutic agents [1–3]. Considerable efforts have been made to boost host immune responses in patients with cancer. These include administration of immune-stimulating cytokines such as interferon (IFN) and interleukin-2, active immunization to enhance endogenous immune responses such as dendritic cell (DC)-based vaccination, adoptive cell-transfer therapy using autologous cancer-specific cytotoxic T-cells (CTLs) activated and expanded in vitro or T cells that are genetically engineered to express chimeric antigen receptors that can recognise cancer cell-specific antigens [1, 4–9]. The limitation of boosting immune response only for cancer therapy is the severe side-effects due to off-target effects and the low response rate due to up-regulation of immune inhibitory factors on cancer cells [10].

Advances in understanding the role of immune checkpoints in suppression of T-cell activation have led to the development of immune checkpoint inhibitors in the treatment of cancer. Indeed, the blocking antibody (Ab) against T-lymphocyte-associated protein-4 (CTLA-4) ipilimumab (marketed as Yervoy) and the Abs against programmed death-1 (PD-1) nivolumab (marketed as Opdivo, Bristol-Myers Squibb) and pembrolizumab (marketed as Keytruda, also known as MK-3475, Merck) have been approved by the US Food and Drug Administration (FDA) to treat metastatic melanoma and non-small-cell lung cancer (NSCLC) [11, 12]. More recently, the Ab against programmed death-ligand-1 (PD-L1) MPDL3280A (Roche) has been approved by the FDA for the treatment of PD-L1-positive NSCLC. Potential applications of these immune checkpoint antibodies in other types of cancers are being evaluated in preclinical and clinical settings. Although immune checkpoint antibodies can achieve long-lasting tumour regression that is evidenced by improved patient progression-free survival and overall survival (OS), the low response rate remains a major obstacle that forestalls the lack of benefits in the majority of cancer patients [13]. In this regard, it is of significance that Abs against PD-1/PD-L1 are more active with higher response rates and less adverse effects than ipilimumab. The distinct roles of CTLA-4 and PD-1 in immune regulation (early
T-cell activation phase in lymphoid tissues versus antigen-experienced T-cells in peripheral (tumour) tissues have promoted studies with combined blockade of CTLA4 and PD-1/PD-L1, and have shown encouraging results. For example, combining nivolumab and ipilimumab resulted in significantly improved 2-year OS rate in patients with advanced melanoma [14]. However, adverse effects were also increased with 55% patients experiencing grade 3 and grade 4 toxicity.

As an important mechanism that negatively regulates T-cell activation to prevent autoimmune responses under physiological conditions, it is not surprising that the expression of PD-L1 is commonly elevated in cancer cells. However, how and when PD-L1 is up-regulated during the pathogenesis of cancer remain less well understood. Recent studies have pointed to a role of oncogenic activation of pro-survival signalling pathways in up-regulation of PD-L1 in cancer cells, which is emerging as a common mechanism by which cancer cells fight against the immune system [15–17]. In this review, we summarise current knowledge about regulation of PD-L1 by oncogenic pathways in cancer cells and discuss the mechanisms involved. The potential practical implications of the interaction between pro-survival signalling pathways and the immune system will also be deliberated.

### the PD-1/PD-L1 axis

Well-orchestrated T-cell activation is necessary for defending the host against pathogens and preventing autoimmune responses. A T-cell must receive two sets of signals from an antigen-presenting cell (APC): the antigen-specific signal mediated by T-cell receptor (TCR) recognition of complexes of major histocompatibility complex with the antigen on the surface of an APC, and a second co-stimulatory signal mediated by interaction of CD28 on the T-cell with CD80 (B7.1) or CD86 (B7.2) on the APC (Figure 1) [18]. Co-stimulation is mediated by a tightly controlled interplay of stimulatory and inhibitory receptor and ligand pairs [19, 20]. Pairs of inhibitory receptors and ligands expressed on T-cells, APCs and other types of cells are collectively termed ‘immune checkpoints’. Among them are CTLA-4 and PD-1. CTLA-4 exclusively expressed on T-cells negatively regulates T-cell activation at early phases by competing with CD28 for binding with CD80 and CD86 [21, 22]. It is up-regulated after antigen-specific activation of naïve or memory T-cells in lymphoid tissues, leading to decreased effector function of T-cells. On the other hand, PD-1 is mainly expressed on antigen-experienced memory T-cells in peripheral tissues [19, 23]. It can also be expressed on B-cells, monocytes, DCs and NK cells. PD-1 is rapidly up-regulated on cells after exposure to cognate antigens, but is down-regulated upon antigen clearance. The engagement of PD-1 on the T-cell surface with its ligand PD-L1 or PD-L2 inhibits T-cell proliferation and effector functions, induces apoptosis and promotes differentiation of CD4+ T-cells into Foxp3 regulatory T-cells [14]. The physiological significance of PD-1 in controlling T-cell activation has been demonstrated by knockout of PD-1 in mice, which resulted in autoimmune diseases such as lupus-like arthritis and glomerulonephritis [24].

Structurally, PD-1 is a type 1 transmembrane glycoprotein consisting of an immunoglobulin V (IgV)-type extracellular domain that shares 21%–22% sequences identical to other members of the CD28 family [25], a transmembrane region, and an intracellular tail that contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which, upon phosphorylation, negatively regulates TCR signals through phosphorylating Src homology phosphatase-1 (SHP-1) and SHP-2. PD-1 exists as a monomeric receptor due to the lack of membrane-proximal cysteine residue necessary for homodimerisation. The gene PDCD1 encoding PD-1 is located in chromosome 2 in human and contains five exons [26].

PD-1 has two ligands, PD-L1 and PD-L2, both of which are members of the B7 family of transmembrane proteins [27, 28]. While the expression of PD-L2 is restricted largely to APCs, PD-L1 is expressed on many cell types, including T-cells, B-cells, monocytes, APCs and epithelial cells, and is up-regulated in response to proinflammatory cytokines such as IFNγ.

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**Figure 1.** Antigen activation and checkpoint protein inhibition of T-cells. APCs present antigens to TCR, which activates signalling pathways to cause T-cell activation with co-stimulating signals from CD86/CD28 axis. CTLA-4 also binds to CD86, but leading to T-cell inactivation. PD-L1 on both APCs and cancer cells binds to PD-1 to inhibit signalling pathways that activate T-cells. APC, antigen-presenting cell; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; TCR, T-cell receptor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4.
and IL4 through signal transducer and activator of transcription-1 (STAT1) and IFN regulatory factor-1 (IRF1) [29]. PD-L1 comprises a transmembrane region and two extracellular domains, IgC and IgV [27, 30]. The cytoplasmic domain of PD-L1 is short and whether it transmits intracellular signals remains to be established. The gene CD274, encoding PD-L1 protein, contains seven exons and is located in chromosome 9 in humans [26]. Each exon encodes a different part of PD-L1 protein as shown in Fig 2 [31]. The functional importance of PD-L1 has been evidenced by autoimmune diseases in mice deficient in PD-L1 [32, 33]. In humans, altered PD-L1 expression is associated with systemic lupus erythematosus.

Similar to many other B7 family co-stimulatory proteins, PD-L1 can exist as a soluble form (sPD-L1) in the circulation of healthy individuals. It is mainly produced by myeloid-derived cells including monocytes, macrophages and DCs [34]. On the other hand, T-cells do not produce significant amounts of sPD-L1, even when activated. Significantly, sPD-L1 retains its IgV ligand-binding domain necessary for interaction with PD-1 and can suppress T-cell activation. The physiological significance of sPD-L1 remains to be established, but it is intriguing that the expression of sPD-L1 is commonly elevated in elderly individuals. Cleavage of membrane PD-L1 (mPD-L1) from the cell surface by matrix metalloproteinases has been implicated in the production of sPD-L1 [34]. sPD-L1 has been found in several human cancer cell lines including NSCLC cell line H1299 cells, lymphoma cell line U-937, ovarian carcinoma cell line HO8910, lung adenocarcinoma cell line SPICA-1 and glioblastoma cell line U251 [34]. The increased sPD-L1 levels in blood are associated with metastasis and poor prognosis in breast cancer and diffuse large B-cell lymphoma [35, 36].

**PD-L1 expression in cancer as a biomarker**

As an immune suppression mechanism, the expression of PD-L1 is elevated in many types of cancer and is often correlated with poor patient prognosis and predictive of responses to Abs against PD-1/PD-L1 [37–39]. Nevertheless, tumours that do not express detectable levels of PD-L1 on the cell surface can also respond to Abs against PD-1, suggesting that the predictive value of PD-L1 expression may not be uniformly applicable to all types of cancer. On the other hand, this may be associated with unresolved technical and biological issues that preclude an accurate detection of PD-L1 with a standardised criterion for quantitation of PD-L1 expression in clinical samples. Moreover, it is well recognised that there are wide intra- and inter-tumoural variations in the expression of PD-L1, which indicates that sampling of tumour tissues may also impinge on the outcome of PD-L1 detection [22]. Regardless, there is increasing evidence showing that the expression of PD-L1 in cancer cells is mediated by oncogenic activation of signalling pathways and is also regulated by factors in the tumour microenvironment [40]. In metastatic melanoma, both PD-L1 and tumour infiltrating immune (mononuclear) cells (TIMC) are associated with prognosis. While expression of TIMC without PD-L1 leads to beneficial treatment outcome, expression of both TIMC and PD-L1 has no such advantage, indicating that PD-L1 is negatively associated with prognosis [41]. Under the absence of TIMC, expression of PD-L1 does not affect prognosis.

Expression of PD-L1 is associated with poor prognosis in many cancers [42–44]. For example, in melanoma, PD-L1 could be a marker that defines a subset of melanoma with distinct genetic and morpho-phenotypic features, increased aggressiveness and invasiveness [42]. PD-L1 affects cancer treatment outcomes through various mechanisms. PD-L1 in ovarian cancer cells inhibited CTL function and promoted cancer cell dissemination [43]. PD-L1 may be involved in cell proliferation and progression, which could also be a factor leading to poor prognosis [44]. It has been demonstrated that PD-L1 up-regulation due to decreased miR-200 results in dysfunction of CD8 T-cells, which coupled with epithelial-mesenchymal transition (EMT) to increase metastasis [45]. PD-L1 and EMT can regulate each other to form feed-forward regulation [46]. Expression of PD-L1 is associated with drug resistance to anti-cancer therapy such as BRAF inhibitor resistance in metastatic melanoma patients [41]. This could be caused by both PD-L1-induced immune inhibition and PD-L1-promoting cell proliferation.
regulation of PD-L1 expression in cancer cells by the MAPK pathway

The MAPK signalling pathway plays a critical role in cell survival and proliferation [47–49]. Under physiological condition, it is activated by extracellular signals through receptor tyrosine kinases. However, it is aberrantly activated in many types of cancers through oncogenic mutations in its key components. Among them is activating mutations in BRAF with the most common mutation being a glutamic acid for valine substitution at position 600 (V600E), which accounts for over 90% of BRAF mutations found in cancer [50].

The initial evidence that oncogenic activation of the MAPK pathway was associated with cancer cell immune evasion came from the finding that treatment with the mutant BRAF inhibitors was associated with a rapid increase in T-cell infiltration into the melanoma microenvironment [51, 52]. This was followed by the observation that BRAF inhibitors caused down-regulation of PD-L1 expression, which was nevertheless recovered with rebound activation of the pathway and could be reduced by inhibition of downstream kinases using an MEK inhibitor or by knockdown of ERK1/2 [15]. These findings provided strong evidence that activation of the MAPK pathway drives the expression of PD-L1 in melanoma cells. Not surprisingly, MAPK activation also appears responsible for up-regulation of PD-L1 by oncogenic activation of EGFR in NSCLC [53]. Whether other growth factors drive PD-L1 expression in cancer cells in humans remains to be clarified. However, hepatocyte growth factor has been shown to up-regulate PD-L1 in a mouse melanoma model that was abolished in mice deficient in RAS, an upstream kinase of the MAPK pathway [54, 55].

Of note, the MAPK pathway is also involved in up-regulation of PD-L1 in cancer cells upon treatment with chemotherapeutic drugs. For example, paclitaxel induces PD-L1 expression that is abolished by the MEK inhibitor U0126 [56]. Moreover, low concentration of cisplatin also triggers the expression of PD-L1 through MAPK activation [57]. These results suggest that activation of the MAPK pathway protects cancer cells from destruction by chemotherapeutic drugs not only by inhibition of cell death signalling, but also through inhibition of the immune response via up-regulation of PD-L1.

transcriptional mechanisms involved in the regulation of PD-L1 expression in cancer cells

Signalling pathways drive PD-L1 expression in cancer cells primarily by transcriptional up-regulation. A number of transcriptional factors have been shown to be involved, pointing to their important roles in evasion of cancer cells from the immune system.

HIF-1 (hypoxia-inducible factor alpha)

Increased HIF-1 levels are associated with increased PD-L1 expression and cause down-regulation of T-cell function [66–71], suggesting that hypoxic environments, which leads to increased expression of HIF-1, can also result in immune suppression in addition to promoting cell proliferation and blocking apoptosis. HIF-1 is a major oncogenic factor [72] and inhibition of HIF-1 has been applied for the treatment of cancer [73–75]. It regulates PD-L1 through binding to a hypoxia response element (HRE) of the PD-L1 promoter to activate PD-L1 transcription [76]. There are two HRE-binding sites HRE-1 and HRE-4, with HRE-4 having higher affinity to HIF-1 than HRE1.

STAT3

STAT3 has also been identified to regulate PD-L1 transcriptionally. Mutations of oncogene chimeric nucleophosmin/anaplastic lymphoma kinase (ALK) increased PD-L1 and have been abolished by siRNA against STAT3 [77]. STAT3 has been demonstrated to bind to the PD-L1 promoter to regulate its expression transcriptionally. Latent membrane protein-1 (LMP1) of Epstein–Barr virus increased PD-L1 expression with concomitant increase of phosphorylated STAT3 (pSTAT3) and inhibition of pSTAT3 by the JAK3 inhibitor CP-690550 reduced LMP1-induced PD-L1 expression [78].

NF-κB

NF-κB, a common transcriptional factor, has been shown to regulate PD-L1. However, details of the associated mechanism are not well studied. NF-κB is involved in LMP1-induced PD-L1 expression as the NF-κB inhibitor caffeic acid phenethyl
ester decreased PD-L1 induction [78]. NF-κB is also a major mediator of INF-gamma-induced PD-L1 expression [65]. The NF-κB inhibitor but not MAPK, PI3K and STAT3 inhibitors abolished INF-induced PD-L1 expression.

epigenetic regulation of PD-L1

Epigenetic regulation has also been shown to involve in PD-L1 expression in cancer cells. Several microRNAs (miRs) are involved in the regulation of PD-L1 expression. MiRs are 22–24 nucleotides in length and are non-coding single-stranded RNAs, which regulate gene expression [79]. They can bind to target gene mRNAs to degrade the mRNAs or prevent their translation. MiR-513, miR-570, miR-34a and miR-200 have an inverse relationship with PD-L1 expression [45, 80–82]. These miRs can complement with three-untranslated regions of PD-L1 to repress PD-L1 protein expression [81]. Introduction of miR-513 into Jurkat cells abolished IFN-gamma-induced PD-L1 expression [83, 84] while introduction of anti-miR-513 into cholangiocytes increased PD-L1 expression. MiR-570 has a similar effect pattern as mutation of the PD-L1 three-untranslated region which resulted in disruption of the associated miR-570, leading to over-expression of PD-L1 [85]. MiR-200-regulated PD-L1 expression has been shown to co-operate with miR-200-caused EMT to increase cancer metastasis [45]. MiR-197 decreases PD-L1 expression indirectly by targeting PD-L1 regulator STAT3 [86].

regulatory mode of PD-L1

Overall MAPK and PI3K/Akt signalling pathways as well as transcriptional factors HIF-1 and STAT3 are involved in the regulation of PD-L1 expression. Both pathways are well-known pro-survival pathways, promoting cancer development through a wide range of downstream targets. Therefore, the regulation of PD-L1 by these pathways may be coupled with other cancer-promoting activities of these pathways, adding a new dimension to the roles of these pathways in cancer. They not only promote cancer cell proliferation but also enable cancer cells to escape immune attack.

A regulatory mode for PD-L1 regulated by various signalling pathways is summarised (Figure 3). STAT3 and HIF-1 can act on the promoter directly while MAPK/c-Jun facilitates STAT3. Akt also regulates PD-L1 at the post-transcriptional level. NF-κB is known to regulate PD-L1. As it is a transcription factor, it is able to act on the PD-L1 promoter directly. Both Akt and STAT3 are known to regulate NF-κB [87, 88]. However, this is not studied in the setting of PD-L1 regulation. MiR-513 and miR-570 can attack PD-L1 mRNAs directly. A stimulus could regulate PD-L1 expression through various modes. EBV LMP1 can act on STAT3, NF-κB and MAPK/API to cause PD-L1 increase [78]. Inhibition of each pathway can reduce PD-L1 expression [78].

clinical implications of the regulation of PD-L1 expression

Increased PD-L1 has been associated with poor prognosis in several cancers including melanoma, ovarian cancer, lung cancer, nasopharyngeal and renal cell cancer [39, 89–92]. Inhibition of PD-L1 with antibodies improved OS rates in patients with these cancers. Excluding currently used antibodies for such therapies, inhibition of PD-L1 expression could also have a therapeutic effect. As PD-L1 expression is regulated by MAPK and PI3K/Akt signalling pathways, inhibition of these pathways should reduce PD-L1. Indeed, receptor tyrosine kinase inhibitors resulted in better treatment outcome in lung cancers with high-expression of PD-L1 [93, 94]. Therefore, inhibition of signalling pathways could have dual effects, inhibiting cell proliferation and PD-L1

Figure 3. A regulatory mode for PD-L1 expression in cancer cells. Both MAPK and PI3K signalling pathways are involved in the regulation of PD-L1 expression. The pathways are activated by gene mutations and growth factors. Activation of MAPK leads to increased activity of c-Jun, which acts together with STAT3 to increase transcription of PD-L1. Activation of Akt increases translation of PD-L1 mRNAs into proteins, which in turn increase activation of Akt. Activated Akt may also act on NF-κB. Hypoxia stimulates transcriptional factors HIF-1, which binds to HRE to increase PD-L1 expression. Transcriptional factors STAT3 and NF-κB can also act on PD-L1 promoter directly. miRNA-513 and miRNA-570 can degrade PD-L1 mRNAs. PD-L1, programmed death-ligand-1; HIF-1, hypoxia-inducible factor-1; HRE, HIF-responsive element; STAT3, signal transducer and activator of transcription 3; * indicates activating mutation. ^ indicates inactivating mutation.
expression. However, signalling molecules involved in PD-L1 regulation may differ from that for cell proliferation. In the PI3K/Akt pathway, Akt inhibition decreased PD-L1 expression while mTOR did not. Thus, selection of molecules for targeted therapy is of importance for treatment efficacy.

Another issue with inhibiting PD-L1 expression is drug resistance. Primary and acquired drug resistance have been major problems in targeted therapy. For example, in melanoma anti-mutated BRAF therapy led to a 50%–80% response rate [95, 96]. However, almost all patients obtained acquired drug resistance after 6 months, resulting in treatment failure. Resistance to inhibitors may also be the case in PD-L1 regulation, but it is not well studied.

It has been proposed that immunotherapy can be used to combine with targeted therapy [97]. Decreasing PD-L1 expression through signalling inhibition may be combined with anti-PD-L1 or anti-PD-1 therapy. This may increase treatment efficacy by controlling the PD-L1/PD-1 axis at very low levels. Indeed, the use of the signalling inhibitor trametinib to reduce PD-L1 expression together with anti-PD-1 antibodies showed a superior effect [98]. This raises a question in the combination therapy: should anti-PD-L1/anti-PD-1 be used simultaneously or sequentially? Based on the understanding of the regulatory role of PD-L1, it may be better to apply combination therapy simultaneously. This is because signalling inhibitors may take time to reduce PD-L1 expression and anti-PD-L1/PD-1 antibodies could decrease the axis at a very early stage. In late stage of the treatment, inhibition of signalling pathways may be sufficient to cause PD-L1 reduction.

conclusions
Up-regulation of PD-L1 has been demonstrated to be caused by activation of pro-survival pathways MAPK and PI3K/Akt as well as transcriptional factors HIF-1, STAT3 and NF-kB. All of them are well known to promote cancer development by increasing cell proliferation and decreasing apoptosis. Therefore, they regulate both cancer growth and immune escape. Inhibition of MAPK and PI3K/Akt pathways, as well as transcriptional factors HIF-1, STAT3 and NF-kB, should also reduce PD-L1 expression. Combination of anti-PD-L1 and anti-PD-1 antibodies may facilitate the reduction of PD-L1/PD-1 caused immunosuppression.

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references


47. Wang X, Li J, Dong K et al. Tumor suppressor miR-34a targets PD-L1 and has a tumour suppressive role in human melanoma. Nat commun 2013; 4: 1508.

48. Wang X, Li J, Dong K et al. Tumor suppressor miR-34a targets PD-L1 and has a tumour suppressive role in human melanoma. Nat commun 2013; 4: 1508.


