LETTER

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Cyclin D–CDK4 kinase destabilizes PD-L1 via Cul3^{SPOP} to control cancer immune surveillance

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Treatments that target immune checkpoints, such as the one mediated by programmed cell death protein 1 (PD-1) and its ligand PD-L1, have been approved for treating human cancers with durable clinical benefit[1,2]. However, many cancer patients fail to respond to anti-PD-1/PD-L1 treatment, and the underlying mechanism(s) is not well understood[3–5]. Recent studies revealed that response to PD-1/PD-L1 blockade might correlate with PD-L1 expression levels in tumor cells[6,7]. Hence, it is important to mechanistically understand the pathways controlling PD-L1 protein expression and stability, which can offer a molecular basis to improve the clinical response rate and efficacy of PD-1/PD-L1 blockade in cancer patients. Here, we report that PD-L1 protein abundance is regulated by cyclin D-CDK4 and the Cullin 3^{SPOP} E3 ligase via proteasome-mediated degradation. Inhibition of CDK4/6 *in vivo* elevates PD-L1 protein levels, largely by inhibiting cyclin D–CDK4-mediated phosphorylation of SPOP and thereby promoting SPOP degradation by APC/C^{Cdh1}. Loss-of-function mutations in *SPOP* compromise ubiquitination-mediated PD-L1 degradation, leading to increased PD-L1 levels and reduced numbers of tumor-infiltrating lymphocytes (TILs) in mouse tumors and in primary human prostate cancer specimens. Notably, combining CDK4/6 inhibitor treatment with anti-PD-1 immunotherapy enhances tumor regression and dramatically improves overall survival rates in mouse tumor models. Our study uncovers a novel molecular mechanism for regulating PD-L1 protein stability by a cell cycle kinase and reveals the potential for using combination treatment with CDK4/6 inhibitors and PD-1/PD-L1 immune checkpoint blockade to enhance therapeutic efficacy for human cancers.

Deregulated cell cycle progression is a hallmark of human cancer, and targeting cyclin-dependent kinases (CDKs) to block cell proliferation has been validated as an effective anti-cancer therapy[8]. Although it has been reported that PD-L1 expression can be regulated at both transcriptional[9,10] and post-translational levels[11,12], it remains unclear whether PD-L1 stability is regulated under physiological conditions such as during cell cycle progression. We found that PD-L1 protein abundance fluctuated during cell cycle in multiple human cancer cell lines, peaking in M/early G1 phases, followed by a sharp reduction in late G1/S phases (Fig. 1a-d; Extended Data Fig. 1a-g). Elevated PD-L1 protein abundance was also observed in multiple mouse tumor-derived cell lines arrested in M phase by nocodazole or taxol[13] (Extended Data Fig. 1h-m).

Cyclin-dependent kinases play crucial roles in regulating the stability of cell cycle-related proteins during cell cycle progression[14,15]. Therefore, we adopted a genetic method to ablate each major cyclin and found that ablating all three *D-type cyclins* (*D1*, *D2* and *D3*), but not *cyclin A* (*A1* and *A2*) nor *cyclin E* (*E1* and *E2*), strongly elevated PD-L1 protein abundance in mouse embryonic fibroblasts (MEFs) (Fig. 2a and Extended Data Fig. 2a-e). Using MEFs lacking individual D-type *cyclins*, we observed that depletion of *cyclin D1*, and to a lesser extent *cyclin D2* or *D3*, upregulated PD-L1 protein levels (Fig. 2b, c). Conversely, reintroduction of cyclin D1, and to a lesser extent cyclin D2 or D3, suppressed PD-L1 protein abundance in *cyclin D1*$^{-/-}$*D2*$^{-/-}$*D3*$^{-/-}$ MEFs (Extended Data Fig. 2f). In further support of a physiological role for cyclin D1 in negatively regulating PD-L1 protein level *in vivo*, mammary tumors arising in cyclin *D1*$^{-/-}$ MMTV-*Wnt-1* or MMTV-*c-Myc* mice displayed elevated PD-L1 protein levels, as compared to tumors arising in *cyclin D1*$^{+/+}$ animals (Fig. 2d and Extended Data Fig. 2g).

Depletion of cyclin D catalytic partner, the *cyclin-dependent kinase 4 (CDK4)* [16], but not *CDK6*[16] nor the cyclin A and cyclin E binding-partner, *CDK2* [17], also increased PD-L1 protein abundance in cells (Fig. 2e, f; Extended Data Fig. 2h-j). Conversely, ectopic expression of wild-type CDK4, but not kinase-dead N158F mutant, decreased PD-L1 levels (Extended Data Fig. 2k, l). Furthermore, treatment of multiple cancer cell lines with two different selective inhibitors of CDK4/6 kinase, palbociclib or ribociclib[8], upregulated PD-L1 protein abundance and stability even in pRB knock-down cells (Fig. 2g, h; Extended Data Fig. 2m-q).

Rb is frequently inactivated in human cancers[18,19]. In agreement with previous reports[20,21], we found that *Rb*-deficient cancer cells often displayed high levels of cyclin D-CDK4/6 inhibitor, p16^{INK4a}. Consistent with the notion that cyclin D1-CDK4 kinase suppresses PD-L1 levels, we observed that upregulation of 16^{INK4} correlated with elevated PD-L1 levels. Moreover, in *Rb*-proficient/p16-low cancer cell lines, higher PD-L1 levels correlated with relatively low CDK4 expression (Extended Data Fig. 2r). In addition, ectopic expression of p16^{INK4} in *Rb*-proficient/p16-low cell lines (MCF7 and T47D) or *Rb*-deficient/p16-low cell line (HLF) elevated PD-L1 protein abundance (Extended Data Fig. 2s-u), while depletion of p16^{INK4} in *Rb*-deficient/p16-high cell lines (MDA-MB-436, BT549, and HCC1937) had an opposite effect (Extended Data Fig. 2v-x), further documenting an inverse correlation between the CDK4 activity and PD-L1 expression.

To extend these observations to an *in vivo* setting, we treated MMTV-*ErbB2* mice bearing autochthonous breast cancers, or mice carrying allografts of murine MC38 or B16-F10 cancer cell lines with palbociclib, and monitored PD-L1 levels. Inhibition to CDK4/6 led a

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