Genes & Diseases (2023) 10, 848–863

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

REVIEW ARTICLE

Emerging role of ubiquitination/deubiquitination modification of PD-1/PD-L1 in cancer immunotherapy

Peng Ding [a,b,1], Zhiqiang Ma [b,1], Yizeng Fan [c,1], Yingtong Feng [a,1], Changjian Shao [a], Minghong Pan [a], Yimeng Zhang [d], Di Huang [b], Jing Han [d,***], Yi Hu [b,**], Xiaolong Yan [a,*]

[a] *Department of Thoracic Surgery, Tangdu Hospital, The Air Force Military Medical University, Xi'an, Shaanxi 710038, China*
[b] *Department of Medical Oncology, Senior Department of Oncology, The Fifth Medical Center of PLA General Hospital, Beijing 100853, China*
[c] *Department of Urology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China*
[d] *Department of Ophthalmology, Tangdu Hospital, The Air Force Medical University, Xi'an, Shaanxi 710038, China*

Received 22 November 2021; received in revised form 1 January 2022; accepted 8 January 2022
Available online 18 February 2022

KEYWORDS
Cancer;
Deubiquitination;
Immunotherapy;
PD-1;

Abstract As members of the immune checkpoint family, PD-1 and its ligand PD-L1 play critical roles in maintaining the balance between autoimmunity and tolerance. The interaction of PD-1/PD-L1 is also involved in tumor evasion inside the tumor microenvironment, caused by reduced T cell activation, proliferation, cytotoxic secretion, and survival. Previous research has shown that the expression level of PD-1/PD-L1 may be regulated by ubiquitin-mediated

Abbreviations: β-TrCP, beta-transducin repeat-containing protein; Cbl-b, casitas B lymphoma-b; c-Cbl, casitas B lineage lymphoma; COP1, constitutively photomorphogenic 1; CSN5, constitutive photomorphogenesis 9 signalosome 5; DCUN1D1, defective cullin neddylation 1 domain-containing 1; DUB, deubiquitinating enzyme; FBXO38, F-box only protein 38; FBXW7, F-box with 7 tandem WD40 repeats; HRD1, HMG-CoA reductase degradation protein 1; KLHL22, kelch like family member 22; OTUB1, OTU domain-containing ubiquitin aldehydebinding protein 1; PD-1, programmed death-1; PD-L1, programmed death-1 ligand; PTM, post-translational modification; RBX1, RING-box protein 1; SPOP, speckle-type POZ protein; STUB1, STIP1 homology and U-box– containing protein 1; UPS, ubiquitin-proteasome system; USP7, ubiquitin-specific protease 7; USP9X, ubiquitin-specific peptidase 9, X-linked; USP22, ubiquitin-specific protease 22.

* Corresponding author. 1 Xinsi Road, Xi'an, Shaanxi 710038, China.
** Corresponding author. 28 Fuxing Road, Haidian, Beijing 100853, China.
*** Corresponding author. 1 Xinsi Road, Xi'an, Shaanxi 710038, China.
E-mail addresses: hanjing.cn@163.com (J. Han), huyi301zlxb@sina.com (Y. Hu), yanxiaolong@fmmu.edu.cn (X. Yan).
Peer review under responsibility of Chongqing Medical University.
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.gendis.2022.01.002

PD-L1;
Ubiquitination

proteasome degradation, which is an important mode of post-translational modification (PTM). PD-1/PD-L1 ubiquitin modification research in tumor immunotherapy is the subject of the present review, which aims to assess the most recent developments in this area. We offer a short explanation of PD-1/PD-L1 as well as some basic background information on the UPS system and discuss many routes that target E3s and DUBs, respectively, in the regulation of PD-1/PD-L1 in tumor immunotherapy. In addition, we offer numerous innovative prospective research areas for the future, as well as novel immunotherapy concepts and ideas. Taken together, the information compiled herein should serve as a comprehensive repository of information about tumor immunotherapy that is currently available, and it should be useful in the design of future studies, as well as the development of potential targets and strategies for future tumor immunotherapy.

Introduction

As members of the immune checkpoint family, programmed death-1 (PD-1) and its ligand programmed death-1 ligand (PD-L1) play critical roles in maintaining the balance between autoimmunity and tolerance in body.[1] Regulated by ubiquitination, a prominent form of post-translational modification (PTM), abnormal expression of PD-1/PD-L1 and excessive immunosuppression mediated by their interaction have been demonstrated have close relationship with many diseases[2,3] including cancer, which can suppress T cell activation, proliferation, cytotoxic secretion and T cell survival to facilitate tumor evasion within the tumor microenvironment.[4] Targeting PD-1/PD-L1 or key molecules in ubiquitination process to inhibit the development and progression of cancer has attracted great attention to researchers, which provides a prosperous future for cancer immunotherapy.[5]

The present study is concerned with evaluating the most recent research development in the field of ubiquitination of PD-1/PD-L1 in tumor immunotherapy. Firstly, a quick explanation of PD-1/PD-L1 as well as a broad background on the ubiquitin-proteasome system (UPS) are presented. Following that, we discuss alternative mechanisms that target E3s and deubiquitinating enzymes (DUBs), respectively, in the regulation of PD-1/PD-L1 in tumor immunotherapy. Finally, we offer numerous innovative prospective research areas for the future, as well as novel immunotherapy concepts. Collectively, the material assembled below should serve as a thorough repository of knowledge that is currently available in this field, and it should contribute to the design of future research as well as the identification of possible targets and techniques for future cancer immunotherapy.

General background on PD-1/PD-L1

PD-1, also known as CD279, is a type I transmembrane protein with a molecular weight of 55 kDa from B7/CD28 family.[6,7] Inducible PD-1 expression has been seen on activated T ($CD4^+$ and $CD8^+$), natural killer (NK), and B lymphocytes, as well as on activated monocytes, dendritic cells, and macrophages.[8–10] PD-1 is an inhibitor of both innate and adaptive immune responses,[9] and it is critical in the maintenance of immunological tolerance as well as the suppression of inefficient or damaging immune responses.[11] Through interference with the immune response, it may also aid in the growth and evasion of various solid cancers such as melanoma, breast cancer, and non-small-cell lung cancer.[11,12]

As one of the ligands of PD-1, the type I transmembrane protein PD-L1 (also referred to as CD274 or B7—H1) has a molecular weight of 33 kDa and interacts with PD-1 produced in numerous cell types to have immunological effects.[13] PD-L1 is often found on immune cells, such as certain activated T and B cells, dendritic cells, macrophages, and epithelial cells, among other places.[14,15] It has been shown to be upregulated in many of these cell and tissue types particularly under inflammatory conditions.[15] PD-L1 is also found in the endoplasmic reticulum, Golgi apparatus, nucleus, and cytoplasm.[16,17] Oncogenic PD-L1 has been shown to be overexpressed in a variety of solid tumors including breast, bladder, melanoma, head and neck cancer, non-small cell lung cancer, and several hematologic malignancies.[12,13] PD-L1 is involved in tumor progression, activating tumor proliferative and survival signaling pathways by interacting with its receptor PD-1.[18] Additionally, PD-L1 has been shown to induce epithelial-to-mesenchymal transition (EMT) and stem cell-like phenotypes, demonstrating non-immune proliferative effects,[19] further promoting cancer progression.

In the last decade, cancer immunotherapies that particularly target the PD-1/PD-L1 axis have achieved tremendous advances and accomplishments.[20] Several cancers, including non-small cell lung carcinoma,[21] melanoma[22] and others, are already being treated with PD-1/PD-L1 inhibitors that have been licensed by the FDA for use in clinical trials.

General background on UPS system

Post-translational modification (PTM) is indispensable in maintaining normal cell function by regulating protein activity and stability, inhibiting the distribution and activity of PD-1/PD-L1 and the molecules that interact with them in their native and mutant forms.[23] Ubiquitination is one of the important PTMs. The ubiquitin-proteasome system

(UPS), as the primary undertaking of ubiquitination, acts as a fundamental PTM mechanism that regulates protein degradation and operates in numerous cellular activities in eukaryotic cells under healthy and pathological situations.[24] Many important components form the UPS, for instance, ubiquitin, the 26S proteasome, ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), ubiquitin-protein enzymes (E3s), and deubiquitinating enzymes (DUBs).[25]

Ubiquitin acts as a modifier by binding to certain protein substrates, and 26S proteasomes are responsible for the proteolysis of substrates that have been tagged with ubiquitin. Ubiquitins may be linked together by their lysine residues, which are found on their surface. Lys11 and Lys48-linked polyubiquitin chains are the most important signals for degradation by the 26S proteasomes.[26] A critical part in the multistep cascade process in which ubiquitin is conjugated to its substrate is played by the E1s, E2s, and E3s enzymes. The carboxyl group (-COOH) of ubiquitin's C-terminus and the Cys residue in the active catalytic region of E1s are linked together by thioester linkage, which is formed by the energy released during ATP hydrolysis. In the next step, the ubiquitin moiety is transported to E2s for a brief period, where it forms an ester bond between E2s and the ubiquitin moieties. Last but not least, the charged E2s work in concert with the E3s to transport the activated ubiquitin to the lysine residue on the target substrates.[27] This multistep mechanism also turns ubiquitin polymerization into the polyubiquitin chain, which is important for cell survival.[28] Because they can remove the ubiquitin chain from substrates, DUBs may stop E3s from degrading or altering the target substrates in any other way due to ubiquitination[29] (Fig. 1).

Connection between PD-1/PD-L1 and ubiquitination/deubiquitination

To understand the PD-1/PD-L1 signaling pathways, ubiquitination and deubiquitination have been intensively studied.[30] Stabilization and de-stabilization of PD-1/PD-L1 have shown a significant impact on so-called inflammation-mediated anti-tumor immunity.[31] As previously mentioned, the relationship between PD-1/PD-L1 and ubiquitination/deubiquitination implies that targeting any of these two pathways may be a viable alternative method to enhance immune checkpoint treatment in the anticancer process. E3s and DUBs have received a great deal of attention from scientists because they are important components in the ubiquitination-related process. This implies that targeting E3s and DUBs is a new technique for improving anticancer immune responses.[32]

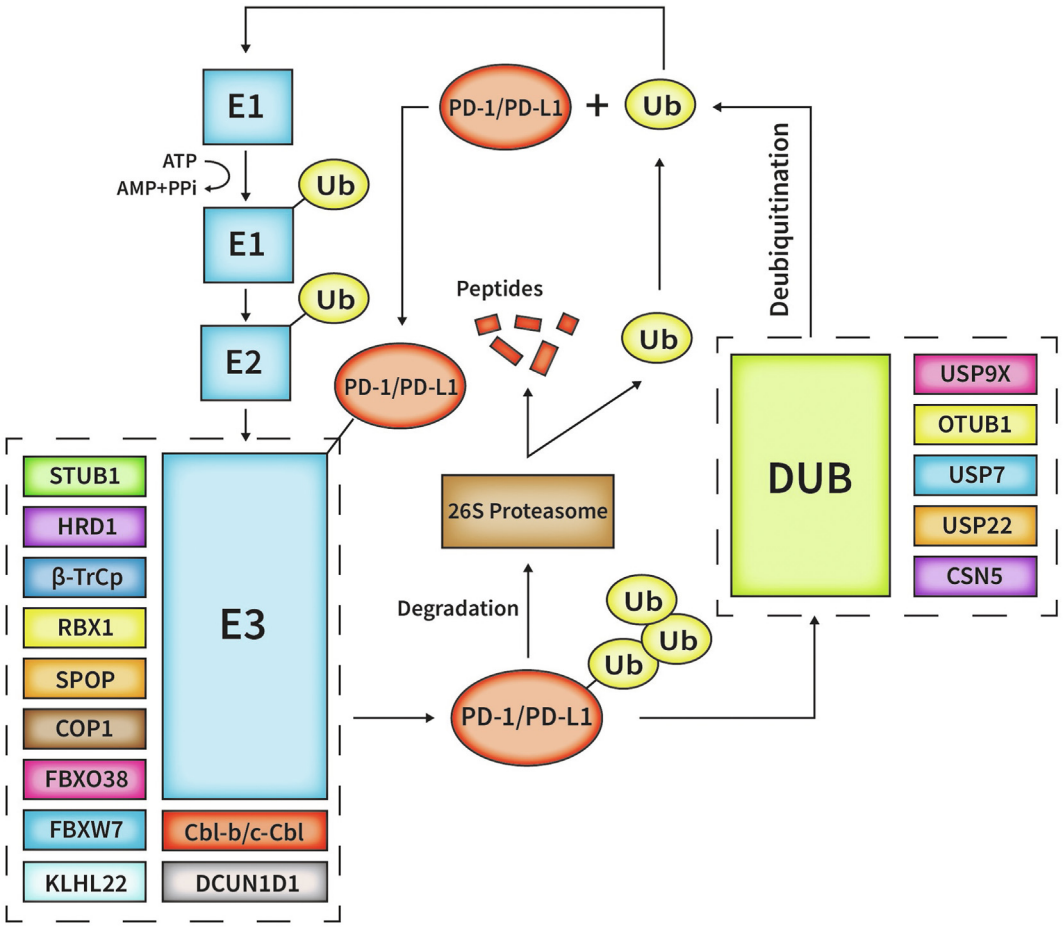

Figure 1 Schematic elucidation of ubiquitination/deubiquitination conjugation cascade and some representative components related to the regulation of PD-1/PD-L1.

E3s in regulation of PD-1/PD-L1 in tumor immunotherapy

RBX1

RING-box protein 1 (RBX1, also known as ROC1) is essential for Skp1, Cullins, F-box proteins (SCF) E3 ubiquitin ligase, which was reported to be over-expressed in human tumor tissues, contributing to tumor progression and poor prognosis by regulating cell proliferation, senescence and apoptosis.[33] An earlier study indicated that 2,5-dimethylcelecoxib (DMC) may enhance ubiquitin degradation of hepatitis B virus X (HBx)-induced PD-L1 in hepatocellular carcinoma cells through stimulating the AMPK pathway in hepatocellular carcinoma cells. It has been shown that DMC may increase the phosphorylation of 5′ AMP-activated protein kinase (AMPKα), resulting in aberrant production and accumulation of PD-L1 in the endoplasmic reticulum. RBX1 is involved in this abnormal protein processing and facilitated the ubiquitin degradation of PD-L1. Besides, combined use of DMC and atezolizumab shows more significant blocking effect on PD-L1 signaling pathway, reflecting the potential application prospects of inhibiting RBX1 in tumor immunotherapy to some extent.[34] However, DMC does not directly act on RBX1 to regulate the stability of PD-L1 but promotes substrate ubiquitination through AMPK pathway. Studies have reported that some molecules such as miR-135b and miR-378 can regulate tumor proliferation, invasion and migration by targeting RBX1 directly,[35,36] but it is not clear whether the downstream molecule of RBX1 is PD-L1 or PD-1. The exploration and elucidation of RBX1 signaling pathway might provide new ideas for multitarget or combined immunotherapy of various cancers.

COP1

Consistently photomorphogenic 1 (COP1), a ubiquitin E3 ligase, plays significant roles in many biological processes, for instance, tumorigenesis,[37] gluconeogenesis[38] and DNA damage response[39] by targeting specific substrates including c-Jun, p53, TORC2 and so on.[40] Reduced COP1 inhibits c-Jun ubiquitination, which slows down c-Jun degradation while simultaneously increasing c-Jun stability. C-Jun N-terminal kinase (JNK) may phosphorylate and activate accumulated c-Jun, which can then translocate into the nucleus and strongly block the production of histone deacetylase 3 (HDAC3), resulting in the induction of histone H3 acetylation of the promoter region of PD-L1.[40] As a result of increased histone acetylation, the chromatin structure became more flexible, encouraging PD-L1 transcription and, thus, promoting PD-L1 expression.[41] As a result, COP1 is a component of a new regulatory network that contributes to the rise in PD-L1 expression in drug-resistant cancer cells, and it may represent a prospective therapeutic target in cancer immunotherapy. However, no small molecule inhibitors that directly target human COP1 have been reported. More advance techniques and drug screening approach may be necessary to develop immunosuppressors of COP1 for cancer therapy.

FBXW7

F-box with seven tandem WD40 repeats (FBXW7, also known as Fbw7, Sel-10, hCdc4, hAgo, or Archipelago) is a crucial component of the SCF E3 ubiquitin ligase.[42] Numerous studies have also showed that deregulation of oncoproteins can be caused by inactivation or downregulation of FBXW7 and is possible to lead to tumorigenesis in human.[43,44] As a substrate of FBXW7, heat-shock factor1 (HSF1) modulates heat shock response and encourages malignant transformation. When FBXW7 is downregulated or mutated in various malignancies, HSF1 degradation is diminished. This may lead to an increase in HSF1 levels and an increase in melanoma's spreading potential.[45] In a prior research, it was discovered that HSF1, FBXW7, and PD-L1 were all linked. When the proto-oncogene Proviral insertion in murine malignancies (*PIM2*) phosphorylates HSF1 at Thr120 and prevents it from binding to the enzyme FBXW7, it disturbs the process by which HSF1 is degraded by the ubiquitin-proteasome. The accumulated HSF1 may bind to the PD-L1 promoter and facilitate the expression of PD-L1, stimulating tumor growth in breast cancer.[46] Thus, it might provide a molecular basis and therapeutic target for treating tumors by interfering with the *PIM2*/HSF1/FBXW7/PD-L1 axis. In addition, another study has reported that inactivation or loss-of-function mutation of FBXW7 promotes anti-PD-1 therapy resistance via downregulation of viral sensing pathways.[47] Therefore, reactivation of FBXW7 or restoration of above signaling pathways might improve clinical efficacy in checkpoints immunotherapy.

DCUN1D1

Defective cullin neddylation 1 domain-containing 1 (DCUN1D1) functions as a component of the neddylation of E3 ligases complex.[48] A growing body of evidence supports the notion that DCUN1D1 is involved in a broad variety of development and metastasis processes in some cancers, including prostate cancer,[49] colorectal carcinoma[50] and glioma.[51] When comparing cervical cancer tissues to neighboring tissues, DCUN1D1 expression is much higher in the cancerous tissues, and high levels of DCUN1D1 expression are related with advanced clinical stage, lymph node metastasis, and a shorter overall survival time.[52] Colorectal carcinoma patients who exhibit high level of DCUN1D1 expression may have a poorer clinical outcome.[50] DCUN1D1 has been implicated in the oncogenic process in non-small cell lung cancer, and it has been shown to be a poor prognosticator in earlier research. This study found that increasing the level of DCUN1D1 and activating focal adhesion kinase (FAK) signals in lung cancer cell lines result in a significant increase in PD-L1 expression, indicating that DCUN1D1 may act as an endogenous stimulator of PD-L1 expression in non-small cell lung cancer.[53] However, the specific mechanism or protein interaction points by which DCUN1D1 upregulates PD-L1 has not been clarified. Whether DCUN1D1 can act as a target in tumor immunotherapy still needs to be confirmed by further experiments.

FBXO38

F-box only protein 38 (FBXO38) is one of the E3 ligases that is responsible for the ubiquitination of Lys48-linked proteins and the subsequent destruction by the proteasome.[54] FBXO38 is involved in PD-1 regulation and cancer immunotherapy. A previous study demonstrated that PD-1 on the surface of $CD8^+$ T cells is closely regulated by protein degradative ubiquitination that mediates by FBXO38.[55] FBXO38 directly induces Lys48-linked polyubiquitination at Lys233 of PD-1, leading to PD-1 ubiquitin degradation. Endogenous PD-1 levels on the cell surface of activated T cells are decreased when FBXO38 is expressed ectopically, while FBXO38 knocking down results in an increase of PD-1 levels on the cell surface. The researchers also discovered that interleukin-2 (IL-2) may target the transcriptional factor signal transducer and activator of transcription 5 (STAT5) and lead to a considerable increase in FBXO38 mRNA abundance, which in turn results in a decrease in the amount of PD-1.[55] Because the findings above reveal that FBXO38 is a critical regulator of PD-1 degradation, innovative therapeutic approaches to reverse T cell depletion and boost anti-tumor responses may be developed in the future. It is possible that targeting the *FBXO38* gene in the PD-1 signaling pathway would open up new opportunities for cancer immunotherapy.

Cbl-b and c-Cbl

Both casitas B lineage lymphoma (c-Cbl) and casitas B lymphoma-b (Cbl-b) are RING finger E3 ligase enzymes that are involved in targeting important protein tyrosine kinases to operate as negative regulators of T-cell receptor expression.[56] It has been shown that they are implicated in several facts of tumor growth, including tumor cell migration,[57] epithelial—mesenchymal transition and metastasis.[58] Evidence shows that the AKT and STAT pathways may be implicated in the downregulation of PD-L1 expression through Cbl-b and c-Cbl,[59] although the particular molecular and protein interactions involved in these pathways have not been clarified to this point. According to another research, miR-940 blocked the STAT5a/Cbl-b interaction and ubiquitination in gastric cancer cells by inhibiting Cbl-b.[60] Besides, the combination between c-Cbl and intracytoplasmic tail of PD-1 facilitates the ubiquitination-proteasomal degradation of PD-1 in macrophages, leading to decreased expression level of PD-1 in macrophages.[47] In addition, Cbl-b is implicated in the down-modulation of $CD8^+$ T cell receptors in response to PD-1/PD-L1 stimulation of T cells. After T cell activation, the combination of PD-1 on the T cell surface and PD-L1 on the APC surface recruits SHP phosphatases and induces the expression of Cbl-b, which results in the ubiquitination and inactivation of significant TCR signal transduction mediators, and the removal of the TCR from the T cell surface, as previously described.[61] Therefore, Cbl-b/c-Cbl and their upstream or downstream components, controlling the PD-1/PD-L1 signaling pathway, and modulating TCR levels are all potential targets for cancer immunotherapy.

SPOP

Speckle-type POZ protein (SPOP) has been discovered as an E3 ubiquitin ligase substrate binding member of the proteasome complex,[62] and it has been shown to have a dual role in carcinogenesis and cancer development.[63] Downregulated or mutant SPOP plays a significant role in driving tumorigenesis in many cancers. A previous research found that the cyclin D—CDK4 (cyclin-dependent kinase 4) and Cullin 3 SPOP E3 ligase are responsible for the regulation of PD-L1 abundance in the body. APC/C^{Cdh1} degradation of SPOP is stimulated by CDK4/6 inhibition, which increases PD-L1 levels *in vivo*. CDK4/6 inhibition *in vivo* has a major effect on PD-L1 levels by reducing cyclin D—CDK4-mediated phosphorylation of SPOP, which promotes SPOP breakdown by APC/C^{Cdh1}. PD-L1 degradation is compromised by a reduced number of or a loss-of-function mutation in SPOP, which results in elevated levels of PD-L1 and a lower number of tumor-infiltrating lymphocytes in primary human prostate cancer tissues and mice tumors, respectively.[64] Phosphorylation of moesin (MSN) by rho-associated protein kinases (ROCK) has been shown to impede the degradation of PD-L1 by the proteasome, which in turn stabilizes its protein levels.[65] Enhanced production of ALDH2 caused by alcohol exposure may also prevent the ubiquitination modification of PD-L1 by SPOP at the residues K280 and K281, resulting in increased protein stability of PD-L1.[66] As a result, SPOP and its regulatory molecules may prove to be attractive targets for cancer immunotherapy in combination with PD-L1/PD-1 blockades in the future.

β-TrCP

Beta-transducin repeat-containing protein (β-TrCP) is the substrate recognition subunit of the $SCF^{\beta-TrCP}$ E3 ubiquitin ligase complex.[67] In the presence of glycogen synthase kinase 3β (GSK3β), β-TrCP has been shown to catalyze the ubiquitination of the PD-L1 protein. GSK3β has been shown to have important roles in embryonic development and cancer via the Wnt signaling pathway, among other things.[68] As an example, GSK3β phosphorylates β-catenin, which then incorporates β-TrCP for the degradation of proteins like PD-L1 by ubiquitination. Conversely, mutations in GSK3β phosphorylation or inhibition of β-TrCP significantly blocks PD-L1 ubiquitination, thus maintaining the stability of PD-L1 [30]. Besides, activation of AKT caused by epidermal growth factor receptor (EGFR) significantly inhibits GSK3β activity through Ser9 phosphorylation, further suppressing the ubiquitination of PD-L1 by β-TrCP, so as to maintain the structure stability of PD-L1 [69]. It has been demonstrated in a few trials that certain specific inhibitors which inactive GSK3β may be utilized to decrease the ubiquitination of PD-L1 and enhance its stability, hence increasing the effectiveness of immunotherapy. To name a few, olaparib and resveratrol have both been shown to inhibit GSK3β activity, allowing them to exert further effect on the interaction between β-TrCP and PD-L1 [30;70]. Another study found that inhibiting the mammalian target of rapamycin complex 1 (mTORC1)/p70 S6 kinase (p70S6K) signaling pathway, either by using mTOR/p70S6K inhibitors

or by raptor knockdown of p70S6K, facilitates β-TrCP degradation, which is associated with increased PD-L1 protein stabilization, even though the precise mechanism by which the signal pathway regulates β-TrCP is still unknown.[71] In other words, targeting β-TrCP stability in combination with PD-L1/PD-1 blockades may give a novel way to improve the effectiveness of cancer immunotherapy in the long term.

STUB1

STIP1 homology and U-box-containing protein 1 (STUB1), with its E3 ligase activity, is related with the ubiquitination of various substrates, for instance, FOXP3,[72] SMAD3,[73] unfolding protein response[74] and so on. Several studies have indicated that STUB1 serves as a tumor suppressor as a consequence of its ability to induce the ubiquitination and degradation of particular oncogenic proteins.[75,76] Furthermore, the expression of STUB1 in different carcinomas is often deficient or non-existent.[77] CMTM6, a type 3 transmembrane protein that is extensively expressed, has been shown to be a positive modulator of PD-L1 and to protect PD-L1 from ubiquitination. A recent research found that knocking out *STUB1* produces a greater rise in PD-L1 levels in CMTM6-deficient cells than in CMTM6-competent cells, confirming STUB1 as an E3 ligase that causes PD-L1 instability either directly or indirectly via regulating lysine in the cytoplasmic domain.[78] Another study has reported that the combination of STUB1 to PD-L1 can be enhanced by pyridoxal (vitamin B6), which facilitates K48-linked polyubiquitination of PD-L1, causing increased degradation of PD-L1 and stronger T cells-killing activity against cancer cells.[79] Although the underlying molecular mechanism by which pyridoxal promotes PD-L1 degradation has not been elucidated clearly, pyridoxal still has the potential to act as a plausible alternative for combined immunotherapy. Whether regulating the stability of PD-L1 by targeting STUB1 contributes to cancer immunotherapy needs to be further investigated precisely.

HRD1

HMG-CoA reductase degrading protein 1 (HRD1, also named as SYVN1) is an E3 ubiquitin ligase that is involved in the endoplasmic reticulum-associated degradation (ERAD) process. It transfers ubiquitin from the endoplasmic reticulum-associated UBC7 E2 ligase to the protein substrate, thereby aiding the ubiquitinated degradation of the target substrate.[80] In different malignancies, HRD1 has an oncogenic function via the ubiquitination-mediated degradation of several proteins, including PTEN and sirtuin 2.[81] A previous study reported that HRD1 expression is increased markedly in human colon cancer cells, and its overexpression is involved in many processes including tumor differentiation, tumor invasive depth, TNM stage and distant metastasis.[82] However, a research found that HRD1 may inhibit the proliferation and spread of breast cancer cells by boosting IGF-1R degradation, indicating that the function of HRD1 in malignancies should be investigated further in the future.[83] Metformin, according to another research, stimulates AMPK, which phosphorylates S195 of the protein PD-L1, resulting in aberrant glycosylation and accumulation of PD-L1. The ERAD pathway is responsible for the degradation of PD-L1, and HRD1 is an important player in this process. PD-L1 S195E and 4NQ mutants are more stable when *HRD1* is knocked out, indicating that the E3 ligase HRD1 is involved in the ERAD of PD-L1 [84]. However, the particular mechanism by which HRD1 has an influence on this pathway has not been adequately described, and more research are required. Targeting HRD1 directly or indirect regulation of HRD1 through its signaling pathways may be effective in future cancer immunotherapy strategies.

KLHL22

Studies have reported that kelch like family member 22 (KLHL22), a substrate-specific adaptor of the Cul3-based E3 ligase, can modulate protein levels via ubiquitination process to regulate various cellular activities such as mitosis and tumorigenesis.[85] KLHL22 has been validated to activate mTORC1 and downstream signaling pathways to facilitate carcinogenesis and aging. The expression level of KLHL22 is also elevated in breast cancer cells.[86] Besides, KLHL22 can modulate the activity of GSK-3β to affect PI3K level via Wnt/β-catenin signaling pathway, further regulating the EMT process and cell proliferation in colorectal cancer.[87] A previous study has reported that KLHL22 can decrease the number of PD-1 located on the cell surface by promoting its ubiquitin degradation. Decreased KLHL22 contributes to the overaccumulation of PD-1, which inhibits the normal function of immune system and facilitates tumor development and progression. In addition, as a member of the first-line chemotherapeutic agents, 5-fluorouracil (5-FU) can upregulate PD-1 by suppressing the transcriptional activity of KLHL22 in colorectal cancer cells.[88] Synergistic application of 5-FU and anti-PD-1 drugs may improve the efficacy of immunotherapy and contribute to better prognosis for cancer patients.

DUBs in regulation of PD-1/PD-L1 in tumor immunotherapy

OTUB1

OTU domain-containing ubiquitin aldehydebinding protein 1 (OTUB1) belongs to the ovarian tumor domain protease subfamily of deubiquitinases. It is responsible for negatively controlling ubiquitination in order to keep targeted proteins active and stable.[29] Cancer cells produce greater levels of OTUB1 than normal tissues, and its expression is closely correlated with tumor growth, differentiation, and metastasis in colon cancer patients.[89] A recent research found that OTUB1 may positively influence PD-L1 stability and mediate cancer immune responses by working on the PD-1/PD-L1 axis. This is the first time that this has been shown. On the ICD area, OTUB1 directly removes the Lys 48-linked polyubiquitin chains, and it specifically interacts with PD-L1, preventing the proteasomal degradation of ER-associated PD-L1 presumably before glycosylation and promoting high expression levels of PD-L1 in a variety of malignancies. When OTUB1 is depleted, it has a

considerable effect on the level of PD-L1 in the blood and on the link between PD-1 and tumor cells. Furthermore, the instability of PD-L1 produced by OTUB1 depletion stimulates the infiltration of more $CD8^+$ T cells and the production of more IFN-γ, both of which aid in the development of antitumor immunity against tumors.[90] Another research describes a regulatory mechanism for OTUB1 that occurs upstream of the transcription factor. CircIGF2BP3, a member of the circRNA family, controls the expression of plakophilin 3 (PKP3) by sponging miR-3173-5p and miR-328-3p, which are both expressed in brain. Fragile X mental retardation-related protein 1 (FXR1) and PKP3 collaborate to create RNA-protein complexes, which help to maintain OTUB1's mRNA stability while blocking its destruction by ubiquitination and degradation.[91] Because of this, it is feasible that modulating the OTUB1 regulatory pathway may serve as a novel therapeutic target and will enhance clinical results in patients receiving PD-L1/PD-1 blocking therapy by altering the expression of PD-L1.

USP7

Ubiquitin-specific protease 7 (USP7), or herpes virus associated protease (HAUSP), is a DUB that altering the stability of a large number of targeted proteins.[92] USP7 overexpression has been extensively studied for the ability to cause tumor progression through dysregulation of DNA damage response, cell cycle modulation and apoptosis. For instance, increased USP7 level is related with tumor aggressiveness in chronic lymphocytic leukemia,[93] prostate cancer[94] and breast carcinoma.[95] Targeted inhibition of USP7 reprograms tumor associated macrophages (TAMs) and stimulates the anti-tumor effect of CTLs, which suppresses tumor growth. The inhibition of USP7 also upregulates PD-L1 level in tumor microenvironment. Using the combination of P5091 (an inhibitor of USP7) and PD-1 monoclonal antibody, researchers were able to demonstrate a synergistic anti-tumor impact, which bodes well for the future of lung cancer therapy.[96] Furthermore, in gastric cancer, the amount of PD-L1 is favorably linked with the level of USP7. Inhibition of USP7 may inhibit the proliferation of gastric cancer cells by halting the cell cycle during the G2-M phase, maintaining p53 expression, and downregulating PD-L1 expression. It also weakens the PD-1/PD-L1 connection, making cancer cells more susceptible to T cell death.[97] However, the underlying mechanism of the upregulated PD-L1 level by targeting USP7 is not clear and needs to be further explored. By modulating Foxp3 and Tip60, a prior research found that genetic or pharmacologic targeting of USP7 may block the inhibitory activities of $Foxp3^+$ Tregs and preserve normal T cell responses, reducing tumor development and enhancing the efficiency of anti-PD-1 monoclonal antibodies.[98] Therefore, the further development and clinical testing of USP7 inhibitors combined with PD-L1/PD-1 checkpoint inhibitors may provide a new valuable approach for cancer immunotherapy.

USP22

Ubiquitin-specific protease 22 (USP22) is a member of the USPs subfamily and is considered as an oncogenic protein due to the fact that it is overexpressed in a variety of malignancies.[99] The downregulation of USP22, according to a prior research, restricts the penetration of myeloid cells and enhances the infiltration of natural killer cells and T cells, hence improving tumor eradication in cancer immunotherapy.[100] According to one research, USP22 deubiquitinates PD-L1 by removing its K6, K11, K27, K29, K33, and K63-linked polyubiquitin chains in a kinase-dependent manner, therefore lowering PD-L1 ubiquitination and preserving PD-L1 from degradation by the proteasome. Apart from that, USP22 has the ability to influence PD-L1 by deubiquitinating CSN5 directly, hence increasing the quantity of CSN5 protein and preventing its expression. The loss of USP22 causes PD-L1 to be degraded at the post-translational level, which has been shown to reduce carcinogenesis and increase T cell-mediated cell death.[101] PD-L1 targeted immunotherapy and CDDP-based chemotherapy were both shown to benefit from USP22 reduction in another research, highlighting the complex and important functions of the USP22-PD-L1 axis in cancer treatment.[102] According to a recent research, the tumor-promoting long noncoding RNA (lncRNA) KCNQ1OT1 controls the ubiquitination of PD-L1 and decreases the response of $CD8^+$ T cells via the miR-30a-5p/USP22 pathway. Low expression of miR-30a-5p regulated by KCNQ1OT1 mitigates its inhibitory effect on USP22, thereby facilitating the stabilization of PD-L1 [103]. Further signal investigation and clinical trial into the combined and synergistic effects of USP22 inhibition and PD-L1/PD-1 blockade may provide an avenue for clinical immunotherapy of cancer.

USP9X

As a member of DUBs, ubiquitin-specific peptidase 9, X-linked (USP9X) can remove ubiquitin from protein substrates and various ubiquitin linkages, participating in the regulation of the immune system.[104] According to a recent research, USP9X is a critical positive regulator of TCR signaling-induced nuclear factor-κB (NF-κB) activation. The loss of USP9X affects T cell proliferation, cytokine generation, and the differentiation of T helper cells, all of which are detrimental to the immune system.[105] USP9X has a role in the control of tumor cell proliferation, adhesion, and apoptosis, among other things.[106] It has been shown that USP9X has a significant function in carcinogenesis and has been found to be inappropriately expressed in non-small cell lung cancer, melanoma as well as breast cancer.[107–109] The regulation of β-catenin by USP9X, for example, has been shown to enhance the development of liver cancer.[110] In ERG-positive prostate cancer, USP9X levels are considerably elevated.[111] It is also possible to reduce the tumorigenicity of pancreatic ductal carcinoma by suppressing cancer cell transformation and anoikis using USP9X.[112] The ubiquitination of PD-L1 is inhibited by USP9X, which has been shown to be substantially expressed in oral squamous cell carcinomas (OSCC). OSCC's high USP9X expression is thought to be regulated by a signaling pathway, although the exact mechanism is still a mystery. OSCC cell tumor development can be dramatically reduced by USP9X knockdown.[113] Therefore, targeting PD-L1 by blocking or silencing USP9X may provide an effective

strategy in cancer immunotherapy. Further experiments about the mechanisms that causing high USP9X level in cancers should be focused and carried out.

CSN5

Constitutive photomorphogenesis 9 signalosome 5 (CSN5) possesses deubiquitination activity, regulating exosomal protein sorting[114] and stimulating the invasion or metastasis of tumor.[115] A previous study has reported that CSN5 participates in carcinogenesis progression and is closely related with poor prognosis.[116] Another research found that the CC motif chemokine ligand 5 (CCL5) -p65/STAT3-CSN5-PD-L1 signaling axis, which is triggered by high-cholesterol diet (HCD)-driven macrophage infiltration or lipopolysaccharide (LPS), is associated with considerably lower survival in colorectal cancer. The p65/STAT3 complexes can be formed by CCL5 and leads to the upregulation of CSN5 by binding to CSN5 promoter. Increased expression of CSN5 deubiquitylates PD-L1 and modulates its stability.[117] Besides, the p65-CSN5-PD-L1 pathway can also be activated by TNF-α, triggering cancer cells immunosuppression via stabilization of PD-L1. Inhibiting this signaling pathway promotes the immune response mediated by tumor-infiltrating cytotoxic T cell.[31] Using berberine (BBR), a proven anti-inflammatory drug, a group discovered that it can specifically bind to the glutamic acid 76 of CSN5 and subsequently inactivate CSN5, which results in PD-L1 degradation, resulting in increased tumor-infiltrating T cell immunity and decreased activation of immunosuppressive Tregs and myeloid-derived suppressor cells (MDSCs).[118] Furthermore, studies have shown that compound-15, an inhibitor of CSN5, might destabilize PD-L1, resulting in a reduction in tumor burden.[117] Aside from that, protein disulfide isomerase family A member 6 (PDIA6) has been shown to increase the amount of CSN5 in pancreatic cancer by interfering with the production of disulfide bonds in CSN5, hence enabling the proper folding of mature CSN5.[119] Furthermore, it has been observed that shikonin, a natural product derived from plants, may prevent the activation of the NF-κB/CSN5 signaling pathway, which promotes the degradation of PD-L1 and inhibits the immune evasion of pancreatic cancer cells.[120] As a result, targeting CSN5 in cancer immunotherapy may prove to be a potential therapeutic strategy.

Discussion

As one of the significant inhibitory signaling pathways, the PD-1/PD-L1 interaction not only participates in physiological activities such as stabilizing T cells immune homeostasis and maintaining peripheral tolerance, but also can be utilized by cancers to escape from anticancer immunity in tumor microenvironment.[121] The majority of cancer patients have poor response rates and develop resistance within a short response time despite the fact that antibodies targeting the PD-1/PD-L1 signaling pathway have shown sustained anticancer responses and disease suppression in individuals with specific forms of cancer.[122,123] Scientists investigate regulatory mechanisms such as transcriptional, translational, and post-translational control of the PD-1/PD-L1 pathway to overcome hurdles in anti-PD-1/PD-L1 immunotherapy. Ubiquitination and deubiquitination are essential post-translational modification processes that control the expression level of PD-1/PD-L1 by modulating its stability, which affects immune system function and anticancer immunotherapy impact.

E3s and DUBs are both implicated in the critical process of ubiquitination and deubiquitination in the control of PD-1/PD-L1 stability and expression, according to previous research. Given the activities of E3s and DUBs, scientists are attempting to determine if targeting E3s or DUBs to control PD-1/PD-L1 levels is successful. New evidence suggests that DMC enhances RBX1-mediated ubiquitination degradation of Hbx-induced PD-L1 in human colorectal cancer cells.[34] Another study demonstrates that Cbl-b can be inhibited by miR-940, suppressing STAT5a ubiquitination and maintaining PD-L1 level in gastric cancer.[60] Besides, the interaction of β-TrCP and PD-L1 has been verified to be influenced by resveratrol and olaparib via the inhibition of GSK3β activity.[30,70] Yuan *et al.*[79] reported that pyridoxal (vitamin B6) enables to facilitate the combination of STUB1 and PD-L1, further promoting the polyubiquitination and degradation of PD-L1. Moreover, the circRNA CircIGF2BP3 and lncRNA KCNQ1OT1 facilitate the stabilization of PD-L1 through targeting OTUB1 and USP22 respectively.[91,103] In addition, BBR, compound-15 and shikonin are all reported to target the CSN5 to accelerate the protein degradation of PD-L1 by previous researches.[117,118,120]

Although the anticancer potential of targeting E3s or DUBs has been reported by multitudes of emerging studies, there are still some significant problems that worth being further investigated. For instance, now that targeting E3s or DUBs combined anti-PD-1/PD-L1 drugs may produce a better anticancer effect on cancer immunotherapy, can E3s or DUBs level be used as an indicator to judge whether combined immunotherapy should be given? Improved determining method of E3s or DUBs may improve the accuracy of prediction effectiveness. In addition, it is better to give some numerical or even functional relationship between E3s/DUBs and PD-1/PD-L1 to comprehensively consider them as a whole, so as to explore the degree of correlation between the whole and the effectiveness of cancer immunotherapy, which may have stronger guiding significance in the application of cancer combined immunotherapy.

Besides, E3s and DUBs also play an extremely important role in normal physiological and biochemical processes in human body. Although targeting E3s or DUBs may play a certain therapeutic role in anti-tumor process, it may cause serious side effects on human body. In addition, the same E3s or DUBs may exhibit entirely opposite pro-cancer or anti-cancer roles in different cancer types, for instance, the E3 ligase SPOP suppresses the tumorigenesis in prostate, liver and gastric cancers while exerts oncogenic effect on kidney cancer.[63] It is possible that alternative pathways involving E3s or DUBs, even in the same kind of cancer, will have a distinct influence on the up- or down-regulation of PD-1/PD-L1 depending on their involvement. To that end, further fundamental and clinical studies are needed to validate the effects of targeting E3s or DUBs to modify PD-1/PD-L1 on cancer progression and normal physiological activities, as well as the impacts of targeting PD-1 on cancer progression and normal physiological activities.

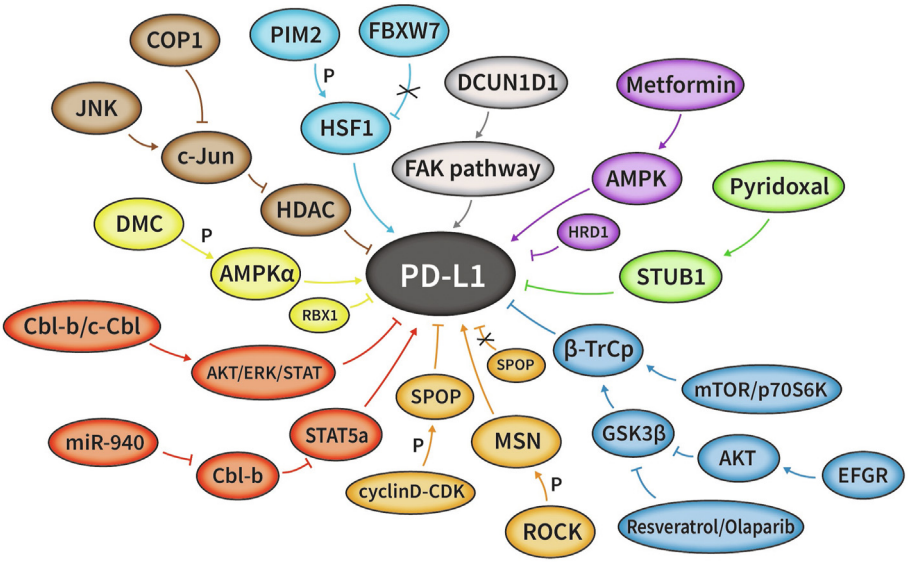

Figure 2 Schematic representation of some E3s and their signaling pathways in regulation of PD-L1. Abbreviations: PD-L1, programmed death-1 ligand; DMC, 2,5-dimethylcelecoxib; AMPKα, 5′ AMP-activated protein kinase α; RBX1, RING-box protein 1; JNK, c-Jun N-terminal kinase; COP1, constitutively photomorphogenic 1; HDAC, histone deacetylase, PIM2, proviral insertion in murine malignancies 2; FBXW7, F-box with 7 tandem WD40 repeats; HSF1, heat-shock factor 1; DCUN1D1, defective cullin neddylation 1 domain-containing 1; FAK, focal adhesion kinase; HRD1, HMG-CoA reductase degradation protein 1; STUB1, STIP1 homology and U-box—containing protein 1; mTOR, mammalian target of rapamycin; p70S6K, p70 S6 kinase; β-TrCP, beta-transducin repeat-containing protein; GSK3β, glycogen synthase kinase 3β; AKT, V-akt murine thymoma viral oncogene homolog; EFGR, epidermal growth factor receptor; ROCK, rho-associated protein kinase; MSN, moesin; SPOP, speckle-type POZ protein; CDK4, cyclin-dependent kinase 4; Cbl-b, casitas B lymphoma-b; c-Cbl, casitas B lineage lymphoma; ERK, extracellular signal-regulated kinase.

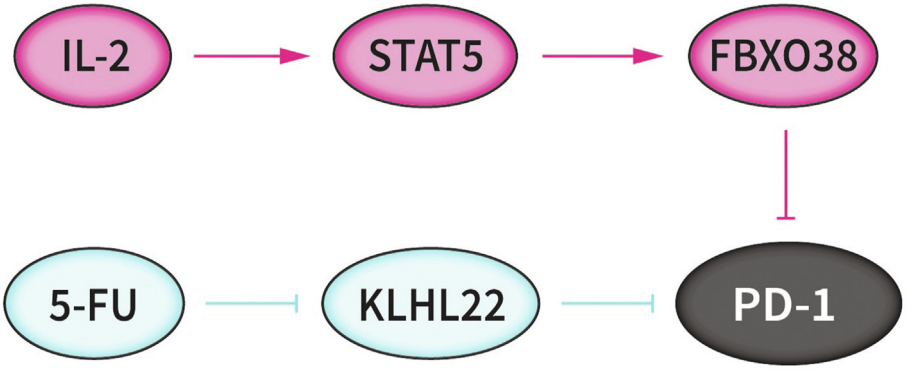

Figure 3 Schematic representation of some E3s and their signaling pathways in regulation of PD-1. Abbreviations: PD-1, programmed death-1; IL-2, interleukin-2; STAT5, signal transducer and activator of transcription 5; FBXO38, F-box only protein 38; KLHL22, kelch like family member 22; 5-FU, 5-fluorouracil.

In addition, E3s and DUBs are numerous, with over 600 E3s and about 100 DUBs that have been reported in previous studies.[124] Is the overwhelming majority of E3s and DUBs, with the exception of the molecules listed in this study, involved in the control of ubiquitination and deubiquitination of PD-1/PD-L1 expression? Is it conceivable to influence the immune system's function as well as the incidence and growth of tumors by manipulating the stability and expression levels of PD-1/PD-L1? The functional investigation of various E3s and DUBs molecules, as well as their interactions with immune checkpoints such as PD-1/PD-L1, opens the door to new options in cancer immunotherapy treatment.

More importantly, despite the fact that various E3s and DUBs have been shown to affect the stability of PD-1/PD-L1, most studies have not gone into depth about the protein interaction mechanism and action site between E3s, DUBs, and PD-1/PD-L1. Still unanswered concerns exist about the signaling pathways that govern the accumulation and deubiquitination of PD-1/PD-L1, such as what the upstream molecules of E3s and DUBs are and how these upstream molecules interact with the downstream substrates. Detailed

Table 1 Role of E3s or DUBs in the regulation of PD-1/PD-L1 via ubiquitination or deubiquitination.

Molecules	Category/PTM	Cancer Types	Upstream Regulators	Mechanism	Related Molecules or Pathways	Modulation of PD-1/PD-L1	References
RBX1	E3/ Ubiquitination	Hepatocellular carcinoma	DMC	Enhances the phosphorylation of AMPKα, and facilitates the ubiquitination degradation of PD-L1	AMPK pathway	Decreased PD-L1	[34]
COP1	E3/ Ubiquitination	Non-small cell lung cancer	—	Facilitates the ubiquitination degradation of c-Jun, increases the expression of HDAC3, inhibits the expression of PD-L1	JNK, c-Jun, HDAC3	Decreased PD-L1	[41]
FBXW7	E3/ Ubiquitination	Melanoma	*PIM2*	Phosphorylates HSF1, disrupts the ubiquitination and degradation of HSF1, facilitates the expression of PD-L1	*PIM2*, HSF1	Increased PD-L1	[45,46]
DCUN1D1	E3/ Ubiquitination	Non-small cell lung cancer	—	Unknown	FAK pathway	Increased PD-L1	[53]
FBXO38	E3/ Ubiquitination	Melanoma	IL-2	Targets STAT5 and improves FBXO38, facilitates the ubiquitination of PD-1	IL-2, STAT5	Decreased PD-1	[55]
Cbl-b/ c-Cbl	E3/ Ubiquitination	Lung cancer	—	Downregulates the expression of PD-L1	AKT, ERK and STAT pathways	Decreased PD-L1	[59]
		Gastric cancer	miR-940	Inhibits Cbl-b, suppresses the ubiquitination of STAT5a	miR-940, STAT5a	Increased PD-L1	[60]
SPOP	E3/ Ubiquitination	Prostate cancer	Cyclin D, CDK4	Phosphorylates and maintains SPOP, facilitates the ubiquitination of PD-L1	Cyclin D, CDK4	Decreased PD-L1	[64]
		Breast cancer	ROCK	Phosphorylates MSN, competes with SPOP for the ubiquitination degradation of PD-L1	ROCK, MSN	Increased PD-L1	[65]
β-TrCP	E3/ Ubiquitination	—	AKT	Inhibits GSK3β, suppresses the ubiquitination of PD-L1 by β-TrCP	AKT, GSK3β	Increased PD-L1	[69]
		Breast cancer	olaparib, resveratrol	Suppresses GSK3β activity, influences the interaction between β-TrCP and PD-L1	GSK3β	Increased PD-L1	[30,70]
		Breast cancer	mTOR, p70S6K	Maintains β-TrCP, facilitates the ubiquitination of PD-L1	mTOR, p70S6K	Decreased PD-L1	[71]
STUB1	E3/ Ubiquitination	Pancreatic cancer	pyridoxal	Enhances the combination of STUB1	—	Decreased PD-L1	[79]

(*continued on next page*)

Table 1 (*continued*)

Molecules	Category/PTM	Cancer Types	Upstream Regulators	Mechanism	Related Molecules or Pathways	Modulation of PD-1/PD-L1	References
				to PD-L1 and causes destabilization of PD-L1			
HRD1	E3/ Ubiquitination	—	metformin	Activates AMPK to phosphorylate PD-L1, facilitates the ubiquitination degradation by HRD1	AMPK, ERAD pathway	Decreased PD-L1	[84]
KLHL22	E3/ Ubiquitination	Colorectal cancer	5-FU	Suppresses the transcriptional activity of KLHL22	—	Increased PD-1	[88]
OTUB1	DUB/ Deubiquitination	Breast cancer	—	Removes the polyubiquitin chain from PD-L1 and modulates the stabilization of PD-L1	—	Increased PD-L1	[90]
		Non-small cell lung cancer	CircIGF2BP3	Upregulates the expression of PKP3, promotes the mRNA stability of OTUB1 and inhibits the ubiquitination and degradation of PD-L1	CircIGF2BP3, PKP3, FXR1	Increased PD-L1	[91]
USP7	DUB/ Deubiquitination	Lung cancer	P5091	unknown	—	Increased PD-L1	[96]
		Gastric cancer	—	unknown	p53	Increased PD-L1	[97]
USP22	DUB/ Deubiquitination	Non-small cell lung cancer	—	Promotes deubiquitination and stabilily of PD-L1	CSN5	Increased PD-L1	[101]
		Colorectal cancer	KCNQ1OT1	Regulates miR-30a-5p, less inhibits USP22 and stabilizes PD-L1	KCNQ1OT1, miR-30a-5p	Increased PD-L1	[103]
USP9X	DUB/ Deubiquitination	Oral squamous cell carcinoma	—	Inhibits the ubiquitination of PD-L1	—	Increased PD-L1	[113]
CSN5	DUB/ Deubiquitination	Colorectal cancer	CCL5	Forms the p65/STAT3 complex, upregulates CSN5 and deubiquitylates PD-L1	CCL5, p65, STAT3	Increased PD-L1	[117]
		Breast cancer	TNF-α	Inhibits the ubiquitination of PD-L1	TNF-α, p65-CSN5-PD-L1 pathway	Increased PD-L1	[31]
		Non-small cell lung cancer	BBR	Inactivates CSN5, leads to ubiquitination of PD-L1	—	Decreased PD-L1	[118]
		Colorectal cancer	compound-15	Inhibits CSN5 and destabilizes PD-L1	—	Decreased PD-L1	[117]
		Pancreatic cancer	shikonin	Suppresses the activation of NF-κB/CSN5 signaling pathway, promotes the degradation of PD-L1	NF-κB/CSN5 pathway	Decreased PD-L1	[120]

Table 1 (*continued*)

Molecules Category/PTM	Cancer Types	Upstream Regulators	Mechanism	Related Molecules or Pathways	Modulation of PD-1/PD-L1	References
	Pancreatic cancer	PDIA6	Upregulates CSN5 via facilitating the correct folding of mature CSN5	—	Increased PD-L1	[119]

PD-1, programmed death-1; PD-L1; programmed death-1 ligand; RBX1, RING-box protein 1; DMC, 2,5-dimethylcelecoxib; AMPKα, 5′ AMP-activated protein kinase α; COP1, constitutively photomorphogenic 1; HDAC3, histone deacetylase3; JNK, c-Jun N-terminal kinase; FBXW7, F-box with 7 tandem WD40 repeats; *PIM2*, proviral insertion in murine malignancies 2; HSF1, heat-shock factor 1; DCUN1D1, defective cullin neddylation 1 domain-containing 1; FAK, focal adhesion kinase; FBXO38, F-box only protein 38; IL-2, interleukin-2; STAT5, signal transducer and activator of transcription 5; Cbl-b, casitas B lymphoma-b; c-Cbl, casitas B lineage lymphoma; AKT, V-akt murine thymoma viral oncogene homolog; ERK, extracellular signal-regulated kinase; SPOP, speckle-type POZ protein; CDK4, cyclin-dependent kinase 4; ROCK, rho-associated protein kinase; MSN, moesin; β-TrCP, beta-transducin repeat-containing protein; GSK3β, glycogen synthase kinase 3β; mTOR, mammalian target of rapamycin; p70S6K, p70 S6 kinase; STUB1, STIP1 homology and U-box–containing protein 1; HRD1, HMG-CoA reductase degradation protein 1; ERAD, endoplasmic reticulum-associated degradation; KLHL22, kelch like family member 22; 5-FU, 5-fluorouracil; OTUB1, OTU domain-containing ubiquitin aldehydebinding protein 1; DUB, deubiquitinating enzyme; PKP3, plakophilin 3; FXR1, fragile X mental retardation-related protein 1; USP7, ubiquitin-specific protease 7; USP22, ubiquitin-specific protease 22; CSN5, constitutive photomorphogenesis 9 signalosome 5; USP9X, ubiquitin-specific peptidase 9, X-linked; CCL5, CC motif chemokine ligand 5; STAT3, signal transducer and activator of transcription 3; TNF-α, necrosis factor α; BBR, berberine; NF-κB, nuclear factor-kappaB; PDIA6, protein disulfide isomerase family A member 6.

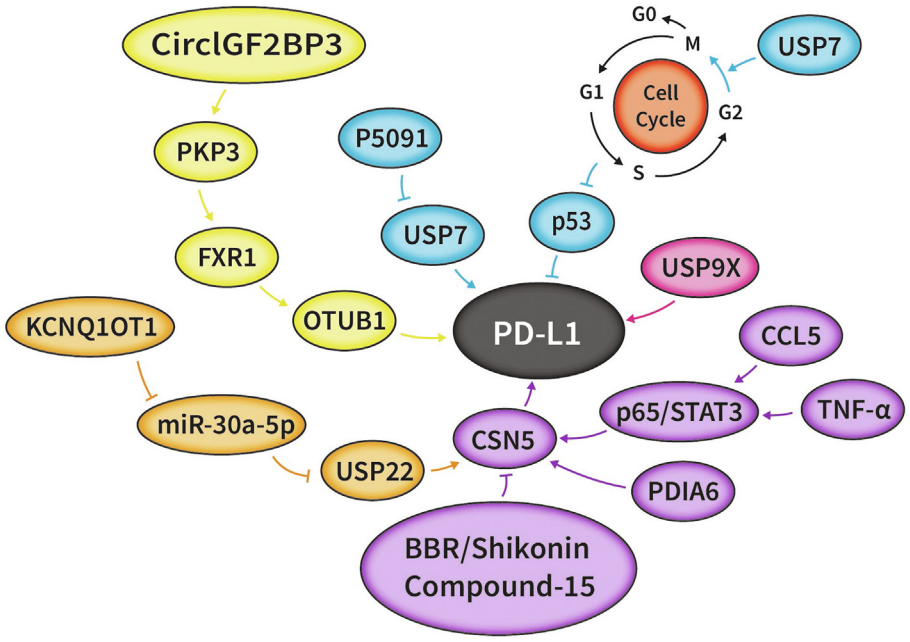

Figure 4 Schematic representation of some DUBs and their signaling pathways in regulation of PD-L1. Abbreviations: DUBs, deubiquitinating enzymes; PKP3, plakophilin 3; FXR1, fragile X mental retardation-related protein 1; OTUB1, OTU domain-containing ubiquitin aldehydebinding protein 1; USP7, ubiquitin-specific protease 7; USP22, ubiquitin-specific protease 22; USP9X, ubiquitin-specific peptidase 9, X-linked; CSN5, constitutive photomorphogenesis 9 signalosome 5; BBR, berberine; PDIA6, protein disulfide isomerase family A member 6; CCL5, CC motif chemokine ligand 5; TNF-α, necrosis factor α; STAT3, signal transducer and activator of transcription 3.

elaboration and in-depth exploration of E3s and DUBs regulation of PD-1/PD-L1 signaling pathway can provide a more solid theoretical basis for finding suitable targeted drugs. The development of drugs that aiming at E3s, DUBs and their upstream molecules is also one of the important research directions of cancer immunotherapy in the future.

Conclusion

In a nutshell, we cannot overstate the importance of the control of the PD-1/PD-L1 signaling pathway by ubiquitination or deubiquitination under all conditions. In addition to being important components of the UPS system, E3s and DUBs can be targeted by a variety of agents, which modulate the process of ubiquitination or deubiquitination (Fig. 2—4), resulting in the subsequent regulation of PD-1 and PD-L1 activity, as well as the subsequent adjustment of immunosuppression and anticancer immunotherapeutic effects (Table 1). A potential future for cancer immunotherapy may lie in the targeting of E3s and DUBs, whether directly or indirectly, despite the fact that some of the mechanisms by which E3s and DUBs regulate the PD-1/PD-L1 pathway have not yet been fully explored and need further studies.

Author contributions

YXL, HJ and HY designed the study. DP, MZQ, FYZ and FYT searched the literature and wrote the manuscript. SCJ, PMH, ZYM and HD searched the literature and made the table, and DP draw the figures. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interests regarding the publication of this manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (82103508, 81871866, 82173252), Shaanxi Special Support Plan-Program for Leading Talents of Science and Technology Innovation (China) (No. 2019 Special Support Plan), the Natural Science Foundation of Shaanxi Province (China) (2016SF-308, 2019SF-033, 2022SF-145) and Project of Tangdu Hospital, the Fourth Military Medical University (China) (No. 2018 Key Talents).

References

1. Zamani MR, Aslani S, Salmaninejad A, et al. PD-1/PD-L and autoimmunity: a growing relationship. *Cell Immunol*. 2016; 310:27—41.
2. Curran CS, Gupta S, Sanz I, et al. PD-1 immunobiology in systemic lupus erythematosus. *J Autoimmun*. 2019;97:1—9.
3. Curran CS, Sharon E. PD-1 immunobiology in autoimmune hepatitis and hepatocellular carcinoma. *Semin Oncol*. 2017; 44(6):428—432.
4. Han Y, Liu D, Li L. PD-1/PD-L1 pathway: current researches in cancer. *Am J Cancer Res*. 2020;10(3):727—742.
5. Burr ML, Sparbier CE, Chan YC, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature*. 2017;549(7670):101—105.
6. Ishida Y, Agata Y, Shibahara K, et al. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J*. 1992;11(11):3887—3895.
7. Riley JL. PD-1 signaling in primary T cells. *Immunol Rev*. 2009; 229(1):114—125.
8. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26: 677—704.
9. Ahmadzadeh M, Johnson LA, Heemskerk B, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood*. 2009; 114(8):1537—1544.
10. Liu C, Jiang J, Gao L, et al. A promoter region polymorphism in PDCD-1 gene is associated with risk of rheumatoid arthritis in the Han Chinese population of southeastern China. *Int J Genomics*. 2014;2014:247637.
11. Salmaninejad A, Khoramshahi V, Azani A, et al. PD-1 and cancer: molecular mechanisms and polymorphisms. *Immunogenetics*. 2018;70(2):73—86.
12. Ohaegbulam KC, Assal A, Lazar-Molnar E, et al. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. *Trends Mol Med*. 2015;21(1):24—33.
13. Ritprajak P, Azuma M. Intrinsic and extrinsic control of expression of the immunoregulatory molecule PD-L1 in epithelial cells and squamous cell carcinoma. *Oral Oncol*. 2015;51(3):221—228.
14. Parry RV, Chemnitz JM, Frauwirth KA, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol*. 2005;25(21):9543—9553.
15. Sharpe AH, Wherry EJ, Ahmed R, et al. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol*. 2007;8(3):239—245.
16. D'Arrigo P, Russo M, Rea A, et al. A regulatory role for the co-chaperone FKBP51s in PD-L1 expression in glioma. *Oncotarget*. 2017;8(40):68291—68304.
17. Satelli A, Batth IS, Brownlee Z, et al. Potential role of nuclear PD-L1 expression in cell-surface vimentin positive circulating tumor cells as a prognostic marker in cancer patients. *Sci Rep*. 2016;6:28910.
18. Dong P, Xiong Y, Yue J, et al. Tumor-intrinsic PD-L1 signaling in cancer initiation, development and treatment: beyond immune evasion. *Front Oncol*. 2018;8:386.
19. Nunes-Xavier CE, Angulo JC, Pulido R, et al. A critical insight into the clinical translation of PD-1/PD-L1 blockade therapy in clear cell renal cell carcinoma. *Curr Urol Rep*. 2019;20(1):1.
20. Chen X, Pan X, Zhang W, et al. Epigenetic strategies synergize with PD-L1/PD-1 targeted cancer immunotherapies to enhance antitumor responses. *Acta Pharm Sin B*. 2020;10(5):723—733.
21. Wang C, Yu X, Wang W. A meta-analysis of efficacy and safety of antibodies targeting PD-1/PD-L1 in treatment of advanced nonsmall cell lung cancer. *Medicine*. 2016;95(52):e5539.
22. Luke JJ, Flaherty KT, Ribas A, et al. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol*. 2017;14(8):463—482.
23. Jayaprakash NG, Surolia A. Role of glycosylation in nucleating protein folding and stability. *Biochem J*. 2017;474(14): 2333—2347.
24. Pohl C, Dikic I. Cellular quality control by the ubiquitin-proteasome system and autophagy. *Science*. 2019;366(6467): 818—822.
25. Puvar K, Luo ZQ, Das C. Uncovering the structural basis of a new twist in protein ubiquitination. *Trends Biochem Sci*. 2019; 44(5):467—477 [PubMed].
26. McKeon JE, Sha D, Li L, et al. Parkin-mediated K63-polyubiquitination targets ubiquitin C-terminal hydrolase L1 for degradation by the autophagy-lysosome system. *Cell Mol Life Sci*. 2015;72(9):1811—1824.
27. Eldridge AG, O'Brien T. Therapeutic strategies within the ubiquitin proteasome system. *Cell Death Differ*. 2010;17(1): 4—13.

28. Ciechanover A. The unravelling of the ubiquitin system. *Nat Rev Mol Cell Biol*. 2015;16(5):322—324.
29. Komander D, Clague MJ, Urbé S. Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol*. 2009;10(8):550—563.
30. Li CW, Lim SO, Xia W, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. *Nat Commun*. 2016;7:12632.
31. Lim SO, Li CW, Xia W, et al. Deubiquitination and stabilization of PD-L1 by CSN_5. *Cancer Cell*. 2016;30(6):925—939.
32. Fujiwara M, Anstadt EJ, Clark RB. Cbl-b deficiency mediates resistance to programmed death-ligand 1/programmed death-1 regulation. *Front Immunol*. 2017;8:42.
33. Wang W, Qiu J, Liu Z, et al. Overexpression of RING box protein-1 (RBX1) associated with poor prognosis of non-muscle-invasive bladder transitional cell carcinoma. *J Surg Oncol*. 2013;107(7):758—761.
34. Chen Z, Chen Y, Peng L, et al. 2, 5-dimethylcelecoxib improves immune microenvironment of hepatocellular carcinoma by promoting ubiquitination of HB_x-induced PD-L1. *J Immunother Cancer*. 2020;8(2):e001377.
35. Ho CS, Noor SM, Nagoor NH. miR-378 and miR-1827 regulate tumor invasion, migration and angiogenesis in human lung adenocarcinoma by targeting RBX1 and CRKL, respectively. *J Cancer*. 2018;9(2):331—345.
36. Zhang XH, Xin ZM. miR-135b-5p inhibits the progression of malignant melanoma cells by targeting RBX1. *Eur Rev Med Pharmacol Sci*. 2020;24(3):1309—1315.
37. Dornan D, Wertz I, Shimizu H, et al. The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature*. 2004; 429(6987):86—92.
38. Dentin R, Liu Y, Koo SH, et al. Insulin modulates gluconeogenesis by inhibition of the coactivator TORC2. *Nature*. 2007; 449(7160):366—369.
39. Yoneda-Kato N, Tomoda K, Umehara M, et al. Myeloid leukemia factor 1 regulates p53 by suppressing COP1 via COP9 signalosome subunit 3. *EMBO J*. 2005;24(9):1739—1749.
40. Migliorini D, Bogaerts S, Defever D, et al. Cop1 constitutively regulates c-Jun protein stability and functions as a tumor suppressor in mice. *J Clin Invest*. 2011;121(4):1329—1343.
41. Wang H, Fu C, Du J, et al. Enhanced histone H3 acetylation of the PD-L1 promoter via the COP1/c-Jun/HDAC3 axis is required for PD-L1 expression in drug-resistant cancer cells. *J Exp Clin Cancer Res*. 2020;39(1):29.
42. Takeishi S, Nakayama KI. Role of Fbxw7 in the maintenance of normal stem cells and cancer-initiating cells. *Br J Cancer*. 2014;111(6):1054—1059.
43. Zhou Z, He C, Wang J. Regulation mechanism of Fbxw7-related signaling pathways (Review). *Oncol Rep*. 2015;34(5): 2215—2224.
44. Gong J, Zhou Y, Liu D, et al. F-box proteins involved in cancer-associated drug resistance. *Oncol Lett*. 2018;15(6):8891—8900.
45. Kourtis N, Moubarak RS, Aranda-Orgilles B, et al. FBXW7 modulates cellular stress response and metastatic potential through HSF_1 post-translational modification. *Nat Cell Biol*. 2015;17(3):322—332.
46. Yang T, Ren C, Lu C, et al. Phosphorylation of HSF_1 by PIM2 induces PD-L1 expression and promotes tumor growth in breast cancer. *Cancer Res*. 2019;79(20):5233—5244.
47. Lyle C, Richards S, Yasuda K, et al. C-Cbl targets PD-1 in immune cells for proteasomal degradation and modulates colorectal tumor growth. *Sci Rep*. 2019;9(1):20257.
48. Huang G, Kaufman AJ, Xu K, et al. Squamous cell carcinoma-related oncogene (SCCRO) neddylates Cul3 protein to selectively promote midbody localization and activity of Cul3 KLHL21 protein complex during abscission. *J Biol Chem*. 2017; 292(37):15254—15265.
49. Zhang ZH, Li J, Luo F, et al. Clinical significance of SCCRO (DCUN1D1) in prostate cancer and its proliferation-inhibiting effect on Lncap cells. *Eur Rev Med Pharmacol Sci*. 2017; 21(19):4283—4291.
50. Xiao J, Li G, Zhou J, et al. microRNA-520b functions as a tumor suppressor in colorectal cancer by inhibiting defective in cullin neddylation 1 domain containing 1 (DCUN1D1). *Oncol Res*. 2018;26(4):593—604.
51. Broderick SR, Golas BJ, Pham D, et al. SCCRO promotes glioma formation and malignant progression in mice. *Neoplasia*. 2010;12(6):476—484.
52. Jiang Y, Hou R, Li S, et al. MicroRNA-302 inhibits cell migration and invasion in cervical cancer by targeting DCUN1D1. *Exp Ther Med*. 2018;16(2):1000—1008.
53. Li J, Yu T, Yan M, et al. DCUN1D1 facilitates tumor metastasis by activating FAK signaling and up-regulates PD-L1 in non-small-cell lung cancer. *Exp Cell Res*. 2019;374(2):304—314.
54. Jin J, Cardozo T, Lovering RC, et al. Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev*. 2004; 18(21):2573—2580.
55. Meng X, Liu X, Guo X, et al. FBXO38 mediates PD-1 ubiquitination and regulates anti-tumour immunity of T cells. *Nature*. 2018;564(7734):130—135.
56. Bachmaier K, Krawczyk C, Kozieradzki I, et al. Negative regulation of lymphocyte activation and autoimmunity by the molecular adaptor Cbl-B. *Nature*. 2000;403(6766): 211—216.
57. Zhang L, Teng Y, Fan Y, et al. The E3 ubiquitin ligase Cbl-b improves the prognosis of RANK positive breast cancer patients by inhibiting RANKL-induced cell migration and metastasis. *Oncotarget*. 2015;6(26):22918—22933.
58. Li H, Xu L, Li C, et al. Ubiquitin ligase Cbl-b represses IGF-I-induced epithelial mesenchymal transition via ZEB2 and microRNA-200c regulation in gastric cancer cells. *Mol Cancer*. 2014;13:136.
59. Wang S, Xu L, Che X, et al. E3 ubiquitin ligases Cbl-b and c-Cbl downregulate PD-L1 in EGFR wild-type non-small cell lung cancer. *FEBS Lett*. 2018;592(4):621—630.
60. Fan Y, Che X, Hou K, et al. miR-940 promotes the proliferation and migration of gastric cancer cells through up-regulation of programmed death ligand-1 expression. *Exp Cell Res*. 2018; 373(1—2):180—187.
61. Karwacz K, Bricogne C, MacDonald D, et al. PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8+ T cells. *EMBO Mol Med*. 2011;3(10): 581—592.
62. Clark A, Burleson M. SPOP and cancer: a systematic review. *Am J Cancer Res*. 2020;10(3):704—726.
63. Song Y, Xu Y, Pan C, et al. The emerging role of SPOP protein in tumorigenesis and cancer therapy. *Mol Cancer*. 2020;19(1): 2.
64. Zhang J, Bu X, Wang H, et al. Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. *Nature*. 2018;553(7686):91—95.
65. Meng F, Su Y, Xu B. Rho-associated protein kinase-dependent moesin phosphorylation is required for PD-L1 stabilization in breast cancer. *Mol Oncol*. 2020;14(11):2701—2712.
66. Zhang H, Xia Y, Wang F, et al. Aldehyde dehydrogenase 2 mediates alcohol-induced colorectal cancer immune escape through stabilizing PD-L1 expression. *Adv Sci (Weinh)*. 2021; 8(10):2003404.
67. Shaik S, Nucera C, Inuzuka H, et al. SCF(β-TRCP) suppresses angiogenesis and thyroid cancer cell migration by promoting ubiquitination and destruction of VEGF receptor 2. *J Exp Med*. 2012;209(7):1289—1307.
68. Doble BW, Woodgett JR. GSK-3:tricks of the trade for a multitasking kinase. *J Cell Sci*. 2003;116(Pt 7):1175—1186.

69. Cohen P, Frame S. The renaissance of GSK_3. *Nat Rev Mol Cell Biol*. 2001;2(10):769—776.
70. Jiao S, Xia W, Yamaguchi H, et al. PARP inhibitor upregulates PD-L1 expression and enhances cancer-associated immunosuppression. *Clin Cancer Res*. 2017;23(14):3711—3720.
71. Deng L, Qian G, Zhang S, et al. Inhibition of mTOR complex 1/p70 S6 kinase signaling elevates PD-L1 levels in human cancer cells through enhancing protein stabilization accompanied with enhanced β-TrCP degradation. *Oncogene*. 2019;38(35):6270—6282.
72. Chen Z, Barbi J, Bu S, et al. The ubiquitin ligase Stub1 negatively modulates regulatory T cell suppressive activity by promoting degradation of the transcription factor Foxp3. *Immunity*. 2013;39(2):272—285.
73. Xin H, Xu X, Li L, et al. CHIP controls the sensitivity of transforming growth factor-beta signaling by modulating the basal level of Smad3 through ubiquitin-mediated degradation. *J Biol Chem*. 2005;280(21):20842—20850.
74. Murata S, Minami Y, Minami M, et al. CHIP is a chaperone-dependent E3 ligase that ubiquitylates unfolded protein. *EMBO Rep*. 2001;2(12):1133—1138.
75. Tang DE, Dai Y, Lin LW, et al. $STUB_1$ suppresseses tumorigenesis and chemoresistance through antagonizing YAP1 signaling. *Cancer Sci*. 2019;110(10):3145—3156.
76. Luan H, Mohapatra B, Bielecki TA, et al. Loss of the nuclear pool of ubiquitin ligase CHIP/$STUB_1$ in breast cancer unleashes the MZF1-cathepsin pro-oncogenic program. *Cancer Res*. 2018;78(10):2524—2535.
77. Wang Y, Ren F, Wang Y, et al. CHIP/Stub1 functions as a tumor suppressor and represses NF-κB-mediated signaling in colorectal cancer. *Carcinogenesis*. 2014;35(5):983—991.
78. Mezzadra R, Sun C, Jae LT, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature*. 2017;549(7670):106—110.
79. Yuan J, Li J, Shang M, et al. Identification of vitamin B6 as a PD-L1 suppressor and an adjuvant for cancer immunotherapy. *Biochem Biophys Res Commun*. 2021;561:187—194.
80. Liu L, Yu L, Zeng C, et al. E3 ubiquitin ligase HRD1 promotes lung tumorigenesis by promoting sirtuin 2 ubiquitination and degradation. *Mol Cell Biol*. 2020;40(7):e00219—e00257.
81. Liu L, Long H, Wu Y, et al. HRD1-mediated PTEN degradation promotes cell proliferation and hepatocellular carcinoma progression. *Cell Signal*. 2018;50:90—99.
82. Tan X, He X, Fan Z. Upregulation of HRD1 promotes cell migration and invasion in colon cancer. *Mol Cell Biochem*. 2019;454(1—2):1—9.
83. Xu Y, Zhao F, Qiu Q, et al. The ER membrane-anchored ubiquitin ligase Hrd1 is a positive regulator of T-cell immunity. *Nat Commun*. 2016;7:12073.
84. Cha JH, Yang WH, Xia W, et al. Metformin promotes antitumor immunity via endoplasmic-*Reticulum*-associated degradation of PD-L1. *Mol Cell*. 2018;71(4):606—620.
85. Beck J, Maerki S, Posch M, et al. Ubiquitylation-dependent localization of PLK1 in mitosis. *Nat Cell Biol*. 2013;15(4):430—439.
86. Chen J, Ou Y, Yang Y, et al. KLHL22 activates amino-acid-dependent mTORC1 signalling to promote tumorigenesis and ageing. *Nature*. 2018;557(7706):585—589.
87. Song Y, Yuan H, Wang J, et al. KLHL22 regulates the EMT and proliferation in colorectal cancer cells in part via the Wnt/β-catenin signaling pathway. *Cancer Manag Res*. 2020;12:3981—3993.
88. Zhou XA, Zhou J, Zhao L, et al. KLHL22 maintains PD-1 homeostasis and prevents excessive T cell suppression. *Proc Natl Acad Sci U S A*. 2020;117(45):28239—28250.
89. Liu X, Jiang WN, Wang JG, et al. Colon cancer bears overexpression of $OTUB_1$. *Pathol Res Pract*. 2014;210(11):770—773.
90. Zhu D, Xu R, Huang X, et al. Deubiquitinating enzyme $OTUB_1$ promotes cancer cell immunosuppression via preventing ER-associated degradation of immune checkpoint protein PD-L1. *Cell Death Differ*. 2021;28(6):1773—1789.
91. Liu Z, Wang T, She Y, et al. N^6-methyladenosine-modified circIGF_2BP_3 inhibits $CD8^+$ T-cell responses to facilitate tumor immune evasion by promoting the deubiquitination of PD-L1 in non-small cell lung cancer. *Mol Cancer*. 2021;20(1):105.
92. Pozhidaeva A, Bezsonova I. USP7:structure, substrate specificity, and inhibition. *DNA Repair*. 2019;76:30—39.
93. Carrà G, Panuzzo C, Torti D, et al. Therapeutic inhibition of USP7-PTEN network in chronic lymphocytic leukemia: a strategy to overcome TP53 mutated/deleted clones. *Oncotarget*. 2017;8(22):35508—35522.
94. Song MS, Salmena L, Carracedo A, et al. The deubiquitinylation and localization of PTEN are regulated by a HAUSP-PML network. *Nature*. 2008;455(7214):813—817.
95. Wang Q, Ma S, Song N, et al. Stabilization of histone demethylase PHF_8 by USP7 promotes breast carcinogenesis. *J Clin Invest*. 2016;126(6):2205—2220.
96. Dai X, Lu L, Deng S, et al. USP7 targeting modulates anti-tumor immune response by reprogramming Tumor-associated Macrophages in Lung Cancer. *Theranostics*. 2020;10(20):9332—9347.
97. Wang Z, Kang W, Li O, et al. Abrogation of USP7 is an alternative strategy to downregulate PD-L1 and sensitize gastric cancer cells to T cells killing. *Acta Pharm Sin B*. 2021;11(3):694—707.
98. Wang L, Kumar S, Dahiya S, et al. Ubiquitin-specific protease-7 inhibition impairs Tip60-dependent Foxp3+ T-regulatory cell function and promotes antitumor immunity. *EBioMedicine*. 2016;13:99—112.
99. Liang JX, Ning Z, Gao W, et al. Ubiquitin-specific protease 22-induced autophagy is correlated with poor prognosis of pancreatic cancer. *Oncol Rep*. 2014;32(6):2726—2734.
100. Li J, Yuan S, Norgard RJ, et al. Tumor cell-intrinsic USP22 suppresses antitumor immunity in pancreatic cancer. *Cancer Immunol Res*. 2020;8(3):282—291.
101. Wang Y, Sun Q, Mu N, et al. The deubiquitinase USP22 regulates PD-L1 degradation in human cancer cells. *Cell Commun Signal*. 2020;18(1):112.
102. Huang X, Zhang Q, Lou Y, et al. USP22 deubiquitinates CD274 to suppress anticancer immunity. *Cancer Immunol Res*. 2019;7(10):1580—1590.
103. Xian D, Niu L, Zeng J, et al. LncRNA KCNQ1OT1 secreted by tumor cell-derived exosomes mediates immune escape in colorectal cancer by regulating PD-L1 ubiquitination via miR-30a-5p/USP22. *Front Cell Dev Biol*. 2021;9:653808.
104. Murtaza M, Jolly LA, Gecz J, et al. La FAM fatale: USP9X in development and disease. *Cell Mol Life Sci*. 2015;72(11):2075—2089.
105. Park Y, Jin HS, Liu YC. Regulation of T cell function by the ubiquitin-specific protease USP9X via modulating the Carma1-Bcl10-Malt1 complex. *Proc Natl Acad Sci U S A*. 2013;110(23):9433—9438.
106. Kapuria V, Peterson LF, Fang D, et al. Deubiquitinase inhibition by small-molecule WP1130 triggers aggresome formation and tumor cell apoptosis. *Cancer Res*. 2010;70(22):9265—9276.
107. Li X, Song N, Liu L, et al. USP9X regulates centrosome duplication and promotes breast carcinogenesis. *Nat Commun*. 2017;8:14866.
108. Nanayakkara DM, Nguyen MN, Wood SA. Deubiquitylating enzyme, USP9X, regulates proliferation of cells of head and neck cancer lines. *Cell Prolif*. 2016;49(4):494—502.
109. Potu H, Peterson LF, Kandarpa M, et al. Usp9x regulates Ets-1 ubiquitination and stability to control NRAS expression and tumorigenicity in melanoma. *Nat Commun*. 2017;8:14449.

110. Chen MY, Li ZP, Sun ZN, et al. USP9X promotes the progression of hepatocellular carcinoma by regulating beta-catenin. *Ir J Med Sci*. 2020;189(3):865—871.
111. Wang S, Kollipara RK, Srivastava N, et al. Ablation of the oncogenic transcription factor ERG by deubiquitinase inhibition in prostate cancer. *Proc Natl Acad Sci U S A*. 2014; 111(11):4251—4256.
112. Pérez-Mancera PA, Rust AG, van der Weyden L, et al. The deubiquitinase USP9X suppresses pancreatic ductal adenocarcinoma. *Nature*. 2012;486(7402):266—270.
113. Wu J, Guo W, Wen D, et al. Deubiquitination and stabilization of programmed cell death ligand 1 by ubiquitin-specific peptidase 9, X-linked in oral squamous cell carcinoma. *Cancer Med*. 2018;7(8):4004—4011.
114. Liu Y, Shah SV, Xiang X, et al. COP9-associated CSN_5 regulates exosomal protein deubiquitination and sorting. *Am J Pathol*. 2009;174(4):1415—1425.
115. Wu Y, Deng J, Rychahou PG, et al. Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. *Cancer Cell*. 2009;15(5):416—428.
116. Wang L, Zheng JN, Pei DS. The emerging roles of Jab1/CSN_5 in cancer. *Med Oncol*. 2016;33(8):90.
117. Liu C, Yao Z, Wang J, et al. Macrophage-derived CCL5 facilitates immune escape of colorectal cancer cells via the p65/STAT3-CSN_5-PD-L1 pathway. *Cell Death Differ*. 2020; 27(6):1765—1781.
118. Liu Y, Liu X, Zhang N, et al. Berberine diminishes cancer cell PD-L1 expression and facilitates antitumor immunity via inhibiting the deubiquitination activity of CSN_5. *Acta Pharm Sin B*. 2020;10(12):2299—2312.
119. Ma Y, Xia P, Wang Z, et al. PDIA6 promotes pancreatic cancer progression and immune escape through CSN_5-mediated deubiquitination of β-catenin and PD-L1. *Neoplasia*. 2021; 23(9):912—928.
120. Ruan Z, Liang M, Shang L, et al. Shikonin-mediated PD-L1 degradation suppresses immune evasion in pancreatic cancer by inhibiting NF-κB/STAT3 and NF-κB/CSN_5 signaling pathways. *Pancreatology*. 2021;21(3):630—641.
121. Tang H, Liang Y, Anders RA, et al. PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression. *J Clin Invest*. 2018;128(2):580—588.
122. Sharma P, Hu-Lieskovan S, Wargo JA, et al. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*. 2017;168(4):707—723.
123. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350—1355.
124. Berndsen CE, Wolberger C. New insights into ubiquitin E3 ligase mechanism. *Nat Struct Mol Biol*. 2014;21(4):301—307.