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Programmed cell death protein 1 in cancer cells

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Abstract
Programmed cell death protein 1 (PD-1) is frequently detected in certain subsets of tumor cells, and our understanding of PD-1 signaling consequences has expanded to include control of tumor growth, stemness and drug resistance. Nonetheless, tumor cell-intrinsic PD-1 has been comparatively underexplored in relation to PD-1 expressed on the surface of immune cells as an immune checkpoint, despite the imperative need to comprehensively elucidate the underlying mechanisms of action for achieving optimal responses in tumor immunotherapy. Here, we review the roles of the regulation and function of tumor-intrinsic PD-1 from its expression to degradation processes. Our primary focus is on unraveling its enigmatic influence on tumorigenesis and progression as proposed by recent findings, while navigating the labyrinthine network of regulatory mechanisms governing its expression and intricate functional interplay. We also discuss how the elucidation of the mechanistic underpinnings of tumor-intrinsic PD-1 expression holds the potential to explain the divergent therapeutic outcomes observed with anti-PD-1-based combination therapies, thereby furnishing indispensable insights crucial for synergistic anti-tumor strategies.

Keywords Tumor cell-intrinsic programmed cell death protein 1, Tumor growth, Regulatory mechanisms, Tumor immunotherapy, Combined therapy

Background

Programmed cell death-1 (PD-1), also known as CD279, is encoded by the *PDCD1* gene and was initially thought to be constitutively expressed on the surface of immune cells [1]. As a member of the CD28 immunoglobulin superfamily, PD-1 functions as a receptor and immune checkpoint for programmed death ligand-1 (PD-L1, also known as B7-H1) and programmed death ligand-2 (PD-L2), which are expressed on tumor cells [1–6]. In the field of tumor immunotherapy, therapeutic strategies targeting PD-1 and its ligand PD-L1 have become fundamental components [7]. However, in unselected patient populations, the response rate to PD-1/PD-L1 ICB therapy is only modest, approximately 20% [8]. Furthermore, with the rapid clinical adoption of targeted PD-1/PD-L1 blockade agents, atypical responses to ICB therapy have emerged, including initial resistance phenomena, hyperprogression following PD-1/PD-L1 blockade, acquisition of antitumor activity patients with low-immunogenic tumors, and lack of response to PD-1 therapy in patients who are unresponsive to cytotoxic T-lymphocyte antigen-4 (CTLA-4) treatment [9].

From a clinical perspective, the use of PD-L1 levels as a biomarker for predicting the response to anti-PD-1 therapy is not without limitations. It has been observed

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that anti-PD-1 therapy can elicit a positive response even in PD-L1-negative tumors, while not all PD-L1-positive tumors necessarily demonstrate therapeutic advantage [10]. In addition, emerging evidence has shown that tumor-intrinsic PD-1 expression is present in certain types of tumor cells, suggesting an independent role of PD-1 in modulating tumor cell behaviors, separate from the immune system [11–13]. Similar oncogenic effects of tumor-intrinsic PD-1 have also been observed in HCC and pancreatic cancer cells [14–17]. However, a recent investigation into the PD-1/PD-L1 expression in lung cancer cells suggested that tumor-intrinsic PD-1 could potentially inhibit tumor growth [18].

PD-1 is pivotal in maintaining peripheral immune tolerance, and is elevated in activated T cells [19–21]. Extensive studies have confirmed the expression of PD-1 not only on immune cell surfaces but also within select subsets of tumor cells, thereby conferring distinct attributes upon these cells, including stemness and enhanced tumorigenic potential [22]. In the context of tumor cells, several molecules have been identified that exhibit the potential for molecular interactions in cis and trans during PD-1 and PD-L1 signaling processes. For example, PD-L1 on the tumor cell surface can interact with PD-1 expressed on the same cell (cis interaction), leading to cell-intrinsic signaling that activates mTORC1 and promotes cell proliferation and tumor growth [23].

Leveraging therapeutic vulnerabilities arising from tumor-intrinsic PD-1 signals presents promising yet largely unexplored avenue for investigation. In this perspective, we delve into the emerging understanding of tumor-intrinsic PD-1 signals, and propose treatment approaches and response biomarkers. We comprehensively review the mechanisms underlying tumor-intrinsic PD-1 signaling and its significant ramifications. Finally, we discuss the prospects of targeting tumor-intrinsic PD-1 signals in the realm of drug discovery.

PD-1 structure and isoform

PDCD1 gene

PDCD1 gene is located on chromosome 2 and comprises five exons. Exon 1 encodes a succinct signal sequence, while exon 2 encodes the IgV-like domain. Exon 3 contains the sequences for the stalk and transmembrane domain coding. Exons 4 and 5 encode the complete cytoplasmic domain and an extended 3' untranslated region (UTR), respectively.

PD-1 domains

PD-1 is a type I transmembrane protein [23]. Full-length PD-1 is categorized as a type I transmembrane glycoprotein, comprising 268 amino acids, and is a member of the CD28/CTLA-4/ICOS costimulatory receptor family. It consists of three main components (Fig. 1) [24]: (1) an immunoglobulin variable-type (IgV) domain; (2) a stalk region; (3) an extracellular domain (EC); (4) a transmembrane region (TM); and (5) a cytoplasmic tail (IC) [1]. In contrast to B7-1 and B7-2, PD-1 does not possess the analogous cysteine residue that facilitates the formation of non-covalent dimers [23]. As a result, PD-1 primarily exists and interacts as a monomer rather than forming dimers [23]. Recent foundings reported that the resistance to PD-1 blockade observed in patients with HCC can be attributed to the immunosuppressive effects mediated by isoformic PD-1 [25]. This isoform, known as Δ42PD-1, displays varying expression profiles on human immune cells. It contains a specific in-frame deletion of 14 amino acids within exon 2 of the *PDCD1* gene, leading to the disruption of its binding capability with PD-L1/L2

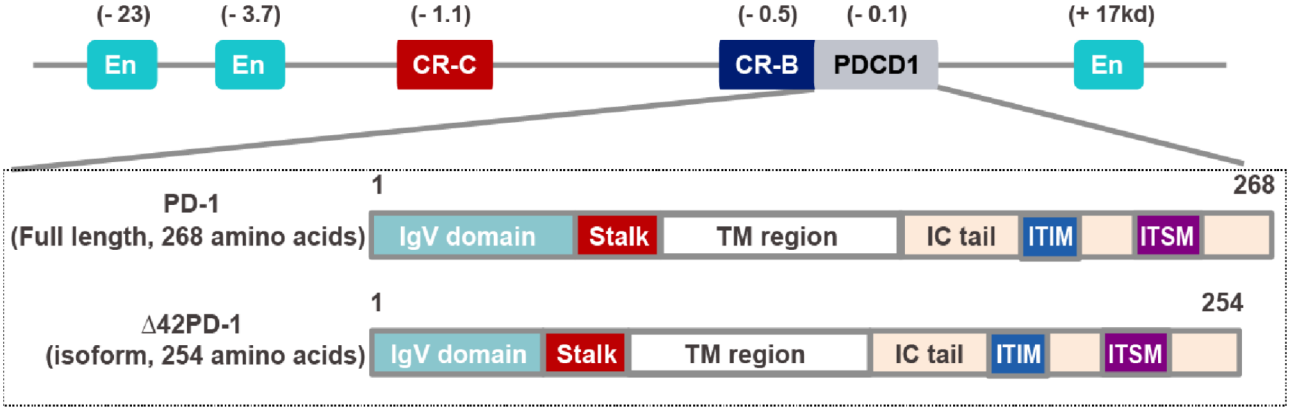

Fig. 1 *PDCD1* gene and programmed cell death-1 (PD-1) isform. The regulatory elements of the *PDCD1* gene, including conserved region C (CR-C) and conserved region B (CR-B), as well as enhancer (En) regions (top). These elements play a role in the transcriptional regulation of PD-1. PD-1 comprises four domains, the order and presence of which are the same between full length PD-1 and its isoform Δ42PD-1 (bottom). The splice isoform of PD-1 (Δ42PD-1) harbors an inframe deletion of 14 amino acids within exon 2 of PD-1 that encodes the IgV-like domain, thus resulting in an abrogated interaction with PD-L1/L2

(Fig. 1) [26]. Nevertheless, the role of Δ42PD-1 in oncology remains enigmatic.

The conventional standpoint posits that the PD-1 protein is expressed as an immunosuppressive receptor on the surface of immune cells [23]. However, accumulated studies have revealed that PD-1 is also prevalent in certain subsets of tumor cell populations and tumor cell lines, where it exerts diverse functions in governing tumor cell biology [22]. In addition, the immune checkpoint molecule CTLA-4, which is traditionally expressed on leukocytes, has been discovered to be expressed and functionally active on cancer cells [27].

The intricate regulatory mechanism

PD-1 signaling promotes the activity of SHP2, mTOR, and ribosomal protein S6 in both T cells and melanoma cells, yet its effects are starkly different. In T cells, PD-1 signaling activates SHP2, which in turn inhibits downstream pathways like PI3K/AKT and mTOR, ultimately suppressing T cell activation and anti-tumor efficacy [23]. By contrast, in melanoma cells, PD-1 signaling activates the same pathways but with an opposing effect: it promotes tumor cell proliferation and survival through PI3K-independent mechanisms [23]. This dichotomy highlights the context-dependent nature of PD-1 signaling.

The contrasting effects of PD-1 on mTOR signaling in melanoma cells versus T cells align with the divergent functions of SHP2. In T cells, SHP2 acts as a negative regulator, impeding tumor progression by inhibiting key signaling pathways [15, 24]. In contrast, in melanoma cells, SHP2 fosters tumor progression by activating the MAPK/ERK pathway, which enhances cell proliferation and survival [15, 24]. Conversely, this dual role of SHP2 underscores the complexity of PD-1 signaling in different cellular contexts.

Similar observations have been made in acute myeloid leukemia (AML). Shanshan Suo et al. identified a subset of PD-1$^+$ AML stem cells (PD-1$^+$AMLLSKs) that exhibited higher levels of SHP2 and p-ERK. These cells showed enhanced proliferation, differentiation, and tumorigenicity due to PD-1 activation of the MAPK/ERK signaling pathway [28]. This finding further illustrates the tumor-promoting role of PD-1 signaling in certain cancer types.

In thyroid cancer, Liotti et al. demonstrated that tumor-intrinsic PD-1 facilitates the recruitment of SHP2 to the plasma membrane, leading to increased SHP2 phosphorylation. This event enhances Ras activity by dephosphorylating tyrosine residue 32 of Ras, thereby triggering the SHP2/Ras/MAPK signaling cascade, which directly impairs T cell growth [29]. This mechanism highlights the role of PD-1 in promoting tumor cell survival and immune evasion.

In brain tumor-initiating cells (BTICs), tumor-intrinsic PD-1 recruits SHP2 and phosphorylates the cytoplasmic tail of PD-1, thereby activating the NF-κB signaling pathway. This activation promotes the proliferation and self-renewal of BTICs in a PD-L1-independent manner [30]. This finding underscores the multifaceted role of PD-1 in tumor biology, extending beyond its well-known function in immune cells.

Epigenetic modulation of PD-1

In T cells, PD-1 expression is tightly controlled by epigenetic mechanisms. For instance, in naive CD8$^+$ T cells lacking PD-1 expression, the conserved region B (CR-B) and C (CR-C) of *PDCD1* gene are heavily methylated, leading to gene silencing [24]. In contrast, in exhausted T cells resulting from chronic infections, demethylation of the PD-1 promoter occurs, resulting in sustained and elevated PD-1 expression [31]. This epigenetic switch is critical for the functional adaptation of T cells to chronic antigen stimulation.

Histone modifications also play a significant role in regulating PD-1 expression in T cells. Active histone marks such as H3K9ac, H3K27ac, H3K4me2, and H3K4me3 are associated with increased PD-1 expression, while repressive marks like H3K9me3, H3K27me3, and H4K20me3 are linked to its downregulation [31, 32]. Additionally, the transcription factor TOX, which is involved in T cell exhaustion, binds to the PD-1 promoter and facilitates chromatin remodeling, thereby further enhancing PD-1 expression [33].

Similar to T cells, tumor cells exhibit distinct epigenetic patterns that regulate PD-1 expression. However, the specific mechanisms and their functional outcomes can differ significantly. For instance, hypomethylation of specific regulatory regions within the *PDCD1* gene has been associated with increased PD-1 expression in some tumor types [34]. Moreover, active histone marks such as H3K4me3 and H3K27ac have been linked to enhanced PD-1 transcription, while repressive marks like H3K27me3 are associated with its downregulation [34].

While both T cells and tumor cells utilize epigenetic mechanisms to regulate PD-1 expression, the functional outcomes are context-dependent. In T cells, PD-1 upregulation is primarily associated with immune tolerance and exhaustion, whereas in tumor cells, PD-1 expression can promote tumor growth, stemness, and resistance to therapy [13, 17, 35]. Understanding these differences is essential for developing targeted therapies. For instance, in T cells, histone deacetylase inhibitors (HDACi) have been shown to increase PD-1 expression by enhancing histone acetylation at the *PDCD1* promoter [36]. Conversely, in tumor cells, similar HDACi treatment may have diverse effects depending on the tumor type and its genetic background. For example, in osteosarcoma and

non-small cell lung cancer (NSCLC), HDACi treatment can upregulate PD-1 expression in a p53-dependent manner, potentially enhancing tumor suppression [36]. However, in colorectal cancer (CRC), PD-1 expression can inhibit tumor growth by suppressing the AKT and ERK1/2 pathways [37].

Transcription and translation

The regulation of *PDCD1* transcription in T cells is highly intricate, involving a multitude of stimuli such as TCR and cytokine signaling, which exert their influence through a repertoire of at least 14 distinct transcription factors [24]. Specifically, PD-1 expression in immune cells is positively regulated by transcriptional activators including c-fos/AP-1, NOTCH, FOXO1, STAT3/4, ISGF3, NFATc1 and NF-κB, and negatively regulated by transcriptional repressors BLIMP-1, EOMES and T-bet [24, 36]. These transcription factors interact with the promoter region of *PDCD1*, encompassing CR-B and CR-C, as well as the enhancer regions [24].

In both neoplastic and normal cells, PD-1 serves as a direct target gene of the transcription factor p53, which undergoes acetylation at the K120/164 residues [36]. Acetylated p53 recruits acetylation-associated co-factors to the promoter region of the *PDCD1* gene, thereby augmenting local chromatin acetylation and selectively promoting PD-1 transcription [36]. Histone deacetylase inhibitors (HDACi) facilitate PD-1 expression in tumor cells in a p53-dependent manner by acting on acetyltransferases p300/CBP and TIP60 [36].

Post-translational modifications and degradation of PD-1

Post-translational modifications, including glycosylation, ubiquitination, and phosphorylation, are key steps in regulating PD-1 function. The deglycosylation of the PD-1 protein, specifically the removal of N-linked glycosylation, is a critical step for its effective ubiquitination and subsequent degradation. This process significantly affects PD-1 protein stability.

Human PD-1 is characterized as a glycoprotein, possessing four core N-glycans, namely N49, N58, N74, and N116 [24]. In tumor cells, murine double minute 2 (MDM2) enhances the association between glycosylated PD-1 and the glycosidase NGLY1 which facilitates the subsequent deglycosylation and ubiquitination-mediated degradation of PD-1 catalyzed by NGLY1 [38]. As an evolutionarily conserved cytosolic glycosidase, NGLY1 catalyzes the removal of N-linked glycosylation from target glycoproteins, participates in the deglycosylation of misfolded glycoproteins, and the subsequent ER-associated degradation (ERAD) process to promote the degradation of target proteins [19]. Glycosylation plays a critical role in preserving the binding capability of soluble PD-L1 to PD-1 [39]. However, glycosylation of PD-1 is not necessary for the interaction between PD-1 and PD-L1 as the glycosylation sites of PD-1 are situated at a distance from the binding interface of PD-1 and PD-L1 [40].

The exact biochemical mechanisms underlying the impact of core fucosylation on the structure and function of PD-1 have yet to be fully elucidated. In recent research conducted by Rui Liu et al., it was revealed that PD-1 can trigger the expression of MARCH5 through BATF-dependent transcription in T cells [19]. This, in turn, enables the ubiquitination and subsequent lysosomal degradation of the γc chain at the K27 position [19]. Moreover, FBW7 has been identified as a facilitator of proteasome-dependent PD-1 degradation in NSCLC cells [41]. The proteasomal pathway-dependent degradation of PD-1 mediated by FBXO38, KLHL22, and c-Cbl has been reported in T cells [23, 24]. However, further investigations are warranted to ascertain whether proteasome-mediated degradation of tumor-intrinsic PD-1 is also implicated in tumor cells.

In the colon cancer cell lines HT29 and HCT116, Nivolumab administration was noted to stimulate the upregulation of both ubiquitin-like modifier activating enzyme 1 (UBA1) and ubiquitination factor E4B (UBE4B) [19]. This compelling evidence supports the notion that Nivolumab treatment modulates the expression of PD-1, a pivotal regulatory molecule, via the intricate process of proteasomal degradation [19].

Cellular distribution and trafficking of PD-1

In T cells, the N49 and N74 residues of PD-1 undergo fucosylation catalyzed by the enzyme FUT8, a core fucosyltransferase identified by through CRISPR-based screening [24, 42]. The fucosylation of N49 and N74 is essential for the appropriate expression of PD-1 on the cell surface and its localization for functional purposes [24, 34]. The mobility group box protein (TOX) essential in T cell exhaustion, binds to PD-1 in the cytoplasm, aiding in the relocation of PD-1 to the cell membrane [24, 32]. This interaction ultimately promotes the relocation of PD-1 from the cytoplasm to the cell surface [24, 43]. Nevertheless, further investigation is required to determine whether these regulatory effects exist in tumor cells as well.

Decoding the process: the discovery of functional tumor-intrinsic PD-1

In 2008, Tobias Schatton et al. a subpopulation of ATP-binding cassette sub-family B member 5-positive ($ABCB5^+$) melanoma cells as malignant melanoma-initiating cells (MMIC), which possess the crucial attributes of self-renewal and differentiation. This establishing them as the predominant cellular subset responsible for initiating melanoma [44]. Subsequent investigations revealed

that tumorigenic ABCB5$^+$ MMIC cells display the capacity to impede IL-2-dependent T cell activation while promoting B7.2-dependent regulatory T cell (Treg) induction. These cells also exhibit preferential expression of the co-stimulatory molecules B7.2 and PD-1 [44]. Meanwhile, the role of PD-1 as an immune checkpoint receptor in immune cells, particularly T cells, has been extensively elucidated [45–47].

Despite the impressive efficacy demonstrated by ICB therapy in the management of malignant neoplasms, a significant proportion of patients persistently manifest non-responsive or recurrent disease after receiving treatment [9, 48]. The occurrence of ICB-related adverse effects, particularly those tied to autoimmune reactions, remains a pressing issue. Moreover, the emergence of acquired resistance to ICB therapy subsequent to an initial treatment response has been documented [9, 48, 49]. Furthermore, in addition to conferring benefits to patients afflicted with highly immunogenic tumors, such as malignant melanoma, PD-1 pathway blockade has also yielded meaningful anti-tumor activity in tumors with low immunogenicity or in those that have historically demonstrated limited responsiveness to immunotherapeutic interventions [9, 13, 50]. These observations highlight the potential of PD-1 pathway blockade to elicit robust anti-tumor responses in a broader spectrum of patients.

Clinical observations have identified instances of atypical responses, wherein patients with melanoma exhibiting low immunogenicity but a comparable immune microenvironment have proven refractory to treatment with anti-CTLA-4 therapy [9]. However, these patients have displayed substantial clinical improvements following anti-PD-1 therapy. These observations hint at the possibility that PD-1 exerts immune-independent complementary, pro-tumorigenic mechanisms. Although responses to PD-1 inhibition have been observed in patients who are refractory to anti-CTLA-4 therapy, these findings do not provide conclusive evidence for non-immune functions of PD-1. While it is plausible that perturbation of tumor-intrinsic PD-1 activity may contribute to therapeutic efficacy, further studies are required to elucidate the precise mechanisms underlying these observations. The potential involvement of non-immune functions of PD-1 in tumor cells remains an area of active investigation and warrants additional research to establish a more definitive role.

Until 2016, Sonja Kleffel et al. undertook a comprehensive characterization of PD-1 mRNA and protein expression in clinical tumor biopsy tissues and melanoma cell lines, revealing the presence of PD-1-expressing tumor cells in these specimens [51]. However, PD-1 expression was not uniformly distributed among all melanoma cells, but rather selectively expressed in a minor subset of melanoma subpopulations that play a crucial role in tumor growth, in parallel with previous observations of PD-1-expressing cells in melanoma-initiating cells [13]. Subsequent investigations by Sonja Kleffel et al. demonstrated that tumor-intrinsic PD-1 exerts a growth-promoting effect on melanoma cells by activating the mTOR-S6 signaling pathway, with this process being contingent upon the presence of its ligand PD-L1 [13]. Consistent withh the interaction between PD-1 and mTOR and PI3K/AKT signaling pathways in T cells, the findings from Sonja Kleffel et al.'s study confirmed the relationship between intrinsic PD-1 and the mTOR and PI3K/AKT signaling pathways in tumors [13]. However, despite the ability of PD-1 signaling to promote the activity of SHP2, mTOR, and ribosomal protein S6 in both T cells and melanoma cells, the former suppresses the anti-tumor efficacy of T cells, while the latter facilitates PI3K-independent melanoma cell growth [23].

PD-1 in malignant progression

Pan-cancer studies on PD-1 have demonstrated that higher *PDCD1* expression levels are associated with a favorable prognosis in several cancer types, including breast cancer (BRCA), cervical cancer (CESC), head and neck squamous cell carcinoma (HNSC), liver hepatocellular carcinoma (LIHC), ovarian cancer (OV), soft tissue sarcoma (SARC), stomach adenocarcinoma (STAD), skin cutaneous melanoma (SKCM), uterine corpus endometrial carcinoma (UCEC), and testicular germ cell tumor (TGCT) [11]. Specifically, in triple-negative breast cancer (TNBC), elevated levels of *PDCD1* expression have been linked to an improved disease-free survival (DFS) [11]. Similarly, in the case of HNSC, studies have demonstrated a significant association between increased *PDCD1* expression and better overall survival (OS), as well as a lower likelihood of disease recurrence [11]. In contrast, high *PDCD1* expression levels were correlated with a worse prognosis in Glioblastoma (GBM), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), lower grade glioma (LGG), acute myeloid leukemia (LAML), and uveal melanoma (UVM). However, in the case of BLCA, the prognostic role of the *PDCD1* gene may be conflicting in different datasets [11]. Nevertheless, it is difficult to determine whether PD-1 originates from tumor cells or other cells in these studies. Furthermore, there are inconsistencies between certain findings and previous research results, such as bladder cancer (BLCA) [12]. These uncertainties necessitate further in-depth investigations for validate the prognostic value of *PDCD1*. The potential outcomes and mechanisms of tumor-intrinsic PD-1 signaling are briefly summarized in Table 1 (placed at the end of the document text file).

Table 1 Tumor cell-intrinsic PD-1 signal potential outcomes and mechanisms

Category	Functional consequence of tumor intrinsic PD-1 signal	Mechanisms	Tumor types	Experimental models and context
Normal biology of PD-1	Inhibit cell growth	HDACi facilitates the expression of PD-1 in a p53-dependent manner by acting on acetyltransferases p300/CBP and TIP60; tumor intrinsic PD-1 acts possibly via inhibiting AKT/mTOR pathway	Osteosarcoma, melanoma, NSCLC, pancreatic carcinoma	In vitro and in vivo of human and mouse models [36]
Normal biology of PD-1	Inhibit cell proliferation, promote cell apoptosis and drive nivolumab-associated tumor growth	Possibly through activating p38, AKT, and MEK/ERK1/2 pathways	CRC	In vitro studies of human cell lines [52]
Normal biology of PD-1	Diminish cell viability	Possibly related to the complex consequences of the phosphatases that interact with activated PD-1	NSCLC	In vitro and in vivo experiments in mouse models [10]
Normal biology of PD-1	Facilitate tumorigenesis	Activating the mTOR-S6 signaling pathway	Melanoma	In vitro and in vivo of human and mouse cell lines [13]
Normal biology of PD-1	Promote tumor stemness and potential synergism with anti-PD-1 antibody	By promoting the expression of a distinct marker profile associated with tumor initiation (e.g. activates the Oct4 promoter)	Melanoma	In vitro and in vivo of human and mouse models [53]
Normal biology of PD-1	Facilitate proliferation, differentiation and tumorigenicity	Activate the MAPK/ERK signaling	AML	In vitro and in vivo experiments in mouse models [28]
Normal biology of PD-1	Facilitate cell proliferation and migration	Triggering the SHP2/Ras/MAPK signaling cascade	TC	In vitro and in vivo experiments in mouse models [29]
Normal biology of PD-1	Facilitate cell cycle progression and apoptosis induction	PD-1 binds the downstream mTOR eIF4E and S6, and subsequently promotes the over-expression of Cyclin D and the down-expression of DDR4 and attenuates the TRAIL pathway	HCC	In vitro studies of human cell lines and in vivo mouse models [17]
Normal biology of PD-1	Facilitate proliferation and inhibits cell apoptosis	Tumor intrinsic PD-1 interacts with MOB1, leads to the inactivation of LATS1 and increases the accumulation and nuclear translocation of unphosphorylated YAP, and subsequently stimulate the Hippo/CYY61/CTGF pathway	PDAC	In vitro studies of human cell lines and in vivo mouse models [16]
Normal biology of PD-1	Accelerate tumor cell growth	Activating the mTOR signaling pathway and triggering the mROS generation	MCC	In vitro studies of human cell lines and in vivo mouse models [54]
Normal biology of PD-1	Foster cell proliferation and metastasis	YB-1 promotes PD-1 expression through the translational activation pathway; tumor intrinsic PD-1 signal activate downstream AKT and MAPK pathways	TNBC	In vitro studies of human cell lines and in vivo mouse models [35]
Normal biology of PD-1	Facilitate cell proliferation and self-renewal	Activating the NF-κB signaling pathway	GBM	In vitro and in vivo of human and mouse models [30]
Effect of inhibition of PD-1	Cotargeting FBW7 enhances antitumor immunity	FBW7 facilitates proteasome-dependent degradation of PD-1 by promoting the K48-linked polyubiquitination of PD-1 protein at Lys233 residue	NSCLC	In vitro and in vivo of human and mouse models [41]
Effect of inhibition of PD-1	Inhibit cell proliferation, promote apoptosis and targeting pd-1 results in radio/chemo resistance	C. tropicalis down-regulate tumor intrinsic PD-1 expression via enhancing cell autophagy levels; tumor intrinsic PD-1 acts by activating the AKT and ERK1/2 pathways	CRC	In vitro studies of human cell lines and in vivo mouse models [37]

Abbreviations: AML, acute myeloid leukemia; MCC, merkel cell carcinoma; YB-1, Y-box binding protein 1; mROS, mitochondrial reactive oxygen species; YAP, Yes-associated protein; LATS1, large tumor suppressor kinase 1; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DDR4, death receptor 4; eIF4E, effectors eukaryotic initiation factor 4E; S6, ribosomal protein S6

Oncogenic implications of tumor-intrinsic PD-1: shedding light on a double-edged sword

The groundbreaking study conducted by Sonja Kleffel et al. provided the first empirical evidence of the biological role of tumor-intrinsic PD-1, demonstrated that PD-1 actively facilitates tumor cell proliferation by inducing S6 phosphorylation [13]. Given that S6 phosphorylation acts as a junction for numerous upstream signaling networks, the possibility of PD-1 in melanoma regulating other alternate signaling networks apart from the mTOR pathway cannot be discounted [55].

The contrasting effects of PD-1 interaction on mTOR signaling in melanoma cells versus T cells coincide with the varied functions of SHP2; it fosters tumor progression

in melanoma cells while impeding it in T cells [15, 24]. Similar SHP2-associated tumorigenic effects of PD-1 have been observed in acute myeloid leukemia (AML) and thyroid cancer [56]. Shanshan Suo et al. found a subset of PD-1$^+$AMLLSKs in bone marrow-derived LSKs (Lin$^-$Sca-l1$^+$c-kit$^+$) stem cells in an AML model [56]. The PD-1$^+$AML LSKs subgroup expressed comparatively higher levels of SHP2 and p-ERK. Moreover, PD-1 activated the MAPK/ERK signaling pathway in this cell subset, making this cell population more proliferative, differentiated, and tumorigenic than PD-1-AML LSKs [56].

High PD-1 expression is positively correlated with thyroid cancer stage and lymph node metastasis [29]. Liotti et al. demonstrated that tumor-intrinsic PD-1 facilitates the recruitment of SHP2 to the plasma membrane, leading to an increased level of SHP2 phosphorylation. This event, in turn, enhances Ras activity by dephosphorylating tyrosine residue 32 of Ras, thereby triggering the SHP2/Ras/MAPK signaling cascade [29].

The utilization of PD-1 inhibitors in conjunction with the multi-target receptor tyrosine kinase inhibitor lenvatinib for the management of unresectable HCC resulted in prolonged overall survival, alongside notable objective response rates and disease control rates [14]. Nonetheless, it is crucial to acknowledge the limited responsiveness of anti-PD-1 therapy in the majority of HCC patients, and the potential occurrence of hyperprogression in certain individuals with advanced-stage HCC [14]. Hui Li et al. conducted a study that elucidated the involvement of the PD-1 receptor within HCC cells [17]. The study revealed that PD-1 engagement initiates binding interactions and subsequent phosphorylation of mTOR's downstream targets, specifically the eukaryotic initiation factor 4E (eIF4E), as well as the ribosomal protein S6 [17]. This chain of interactions culminates in the over-expression of Cyclin D and the down-expression of the death receptor 4 (DR4) protein levels [17]. As a result, cell cycle progression is facilitated, whereas apoptosis induction via the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) pathway is attenuated [17]. Importantly, this mechanism potentially underlies the development of hyper-progression observed in HCC patients following treatment with ICB. The study additionally revealed that the combined administration of mTOR inhibitors and anti-PD-1 blocking antibodies synergistically promotes more sustained tumor regression [14]. Furthermore, the interaction between PD-1 and eIF4E/S6 may exert an influence on the binding affinity of several key molecular pairs, including eIF4E with 4E-BP1 or eIF4G, eIF4G with MNK1/2, and S6K with the eIF3 complex, thereby modulating the phosphorylation status of S6 and eIF4E [14]. Through a comparative analysis of the tumorigenic potential between PD-1$^+$ and PD-1$^-$ sorted SMMC7721 cells, it was ascertained that the PD-1$^+$ subpopulations demonstrated heightened proliferation capacity in vitro. Additionally, in B-NSG mice, the PD-1$^+$ cells exhibited augmented tumor growth in contrast to the PD-1$^-$ cells [14].

In PDAC cells, tumor-intrinsic PD-1 interacts with the downstream MOB1, which subsequently leads to the inactivation of large tumor suppressor kinase 1 (LATS1) and the consequent inhibition of Yes-associated protein (YAP) phosphorylation [16]. This event consequently increases the accumulation and nuclear translocation of unphosphorylated YAP [16]. Furthermore, the activation of PD-1 plays a pivotal role in facilitating tumor cell growth by stimulating the Hippo/CYY61/CTGF pathway [16]. Combination of an anti-PD-1 blocking antibody with an inhibitor targeting the Hippo signaling pathway substantially enhances the anti-tumor efficacy [16].

Most recently, the MCC-PD-1-mTOR-mtROS signaling cascade has been recognized as an intrinsic promoter of tumor proliferation in Merkel cell carcinoma (MCC) [55]. This pathway accelerates tumor cell growth through activation of the mTOR signaling pathway and triggering the mitochondrial reactive oxygen species (mROS) generation [55]. Inhibition of this pathway could potentially enhance therapeutic outcomes in MCC patients [57]. In relation to TNBC, a study conducted by Qian Wu et al. demonstrated that PD-1 is aberrantly up-regulated in TNBC patients and TNBC cell lines [35]. Acting as an effector for Y-box binding protein 1 (YB-1), a gene expression regulator, PD-1 fosters both the proliferation and metastasis of TNBC cells, both in vitro and in vivo [35].

GBM is the predominant and highly malignant primary brain tumor in adults. Clinical trials have revealed that the response rate to nivolumab treatment is lower than 10% [56]. Nonetheless, in patients exhibiting anti-tumor activity following anti-PD-1 blockade, those treated with nivolumab display a longer duration of response compared to patients treated with bevacizumab, an anti-VEGF-A antibody. The response durations are 11.1 months for nivolumab and 5.2 months for bevacizumab, respectively [56]. Further investigation of the underlying mechanisms contributing to this atypical phenomenon was conducted by V. Wee Yong and colleagues through culturing brain tumor-initiating cells (BTICs) derived from both patient and mouse brain tumors, they demonstrating that tumor-intrinsic PD-1 recruits SHP2 and phosphorylates the cytoplasmic tail of PD-1, thereby activating the NF-κB signaling pathway in BTICs [30]. This activation facilitates the proliferation and self-renewal of BTICs in a PD-L1-independent manner [30].

Tumor-suppressive potential of tumor-intrinsic PD-1: a key player in tumor control

It is noteworthy that tumor-intrinsic PD-1 can exhibit divergent pro- and anti-tumor effects in tumor cells, contingent upon the tumor type and microenvironment [18]. In 2018, Shisuo Du and colleagues documented a case of accelerated disease progression in a patient with PD-1$^+$ NSCLC following combination therapy involving palliative radiotherapy and pembrolizumab, a monoclonal antibody targeting PD-1 (NCT02318771) [10]. Shisuo Du et al. conducted in vitro and in vivo experiments to assess the impact of PD-1 inhibition on cell proliferation, colony formation, and tumor growth in mouse lung cancer M109 cells [10]. The study findings demonstrated the expression of PD-1 in NSCLC and its tumor-inhibitory effect [10]. Conversely, the inhibition of PD-1/PD-L1 signaling through treatment may potentially contribute to hyper-progression in NSCLC patients. Moreover, a study by Gao et al. revealed that intrinsic PD-1 in NSCLC cells achieves tumor suppression by inhibiting AKT and ERK1/2, two classical signaling pathways [18].

In a recent study conducted by Cao Zhijie et al., the role of intrinsic PD-1 in lung cancer cells was elucidated, demonstrating its significant immune-independent inhibition of tumor cell growth [36]. By employing a PD-1 knockdown strategy in H1299 cells using a p53-Tet-on expression system, the researchers disrupted PD-1 expression induced by p53 and observed a subsequent attenuation of p53-mediated tumor suppression [36]. This confirmed involvement of tumor-intrinsic PD-1 in the p53-mediated suppression of tumor growth [36]. Furthermore, based on the findings from immunohistochemistry and bioinformatics analysis, it was postulated that PD-1 may exert its inhibitory effects on tumor growth via modulation of the AKT/mTOR signaling pathway [36].

In a recent investigation conducted by Junxing Qu et al., it was corroborated that tumor-intrinsic PD-1 confers a protective effect in colorectal cancer (CRC) [37]. The investigation elucidated that tumor-intrinsic PD-1 exerts a suppressive influence on tumor growth through mechanisms independent of adaptive immunity, by inhibiting the AKT and ERK1/2 signaling pathways [37]. Remarkably, the study demonstrated that the depletion of PD-1 or administration of anti-PD-1 antibodies can facilitate the progression of CRC [37]. It was also observed that a high dosage of *Candida tropicalis* (C. tropicalis) can down-regulate the expression of tumor-intrinsic PD-1 through autophagy, thereby promoting CRC tumor growth [37].

It is important to note that the role of PD-1 appears to be context-dependent, as evidenced by the protective effect of nivolumab treatment on PD-1positive colon cancer cells against chemotherapy and radiotherapy [52]. This highlights the heterogeneity in the function of PD-1 across different contexts [52]. PD-1 positive colon cancer cells exhibited notable enrichment of p53, epithelial-mesenchymal transition (EMT), and stem-like gene sets, including the well-established Wnt/β-catenin signaling pathway [52]. These contradictory results indicate a complex and diverse impact of tumor-intrinsic PD-1. This phenomenon, characterized by a paradoxical function of tumor cell-intrinsic PD-1/L1, might be attributed to the specific characteristics of tumor cells or certain signaling pathways.

The hidden potential: intrinsic PD-1 as a mediator of tumor cell stemness

Cancer stem cells (CSCs), a distinct subpopulation of tumor cells characterized by their ability to self-renew and undergo EMT, are intimately associated with the development of chemoresistance and tumor relapse [57]. In the context of ICB therapy, the investigation into the intricate interplay between CSCs and tumor immune evasion has emerged as a burgeoning and compelling field of research [57]. The Wnt/β-catenin signaling pathway, being the first cascade implicated in melanoma immune evasion via intrinsic mechanisms, has been substantiated across a diverse spectrum of tumor malignancies [52].

Caterina Ierano et al. found that human colon cancer cells including HT29, HCT116, SW620, and LOVO, express PD-1, and tumor-intrinsic PD-1 signaling activated AKT and MEK/ERK1/2 signaling pathways, which inhibited cell proliferation and promoted cell apoptosis [52]. Transcriptional profiles revealed a distinct set of genes that were oppositely regulated in colon cancer and melanoma cells upon treatment with Nivolumab [52]. Consistent with the observations in colorectal cancer, emerging research has revealed a significant association between the expression indices of stemness genes and gastric cancer, highlighting their relevance to tumor progression and PD-L1 expression [52]. These findings provide further validation of the pivotal role played by PD-1/PD-L1 signaling in the advancement of cancer stem cells [52]. A study conducted by V. Wee Yong and colleagues examined the immune checkpoint axis in human GBM samples, uncovering a fascinating observation wherein approximately 8% of cells exhibited a concurrent expression of BTIC markers and PD-1 [30]. Moreover, current research points to the PD-1 receptor's unique and crucial role in fostering stem-like subclones in both melanoma and NSCLC [51, 58]. In cases where initial chemotherapy is conducted without anti-PD-1 treatment, the idea of maintenance PD-1 blockade as a consolidative therapy to address any remaining PD-1$^+$ CSCs could be beneficial [51, 58].

Cancer stem cells in certain tumor types demonstrate heightened expression of PD-L1 relative to non-stem cells [59]. The intrinsic expression of PD-L1 in cancer cells fosters stemness-related features by inducing the up-regulation of well-known stemness genes, such as Oct4 and Nanog [59]. Similarly, in the PD-1$^+$ tumor cell subset, which displays resistance to chemotherapy, upregulation of stemness genes, including ABCG2, OCT3/4, and SOX2, was also observed [60]. The mutual regulation between PD-1 and PD-L1 may play a role in modulating tumor cell stemness characteristics. However, the exact molecular mechanisms governing the regulatory role of PD-1 in stemness properties, as well as its potential dependence on PD-L1, remain to be fully elucidated. Additionally, the immunological microenvironment of the TME continues to be a crucial modulator of tumor-intrinsic PD-1 signaling, potentially fostering CSC advancement subsequent to cytotoxic treatment [58, 61].

The underlying hypothesis is that a subset of PD-1$^+$ tumor cells, presumed to be possessing stem-like properties, constitutes only a minor fraction of cells under normal tumor conditions. However, under certain circumstances, this minority could proliferate and become instrumental in maintaining tumor homeostasis and evoking resistance to chemotherapy. The potential clinical significance of these observations might be particularly pertinent in circumstances where putative CSC are believed to play an active role. An illustrative example is the often-observed disease relapse after an initial chemotherapy response, a problem frequently linked to chemo-resistant PD-1$^+$ CSCs (Fig. 2). In this context, these investigations underline the lymphocyte-independent anti-cancer efficacy of anti-PD-1 blocking antibodies, suggesting their potential for use in combination with cisplatin (CDDP). The absence of immune cells in these experimental studies poses a potential limitation, which needs to be taken into consideration when interpreting

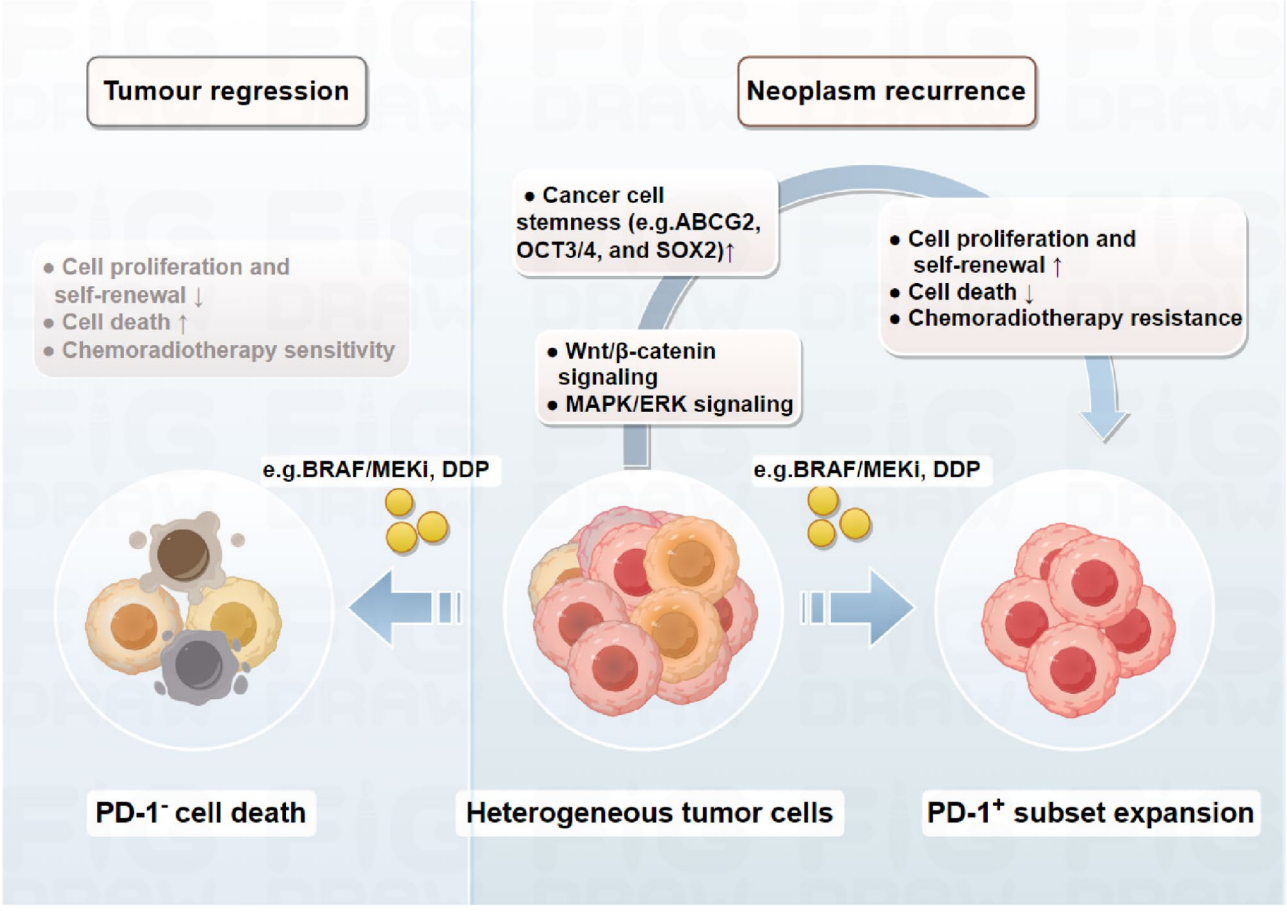

Fig. 2 The hidden potential of intrinsic PD-1 promotes enhance of tumor cell stemness and accelerates drug resistance. The administration of BRAF/MEK inhibitors or chemoradiotherapy can modulate the population of PD-1$^+$ tumor cells by altering signaling pathways that regulate essential cellular processes such as proliferation, cell death, and self-renewal. Specific signaling pathways affected by these treatments include the Wnt/β-catenin signaling pathway and the MAPK/ERK signaling pathway, among others. These pathways play critical roles in determining the fate and behavior of tumor cells, including their stemness properties. Activation of these pathways can lead to the upregulation of stem cell markers such as OCT3/4 within a subset of PD-1$^+$ tumor cells, promoting cancer stemness and contributing to tumor recurrence

these conflicting findings and their applicability to broader populations.

The insidious protagonist: the pivotal role of intrinsic PD-1 in promoting tumor therapy resistance

The findings of Caterina Ierano et al. highlight a novel association between PD-1 targeting and the development of radio/chemo resistance specifically in colon cancer [52]. While further validation in a larger population is warranted, the expression of tumor-cell intrinsic PD-1/total PD-1 may serve as a potential biomarker to guide the selection of ICB therapy in patients with CRC [52].

Rotolo et al. unveiled a notable expansion of PD-1$^+$ melanoma subpopulation subsequent after exposure to BRAF/MEK inhibitors [53]. These PD-1 + subpopulation, identified as melanoma stem cells, exhibited a distinct marker expression profile associated with tumor initiation, suggesting their potential involvement in tumor resistance and recurrence [53]. Indeed, the combination therapy of BRAF/MEK inhibitors with anti-PD-1 antibodies demonstrated augmented efficacy in combatting the malignancy [53]. However, the observed elevation in the proportion of PD-1$^+$ melanoma cells following treatment with BRAF/MEKi is likely attributable to the mechanism involving molecular modulation of PD-1 protein expression, as well as to selective processes favoring the survival of PD-1$^+$ melanoma cells with reduced susceptibility to targeted therapy.

In vitro investigations revealed that Rotolo et al. exclusively noted the suppressive impact of anti-PD-1 antibodies on stem-like pneumospheres [53]. Moreover, they noted that the observed restraint of pS6 in PD-1$^+$ NSCLC cells upon treatment with anti-PD-1 antibodies appears to be independent of AKT signaling, as they observed a modest increase in p-AKT levels following PD-1 blockade instead of the expected reduction [53].

The synergistic therapeutic efficacy of combining PD-1 blockade with epidermal growth factor receptor inhibitor (EGFRi) therapy in the management of recurrent or metastatic HNSCC has been convincingly demonstrated [62]. A subset of cancer cells harboring EGFR mutations has been observed to exhibit stem-like properties and display resistance towards EGFRi [63]. Further areas of inquiry and potential prospects for future studies include delving into the profound effects of varying genetic backgrounds, exemplified by EGFR mutations, as well as investigating the intricate biological variables associated with specific temporal windows wherein tumor homeostasis may undergo alterations. These contradictory results indicate a complex and diverse impact of tumor-intrinsic PD-1.

Efforts are also underway to expand the research on the expression regulation of PD-1, its impact on drug resistance associated with CSC characteristics, and the ubiquitination regulatory mechanisms. However, the endeavor to functionally interpret these processes may become more intricate, yet simultaneously fascinating, when considering recent insights into tumor cell-intrinsic PD-L1 signaling. This signaling regulates numerous key processes, including tumor growth, survival pathways, stemness, immune impacts, DNA damage (Fig. 3). Moreover, as most investigations lack clinical sample validation for tumor-intrinsic PD-1 and fail to examine the precise regulatory mechanisms of PD-1 signaling on tumor cell proliferation. Additionally, there is limited research on the expression regulation of PD-1 in tumor cells. The role and regulatory mechanisms of tumor-intrinsic PD-1 signaling remain a novel area that requires ongoing efforts to characterize its involvement in tumor development.

Potential causes for tumor cell-intrinsic PD-1 paradox

Accumulated studies have elucidated tumor-intrinsic PD-1 exerts a paradoxical impact on tumorigenesis, exhibiting oncogenic implications in the malignant transformation of NSCLC and colon cancer cells, while paradoxically fostering the relentless march of malignancies including but not limited to melanoma [13], HCC [17], PDAC [16], TNBC [35], GBM [30], and TC [29], extending beyond the realms of adaptive immunity. The genetic and epigenetic alterations, tumor immune evasion mechanisms and heterogeneity of signaling pathways may be involved in this interplay.

The intrinsic motivational factors can significantly differ across tumors of diverse origin [64]. Genetic and epigenetic alterations: Genetic and epigenetic modifications in tumor cells intricately modulate the expression of PD-1 and its ligands, particularly PD-L1, as well as other molecules implicated in PD-1 signaling [6]. These alterations dynamically shape the extent to which PD-1 contributes to tumor growth or suppression, thus contributing to the observed heterogeneity [17]. For instance, intrinsic PD-1 assumes a paramount role in promoting cancer cell proliferation in both TC cells [29] and HCC cells [29]. However, genetic alterations, such as RET/PTC rearrangements and BRAF and Ras gene point mutations, are present in over 70% of PTC cases, leading to Ras/MAPK pathway activation in TC cells [29]. These mutations regulate the transcription of crucial genes associated with TC cell proliferation [27]. Another example is colorectal cancers where the adenomatous polyposis coli (APC) mutation is prevalent [15]; in contrast, hepatocellular carcinoma often displays the p53-R249S mutation [15, 65]. These primary mutated oncogenes and tumor suppressor genes are potential upstream regulatory genes of intrinsic PD-1.

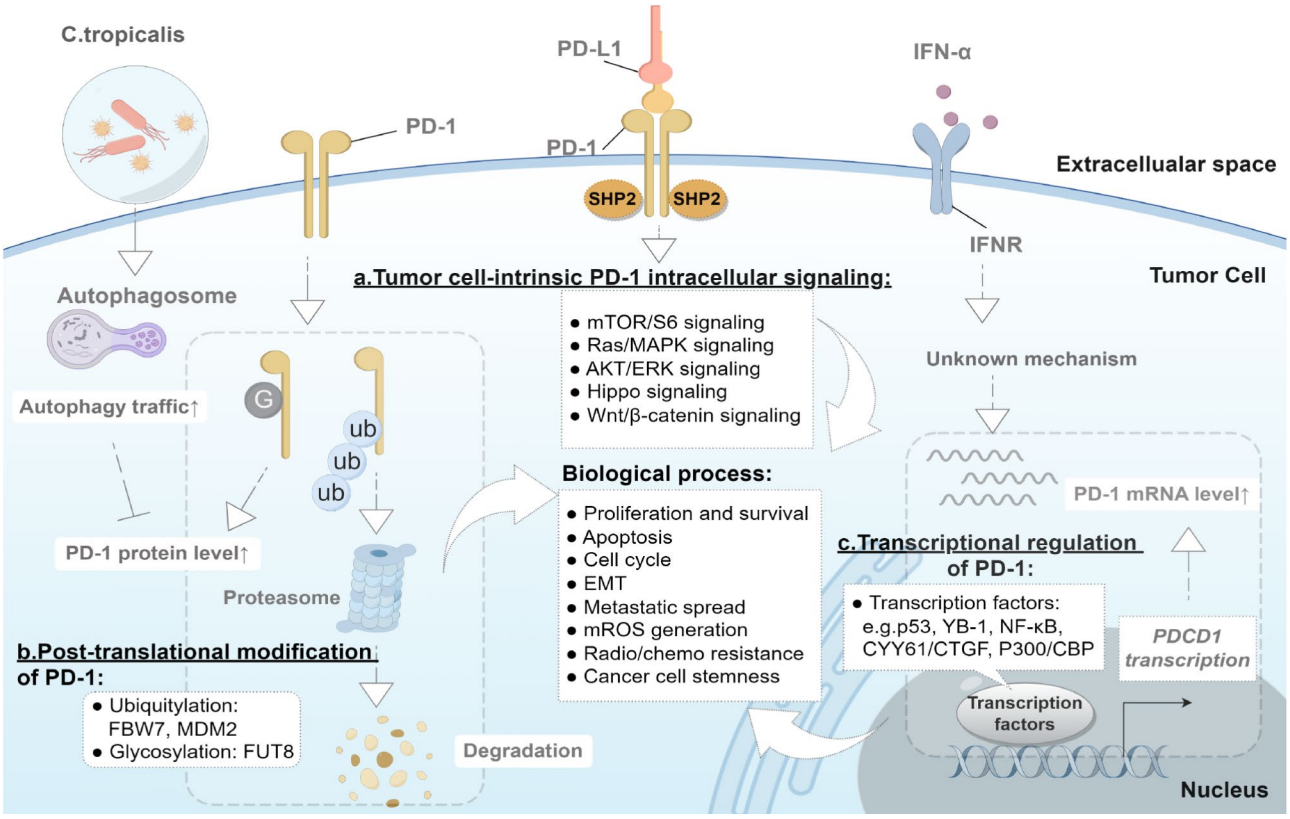

Fig. 3 The intricate regulatory mechanisms of programmed death 1 in tumor cells. (**a**) Tumor cell-intrinsic PD-1 intracellular signaling. The immunoglobulin-like domain of extracellular PD-1 interacts with the immunoglobulin-like extracellular domain of PD-L1, trigger downstream signaling pathways, including mTOR signaling, Ras/MAPK signaling, AKT/ERK signaling, Hippo signaling, Wnt/β-catenin signaling. These signaling pathways play a crucial role in multiple biological processes, such as proliferation, apoptosis, cell cycle progression, EMT, Metastatic spread, generation of mROS, development of radio/chemotherapy resistance, and maintenance of cancer stemness. For example, the activation of PD-1 signaling in tumor cells can lead to increased phosphorylation of downstream molecules in the mTOR pathway, such as ribosomal S6 protein (p-S6). This phosphorylation of key molecules within these signaling pathways can have a range of effects on the behavior and characteristics of tumor cells, contributing to tumor progression, aggressiveness, and resistance to therapeutic interventions. (**b**) Post-translational regulation. One key aspect of post-translational regulation highlighted is the role of FBW7 as an E3 ubiquitin ligase for the PD-1 protein. FBW7 promotes the K48-linked polyubiquitination of PD-1 at the Lys233 residue, marking it for degradation by the proteasome. This process is crucial for controlling the levels of PD-1 protein in tumor cells. Another important post-translational regulatory mechanism involves MDM2, which enhances the association between glycosylated PD-1 and the glycosidase NGLY1. This interaction facilitates the deglycosylation and ubiquitin-mediated degradation of PD-1 by NGLY1. Additionally, the fucosylation of specific residues on PD-1 (N49 and N74) mediated by FUT8 is essential for the functional localization of PD-1. Loss of core fucosylation has been linked to enhanced degradation of PD-1 by the ubiquitin-proteasome system. (**c**) Transcriptional regulation. The transcription of *PDCD1* is regulated by various transcriptional factors include p53, YB-1, NF-κB, CYY61/CTGF and P300/CBP

Tumor immune evasion mechanisms: Tumors employ a panoply of immune evasion strategies, such as the upregulation of alternative immune checkpoint molecules, recruitment of immunosuppressive cells, and secretion of immunosuppressive factors. The intricate interplay between these mechanisms and PD-1 signaling can differ remarkably across tumors, thereby imparting heterogeneity to the impact of PD-1. Heterogeneity of signaling pathways: selective signaling pathways might be engaged. A finding by Kleffel et al. revealed that blocking PD-1 led to growth suppression in melanoma cells expressing PD-1 [13]. They suggested that the contrasting effects of PD-1 blockade on T cells as opposed to melanoma could be ascribed to the differing SHP2 signaling in these two cell types [66]. In melanoma [13] and HCC [17], intrinsic PD-1 facilitates tumor growth by activating the mTOR signaling pathway, a mechanism independent of adaptive immunity. However, PD-1 suppresses tumor growth by damping down the canonical signaling pathways, including the AKT and ERK1/2 pathways, in NSCLC [18, 66]. Further comprehensive investigations are warranted to fully unravel these mechanisms and pave the way for personalized therapeutic interventions.

Expanding the horizons for PD-1 targeting and harnessing the potential of combination therapies with PD-1 blockade

PD-1 has emerged as an eminent immune checkpoint molecule, demonstrating unparalleled success in the field. As of 2023, a total of 11 PD-1 monoclonal antibodies (mAbs) (e.g., nivolumab, pembrolizumab, cemiplimab) and 5 PD-L1 mAbs (e.g., atezolizumab, durvalumab, avelumab) have been approved for clinical utilization [67]. This list continues to expand. These antibodies are deployed either alone or in combination with radiotherapy, chemotherapy, or targeted therapy to combat a variety of malignancies [67].

It is worth noting that the majority of commercially available mAbs targeting PD-1 are unable to detect the isoform Δ42PD-1 [25, 26]. It is reassuring that specific antibodies targeting Δ42PD-1 have been developed. Two mAbs, CH101 and CH34, specifically designed for Δ42PD-1, do not exhibit cross-reactivity with PD-1 [25, 26]. These observations indicate that therapeutic strategies focused on ICB targeting PD-1 and PD-L1 may not effectively address disorders mediated by Δ42PD-1. This aspect should be taken into consideration in anti-PD-1-based tumor therapies, especially for patients with HCC [25].

In contrast to the earlier discovered and clinically employed immune checkpoint molecule CTLA-4, targeted blockade of the PD-1/PD-L1 signaling pathway demonstrates a broader responsive patient population and typically yields more robust antitumor efficacy with lower incidence of immune-related adverse events (irAEs) [68]. This therapeutic approach has exhibited notable effectiveness in the management of HNSCC, melanoma, Hodgkin lymphoma, and microsatellite-highly unstable tumors [7, 9].

The pursuit of alternate non-antibody PD-1 inhibitors has seen a gradual resurgence and emerged as a prominent area over the past few decades. These alternatives encompass peptide inhibitors and non-peptide small molecule inhibitors. Oral formulations offer the advantage of simplified transportation and storage for clinical treatment purposes.

Small-molecule inhibitors targeting PD-1

Apart from the availability of high-affinity PD-1 antibodies, small molecule inhibitors targeting PD-1 have also been formulated specifically for oral administration. However, small molecule inhibitors offer distinct advantages over mAbs in tackling these challenges. They are more amenable to oral administration and can modulate drug half-life to minimize target occupancy. Small molecule inhibitors exhibit superior efficacy in inhibiting tumor growth and migration compared to antibodies, while also demonstrating a higher level of biosafety. At present, there is a paucity of small molecule inhibitors targeting PD-1 within the field of immuno-oncology. PD-1/PD-L1 antibody inhibitors have been authorized for clinical usage with the primary objective of impeding the interaction between PD-L1 and PD-1, thereby enhancing the cytotoxicity of $CD8^+$ cytotoxic T lymphocytes (CTLs).

The association of PD-1 with PD-L1 induces a conformational change in the CC' loop of PD-1, resulting in a 90° rotation from the "open" state to the "closed" state, which extends beyond the binding site [69]. This conformational alteration enables the establishment of four hydrogen bond pairs between PD-1 and the PD-L1 heterodimer [69]. Specifically, three hydrogen bonds are formed by Gln75 of PD-1 with Asp26 and Arg125 of PD-L1, while a single hydrogen bond is established between Thr76 of PD-1 and Tyr123 of PD-L1 [69]. These intermolecular hydrogen bonds serve as the architectural basis for the development of small molecule inhibitors designed to disrupt the PD-1 and PD-L1 interaction [69].

The peptide-based small molecule inhibitor AUNP-12, comprising 29 amino acids, can be designed to incorporate segments derived from the extracellular (EC) domain of the human PD-1 protein, enabling it to impede the progression and dissemination of primary tumors [70]. Additionally, it maintains antitumor immune function for at least 24 h without causing substantial toxicity. The compound possesses the capacity to effectively modulate the occurrence of irAEs due to its comparatively shorter metabolic half-life in relation to mAbs [70]. Moreover, the publication of nonpeptide-based small molecule inhibitors such as BMS-200 [71] and CA-170 [72] has provided potential strategies for addressing immunological antagonistic diseases initiated by the activation of PD-1 and PD-L1 pathways.

However, the feasibility of targeting other components within the PD-1 signaling pathway is yet to be determined. It has been reported that methylene blue (MB) is typically administered in septic shock, and exhibits effective inhibition of PD-1 signaling [73].

Potential combination strategies targeting PD-1

In the realm of cancer treatment, the implementation of combination strategies that include PD-1/PD-L1 blockade is becoming an inevitable trajectory. These strategies are not limited to combinations with chemotherapy, targeted therapy, radiation, and intra-tumoral therapies, but also extend to systemic procedures like the application of bispecific and multi-specific antibodies, innovative immunoconjugates, cancer vaccines, adoptive cell therapy, and microbiome modulation [48, 66, 74].

The efficient delivery characteristics of nanomaterials facilitate the effectiveness of combination therapy, offering an effective approach to overcome the limitations

associated with PD-1 blockade therapy [75, 76]. By employing multidrug co-delivery strategies and constructing sensitive bonds to enable controlled drug release, the integration of nanomaterials with PD-1 blockade therapy demonstrates promising potential [77]. Ordikhani et al. employed the double emulsion evaporation method to fabricate nanoparticles that encapsulated PD-1 antibodies (termed anti-PD-1 NPs) [77]. Additionally, the incorporation of polycationic PRT in the PRT/CpG/OVA nanovaccine yielded enhanced delivery efficiency, characterized by increased internalization by dendritic cells (DCs), efficient escape from endosomes, and facilitated maturation of DCs [78]. Indeed, the combinational approach involving the nanovaccine and anti-PD-1 exhibited a synergistic effect, capitalizing on the cooperative interplay between the nanovaccine's capacity to activate T cells and aPD-1's role in preserving T cell function [76, 78].

Conclusions and prospects

The path to conquering cancer is a difficult journey. Instances where certain cancer patients show resistance to immunotherapy or face hyper-progression of the disease have been disclosed through clinical evidence. The challenge of interpreting the function of tumor-intrinsic PD-1 signaling becomes even more intriguing following recent discoveries. This signaling controls crucial processes such as tumor growth, survival pathways, stemness, immune effects, and DNA damage. The investigation of the modulatory potential of the interaction between tumor-intrinsic PD-1 and anti-PD-1 antibodies on these effects is of paramount importance.

The discovery of tumor-intrinsic PD-1 heralds a significant leap in grasping the underlying reasons for therapy failures. Therefore, it is vital that upcoming research focuses on meticulously unraveling the intricate molecular and cellular mechanisms tied to intrinsic PD-1 in tumor advancement. Specifically, the focus should be on deciphering the regulatory mechanisms that oversee tumor-intrinsic PD-1, depicting the signaling pathways affected by intrinsic PD-1, and demystifying the complex role intrinsic PD-1 plays in tumor immunity, metabolism, metastasis, and drug resistance. The solutions to these inquiries will not only contribute novel insights but also lay the groundwork for new standards in precision-based treatment for cancer patients.

Abbreviations

$ABCB5^+$	ATP-binding cassette sub-family B member 5^+
AML	Acute myeloid leukemia
APC	Adenomatous polyposis coli
APCs	Antigen-presenting cells
BRCA	Breast cancer
BTICs	Brain tumor-initiating cells
C. tropicalis	*Candida tropicalis*
CDDP	Cisplatin
CESC	Cervical cancer
CR-B	Conserved region B
CR-C	Conserved region C
CRC	Colorectal cancer
CRISPR	Clustered regularly interspaced short palindromic repeats
CSCs	Cancer stem cells
CTLA-4	Cytotoxic T-lymphocyte antigen-4
CTLs	Cytotoxic T lymphocytes
DCs	Dendritic cells
DDR4	Death receptor 4
DFS	Disease-free survival
EC	Extracellular domain
EGFRi	Epidermal growth factor receptor inhibitor
eIF4E	Eukaryotic initiation factor 4E
EMT	Epithelial-mesenchymal transition
En	Enhancer
ERAD	ER-associated degradation
HDACi	Histone deacetylase inhibitors
HNSC	Head and neck squamous cell carcinoma
IC	Cytoplasmic tail
ICB	Immune checkpoint blockade
IFN-γ	Interferon-gamma
IgV	Immunoglobulin variable-type
irAEs	Immune-related adverse events
ITSM	Immunoreceptor tyrosine-based switch motif
KIRC	Kidney renal clear cell carcinoma, LGG, lower grade glioma
LAML	Acute myeloid leukemia
LATS1	Large tumor suppressor kinase 1
LIHC	Liver hepatocellular carcinoma, OV, ovarian cancer
LSKs	$Lin^-Sca\text{-}1^+c\text{-}kit^+$
mAbs	Monoclonal antibodies
MB	Methylene blue
MCC	Merkel cell carcinoma
MDM2	Murine double minute 2
MMIC	Malignant melanoma-initiating cells
mROS	Mitochondrial reactive oxygen species
OS	Overall survival, GBM, Glioblastoma, KIBP, kidney chromophobe
PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand-1
PD-L2	Programmed death ligand-2
PDAC	Pancreatic ductal adenocarcinoma
S6	S6 protein
SARC	Soft tissue sarcoma
SHP2	Src homology 2 domain-containing protein tyrosine phosphatase 2
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
TCR	T cell receptor
TGCT	Testicular germ cell tumor
TM	Transmembrane region
TME	Tumor micro-environment
TNBC	Triple-negative breast cancer
TNF	Tumor necrosis factor, IFNs, interferons
TOX	Mobility group box protein
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
tumor-intrinsic PD-1	Tumor cell-intrinsic PD-1
UBA1	Ubiquitin-like modifier activating enzyme 1
UBE4B	Ubiquitination factor E4B
UCEC	Uterine corpus endometrial carcinoma
UTR	Untranslated region
UVM	Uveal melanoma
YAP	Yes-associated protein
YB-1	Y-box binding protein 1

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Chunlian Wei and Meijun Liu reviewed the literature and wrote the article. Chunlian Wei designed the figures and tables. Weifen Zhang critically revised the manuscript. All authors revised the manuscript and approved its final version.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable. The ethical approval is not required for this study. The authors declare that their participation in writing this review as well as its publication is completely voluntary without afecting their actual research work.

Consent for publication
The authors give permission to publish this work.

Competing interests
The authors declare no competing interests.

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