

REVIEW

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# New insights into epigenetic regulation of resistance to PD-1/PD-L1 blockade cancer immunotherapy: mechanisms and therapeutic opportunities

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## Abstract

Programmed cell death protein 1 (PD-1) is a type of immune-inhibitory checkpoint protein, which delivers inhibitory signals to cytotoxic T cells by binding to the programmed death ligand-1 (PD-L1) displayed on the surface of cancer cells. Antibodies blocking PD-1/PD-L1 interaction have been extensively used in treatment of human malignancies and have achieved promising outcomes in recent years. However, gradual development of resistance to PD-1/PD-L1 blockade has decreased the effectiveness of this immunotherapy in cancer patients. The underlying epigenetic mechanisms need to be elucidated for application of novel strategies overcoming this immunotherapy resistance. Epigenetic aberrations contribute to cancerogenesis by promoting different hallmarks of cancer. Moreover, these alterations may lead to therapy resistance, thereby leading to poor prognosis. Recently, the epigenetic regulatory drugs have been shown to decrease the resistance to PD-1/PD-L1 inhibitors in certain cancer patients. Inhibitors of the non-coding RNAs, DNA methyltransferases, and histone deacetylases combined with PD-1/PD-L1 inhibitors have shown considerable therapeutic efficacy against carcinomas as well as blood cancers. Importantly, DNA methylation-mediated epigenetic silencing can inhibit antigen processing and presentation, which promotes cancerogenesis and aggravates resistance to PD-1/PD-L1 blockade immunotherapy. These observations altogether suggest that the combination of the epigenetic regulatory drugs with PD-1/PD-L1 inhibitors may present potential solution to the resistance caused by monotherapy with PD-1/PD-L1 inhibitors.

**Keywords** PD-1/PD-L1, Immune checkpoints, Therapy resistance, Epigenetic regulation, DNA methyltransferase, Histone deacetylase, Non-coding RNA

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## Introduction

Over the past few years, cancer immunotherapy has been at the forefront of research. A breakthrough in the treatment of advanced stage cancers has been the targeting of immunological checkpoints, particularly the interplay between programmed death 1 (PD-1) and programmed death ligand 1 (PD-L1). PD-1 inhibits T cell activation in the tumor microenvironment by binding to two ligands: PD-L1 and PD-L2 expressed on the surface of tumor cells [1–4]. PD-1/PD-L1 inhibitors can boost T cell effector activity against tumor cells by interfering with their interactions. Multiple solid tumors, including non-small cell lung cancer (NSCLC) [5], melanoma [6], triple negative breast cancer (TNBC) [7], liver cancer [8], and cervical cancer [9], as well as blood cancers like Hodgkin's lymphoma (HL) [10] and NK/T-cell lymphoma [11], have been treated with PD-1/PD-L1 inhibitory drugs. However, drug resistance has gradually developed as a result of the widespread use of PD-1/PD-L1 inhibitors in cancer treatments. A majority of patients developed resistance to PD-1/PD-L1 inhibitors even if they initially showed good response to PD-1/PD-L1 therapy [12].

Among NSCLC patients treated with PD-1 inhibitors, almost half of the patients developed drug resistance [13]. The development of NSCLC was shown to be associated with genomic instability promoted by alterations in DNA methylation patterns [14, 15]. Among melanoma patients with resistance to PD-1 antibodies, the therapeutic outcomes were reported to be associated with the expression levels of certain non-coding RNAs [16]. In NSCLC, for instance, it was demonstrated that p53 down-regulates PD-L1 via miR-34, which could serve as a predictive biomarker for PD-1 inhibitor immunotherapy [17, 18]. Histone deacetylase 6 (HDAC6), which has the potential to be a predictive biomarker for melanoma and ovarian cancer [19, 20], also regulates epigenetic resistance to PD-1 immunotherapy in melanoma patients. Numerous studies on the epigenetic modulation of the PD-1/PD-L1 immune checkpoints have shown that a variety of epigenetic inheritance mechanisms play a significant role in the interaction between epigenetic and immune modulation [21, 22]. Here, we summarized the potential clinical benefits of epigenetic regulation on reversing resistance to PD-1/PD-L1 blockade for patients with cancers.

## Epigenetic therapies in cancer

The term 'epigenetics' defines all meiotically and mitotically heritable changes in gene expression that are not coded in the DNA sequence itself. Three epigenetic mechanisms, that is, DNA methylations, histone modifications, and non-coding RNAs, were shown to initiate and sustain epigenetic silencing [23]. Recently, numerous studies have suggested that epigenetic alterations play critical roles in a wide range of cancer types [24, 25].

Cancer-associated epigenetic alterations in tumors reveal potentially reversible targets for existing drugs, and for an increasing repertoire of new drugs [26, 27].

## The types of epigenetic aberrations in cancer

Methylation of the C5 position of cytosine bases in the context of CpG dinucleotides in DNA has long been recognized as an epigenetic silencing mechanism of fundamental importance [28]. The methylation of CpG sites within the human genome is generated or maintained by a number of DNA methyltransferases (DNMTs) that have multifaceted roles, including silencing of transposable elements, defense against viral sequences, and transcriptional repression of certain genes [29]. Aberrant methylation of CpG islands is a hallmark of human cancers and is observed early during carcinogenesis [30]. The aberrant silencing of genes, including tumor-suppressor genes, are linked to focal increases in methylation in promoter-associated CpG islands [31].

Post-translational histone modifications have also been defined as epigenetic processes playing critical roles in cancer development [32]. In general, hyperacetylation of histones activates transcription of genes, whereas hypoacetylation of them inactivates transcription [33]. Histone deacetylases (HDACs) regulate biological processes, which include autophagy, DNA damage repair, metabolism, apoptosis, senescence, and cell cycle control [34, 35, 36]. This transcriptional modulation regulates the expression of tumor suppressor, antigen-processing and presentation machinery, and tumor antigen genes that were silenced during tumorigenesis in cancer cells [37].

The role of non-coding RNAs in post-transcriptional silencing has attracted much interest. However, long noncoding RNAs (lncRNAs), in particular antisense transcripts, can also lead to mitotically heritable transcriptional silencing by the formation of heterochromatin [38, 39]. Therefore, lncRNAs might be a key trigger to direct histone modifications and DNA methylations to specific genomic loci, thereby leading to heritable and stable silencing of genes [40, 41]. The therapeutic activation of abnormally silenced genes thus requires drugs that can target the multifaceted changes. Besides, except for lncRNAs and miRNAs, circular RNAs (circRNAs) have also been identified to regulate the PD-1/PD-L1 pathway and thus participate in immune response and immunotherapy.

## Epigenetic drugs used for cancer treatment

Epigenetic alterations have fundamental roles in cancer progression characterized by reversibility and susceptibility to external factors. They are emerging as promising targets for cancer therapies. Typically, transcriptional repression is linked to the actions of DNMTs and HDACs [42]. Thus, drugs targeting these proteins can augment

expression of involved genes, with many consequences for pathways downstream of this gene activation. When incorporated into DNA, DNMT inhibitors (DNMTi) act as cytidine analogues that block the catalytic actions of DNMTs, thereby triggering DNA demethylation [43, 44]. Cytosine methylation at CpG dinucleotides in DNA varies significantly in almost all cancers [45, 46]. Tumor suppressor genes were reactivated by DNMTis, which induced expression of genes that were silenced by promoter DNA methylation [47]. Multiple types of tumor cells were temporarily exposed to low doses of DNMTis, which resulted in marginal changes in apoptosis or cell cycle, and diminished cancer stem cell functions [48]. Due to promising clinical efficacy of DNMTis, such as 5-azacytidine and 5-aza-2-deoxycytidine (decitabine) for treating hematologic neoplasms, these DNMTi drugs were approved by FDA for treatment of myelodysplastic syndrome, a benign neoplasm, precursor of leukemia and acute myeloid leukemia [49].

For the treatment of cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma, HDAC inhibitors (HDACi) have been approved [50, 51, 52]. HDACi have pleiotropic effects, often dose and compound dependent. Some of them affect the acetylation status of non-histone and/or non-nuclear proteins or cause off-target effects [53], while others clearly cause epigenetic alterations and affect histone acetylation [54, 55]. Given the importance of epigenetic regulation in different cancer types, it is not surprising that the epigenetic targeting is becoming an attractive treatment strategy in cancer therapy. Epigenetic treatment may therefore benefit cancer patients as monotherapy or combination [56].

#### **Resistance to PD-1/PD-L1 immunotherapy is epigenetically regulated**

PD-1 is expressed not only in different kinds of tumor-infiltrating lymphocytes but also in some cancer tissues [57]. PD-1/PD-L1 interaction inhibits immune-surveillance against cancer cells through elimination of specific or non-specific immune responses that eliminate tumor cells [58]. Globally, dozens of PD-1 or PD-L1 inhibitors were approved for marketing as they yielded good clinical responses [59–62]. However, a number of recent studies have shown that anti-PD-1/PD-L1 resistance is linked to poor drug responses in some cancer patients [63, 64]. These findings underscore the need for new approaches to combating anti-PD-1/PD-L1 resistance as well as a deeper comprehension of the underlying mechanisms. The total response rate of anti-PD-1/PD-L1 treatment for cancers is less than 20% [65].

Through inducing IFN- $\gamma$  activity triggered by tumor infiltrating lymphocytes (TILs), cancer cells alter PD-L1 expression [66]. DNA methylation and enhancer of zeste homolog 2 (EZH2) activity were found to induce PD-1

resistance in melanoma by inhibiting IFN- $\gamma$  transcription and the RAS and PI3K pathways' subsequent functions. The IFN- response was triggered by hypermethylation of PD-L1-regulating genes, limiting the activities of PD-1/PD-L1 blockade. On the other hand, cytotoxic T cell exhaustion is largely caused by DNA methylation. These methylation changes do have specific mechanisms which are still unclear [70].

Epigenetic processes other than DNA methylation were also reported to be associated with the resistance to PD-1 inhibitors. Modification by N6-methyladenosine (m6A) of RNA is a kind of reversible regulation. The m6A was found in most mRNA of eukaryotic cells [71]. For the melanoma cells, the fat mass and obesity-associated protein (FTO) is the first m6A demethylase identified [73]. YTH521-B homology (YTH) domain family (YTHDF) proteins tend to accelerate the metabolism of mRNAs modified by m6A. The increasing FTO level decreases m6A modification of PD-1 and degradation of RNA induced by YTHDF so that the tumorigenic cells grow faster. Knocking-out FTO in the melanoma cells through in vitro experiments sensitized to IFN- $\gamma$ , and increased the immune response of PD-1 inhibitor in mice melanomas. These data showed that the regulation of m6A is a potential way to eliminate the resistance to anti-PD-1 therapy [73].

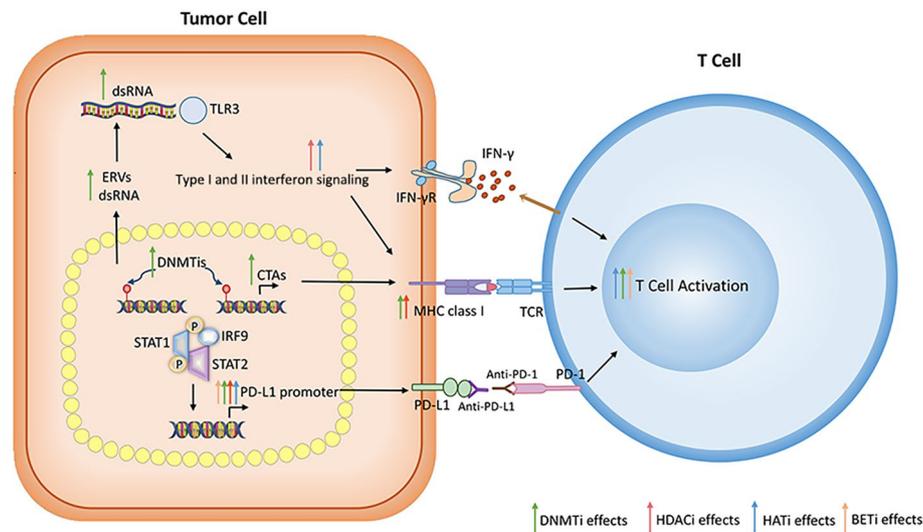
#### **The influence of epigenetic regulatory drugs on PD-1/PD-L1 immunotherapy resistance**

Given that there is an association between epigenetic regulation and cancer development, drugs targeting epigenetic alterations are widely used in clinical trials in combination with PD-1/PD-L1 inhibitors. The following is a list of the most frequently used epigenetic regulatory drugs: BET inhibitors, histone methyltransferase inhibitors, DNA methyltransferase inhibitors, IDH2 or TET2 inhibitors, and histone acetyltransferase inhibitors. Figure 1 demonstrates the effects of epigenetic regulatory drugs on PD-1/PD-L1 immunotherapy.

##### **DNA methyltransferase inhibitors**

DNA methylation in genomes of mammalian cells is usually generated through addition of methyl group of S-adenosylmethionine (SAM) to the upstream of promoter or cytosine residues within CpG (5'-C-phosphate-G-3') islands at the 5' end by DNA methyltransferases (DNMT) [74]. Genome-wide alterations in methylation levels are frequently observed in many malignant tumors with hypermethyations, especially in promoter-associated CpG islands and hypomethyations in rest of the genome [75–77].

DNA methyltransferase inhibitors (i.e., azacitidine and decitabine [5-aza-2'-deoxycytidine]) are used in the treatment of certain blood cancers [78, 79, 80]. Of note,



**Fig. 1** Epigenetic regulatory drugs promote anti-tumor immune signaling to synergize with PD1/PD-L1 immunotherapy

decitabine was reported to increase the response rate to paclitaxel/carboplatin chemotherapy even when it is used in low-doses (7 mg/m<sup>2</sup>/day), which has more than 70% of disease control rate (DCR) in 55 patients with platinum-resistance. Indeed, the combination of cytokine-induced killer (CIK) cell immunotherapy with decitabine almost completely reversed resistance (DCR is 100%), indicating that the efficacy of immunotherapy in cancer treatment may be significantly enhanced by decitabine [81].

The combination of decitabine and PD-1 inhibitor was also evaluated in a Phase 2 trial for classical Hodgkin lymphoma (cHL) [84]. Generally, the objective response rate (ORR) for anti-PD-1 monotherapy of cancer is no more than 50%, and the complete rate (CR) is less than 10% [83]. Although patients with cHL had a high ORR of 80–90% in response to PD-1 inhibitor, CR was still no more than 20% [84]. In a clinical trial with 120 refractory classical Hodgkin lymphoma patients, addition of low-dose decitabine (10 mg/d) to anti-PD-1 antibody camrelizumab yielded an ORR of 95%, and a CR of 71% which was twice superior compared to PD-1 inhibitor monotherapy. Even in patients with anti-PD-1 monotherapy resistance, ORR and CR were estimated to be 62% and CR 28%, respectively, with decitabine plus camrelizumab. The reasons for such improvements observed in therapeutic efficacy were interpreted as the ability of decitabine in increasing sensitivity to PD-1 inhibitors [84, 85].

#### Histone deacetylase inhibitors

Post-translational acetylation of histones is highly dynamic and regulated by the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) [87]. Currently, four HDAC inhibitors have been

approved by FDA: vorinostat, romidepsin, belinostat, and panobinostat. HDAC inhibitors alter RNA polymerase II-driven transcription through acute hyperacetylation of histones and epigenetic regulators at the chromatin interface [33]. This transcriptional modulation includes the re-expression of genes that were silenced during tumorigenesis in cancer cells, including tumor antigens, antigen-processing and presentation machinery genes, and tumor suppressor genes [38]. These HDAC inhibitors were approved by FDA for treatment of different types of lymphoid malignancies: (1) vorinostat and romidepsin for cutaneous T-cell lymphomas [88]; (2) romidepsin and belinostat for peripheral T-cell lymphomas [89]; and (3) panobinostat in combination with dexamethasone for the treatment of multiple myeloma [90].

The anti-PD-1 immunotherapy was considered as a potential way to improve clinical outcomes of Hodgkin lymphoma and multiple myeloma cases because PD-L1 was shown to be highly expressed in tumor cells of these patients [91, 92]. Clinical trials have also been conducted for multiple myeloma and certain other B cell malignancies in some countries [93, 94]; however, the therapeutic efficacy of monotherapy was not satisfactory [95]. In vitro experiments have revealed that HDACs might have an adjuvant function in modulation of sensitivity of the PD-1/PD-L1 signaling [98]. Hence, it is logical to use these two different types of drugs for more effective cancer treatment. Clinical trials involving a combination of PD-1/PD-L1 and HDAC inhibitors were carried out in some cancer types such as Merkel cell carcinoma (MCC). In 2019, four MCC patients with no response to anti-PD-1 monotherapy received a combination of panobinostat and anti-PD-1 therapy, and the results showed that HDACi may increase the effectiveness of anti-PD-1/

**Table 1** Ongoing clinical trials evaluating the combinatory use of epigenetic drugs and PD-1/PD-L1 immunotherapy

Trial number	Official title	Indication	Study Type
NCT04722952	PD-1 inhibitor combined with azacytidine and homoharringtonine, cytarabine, G-CSF for refractory or relapsed AML	Leukemia, AML	Phase 3
NCT04514081	The clinical trial of chidamide + decitabine + camrelizumab versus decitabine + camrelizumab in anti-PD-1 antibody resistant patients with classical hodgkin lymphoma	Hodgkin lymphoma	Phase 2
NCT04510610	Camrelizumab plus decitabine in anti-PD-1 treatment-naive patients with relapsed/refractory classical hodgkin lymphoma	Hodgkin lymphoma	Phase 2 Phase 3
NCT03250962	SHR-1210 alone or in combination with decitabine in relapsed or refractory Hodgkin lymphoma	Hodgkin lymphoma	Phase 2
NCT04353479	PD-1 inhibitor and decitabine combination in elderly patients with relapse and refractory acute myeloid leukemia	Acute myeloid leukemia	Phase 2
NCT04651127	Anti-PD-1 antibody combined with histone deacetylase inhibitor in patients with advanced cervical cancer	Cervical cancer	Phase 1 Phase 2
NCT04512534	Sintilimab combined with chidamide in treating peripheral T cell lymphoma	Peripheral T-cell lymphoma	Phase 2
NCT02936752	Testing the safety and efficacy of the combination of the antibody pembrolizumab and entinostat in patients with myelodysplastic syndrome who are not responding to hypomethylating agents	Myelodysplastic syndrome	Phase 1
NCT04514081	The clinical trial of chidamide + decitabine + camrelizumab versus decitabine + camrelizumab in anti-PD-1 antibody resistant patients with classical hodgkin lymphoma.	Hodgkin lymphoma	Phase 2
NCT04038411	PD-1 antibody, chidamide, lenalidomide, and etoposide for relapsed or refractory NK/T Cell lymphoma	NK/T cell lymphoma	Phase 4
NCT04040491	PD-1 antibody, chidamide, lenalidomide and gemcitabine for peripheral T-cell lymphoma	Peripheral T-cell lymphoma	Phase 4
NCT03993626	A trial of CXD101 in combination with nivolumab in patients with metastatic microsatellite-stable colorectal cancer (CAROSELL)	Malignant colorectal neoplasms	Phase 1 Phase 2
NCT03765229	An exploratory study of pembrolizumab plus entinostat in non-inflamed stage III/IV melanoma	Melanoma	Phase 2
NCT01928576	Phase II anti-PD1 epigenetic therapy study in NSCLC (NA_00084192)	Non-small cell lung cancer	Phase 2
NCT04708470	Phase I/II trial of the combination of bintrafusp alfa (M7824), entinostat, and NHS-IL12 (M9241) in patients with advanced cancer	Metastatic solid tumor	Phase 1 Phase 2
NCT03250273	A clinical trial of entinostat in combination with nivolumab for patients with previously treated unresectable or metastatic cholangiocarcinoma and pancreatic adenocarcinoma	Pancreatic cancer	Phase 2
NCT03161223	Phase 1/2a study of anti-PD-L1 monoclonal antibody durvalumab in combination with pralatrexate and romidepsin, oral 5-aza and romidepsin, romidepsin alone, or oral 5-azacitidine for treatment of patients with relapsed and refractory PTCL	T-cell lymphoma	Phase 1 Phase 2

PD-L1 therapy [99]. Currently, multiple clinical trials including combination of these drugs are carried out in the USA for different cancer types (Table 1).

### Histone methyltransferase inhibitors

Histone methyltransferase (HMT) enzymes methylate residues on specific lysine residues of histones, which activate or repress transcription in a very residue and methyl group number-specific manner. Enhancer of zeste homologue 2 (EZH2), SET domain bifurcated 1 (SETDB1), and disruptor of telomeric silencing 1-like (DOT1L), all of which produce silencing marks, have been utilized as therapeutic targets. As a result, similar to the effects of DNMTis and HDACis, inhibiting these HMTs opens up chromatin structure and activates gene expression. Only small molecule inhibitors of EZH2 have currently been approved for clinical interventions [100].

Melanoma suffers from decreased immunogenicity and loss of antigen presentation when the expression

of EZH2 is increased. Addition of an EZH2 inhibitor to anti-CTLA-4 or IL-2 treatment reversed many of above immunosuppressive effects and significantly improved immune therapy in preclinical models [101]. Zhou et al. identified EZH2 as a therapeutic target for enhancing tumor cell antigen presentation and subsequently decreasing resistance to anti-PD-1 therapy in HNSCC [102].

A histone H3 lysine 79 (H3K79) methyltransferase called DOT1L is recruited by aberrant fusion proteins that are part of the mixed-lineage leukemia (MLL) HMT. This creates a permissive chromatin state that makes it easier for HATs and BRD4 to promote leukemia through driving transcription in an abnormal way. Pharmacological inhibition or genetic depletion of DOT1L can alleviate H3K79 methylation at the promoters of pro-inflammatory cytokines such as IL-6 and IFN- $\beta$ , which is reported to be mediated by DOT1L [103]. Emilie et al. found that the combination of histone deacetylase inhibitor (SAHA)

with DOT1L inhibitors (EPZ5676 or SGC0946) or BET bromodomain inhibitor (PFI-1) were efficient to partially reverse TGF- $\beta$ 1 effects by decreasing PD-L1 expression, suggesting that combination of epigenetic compounds might enhance clinical responses to PD-L1 [104].

### BET inhibitors

Functionally associated with transcriptional co-activators, bromodomain and extra-terminal (BET) proteins positively regulate RNA Pol II-dependent transcription at active enhancer and promoter regions. In a wide range of genetically diverse solid and hematological cancers, BET inhibitors have demonstrated broad efficacy. Suppressing Pol II-driven oncogenic transcription, particularly MYC and MYC-dependent transcriptional programs in hematological malignancies, is where BET inhibitors exert their anti-tumor effects [105].

In genetically diverse tumor models, a number of researchers have demonstrated that promoter- and enhancer-bound BET proteins are necessary for the transcription of immune checkpoint ligands PD-L1 and PD-L2. In addition, ectopic expression of PD-L1 in lymphoma was sufficient to reduce the efficacy of a BET inhibitor (i.e., JQ1) *in vivo*. Importantly, IRF1-driven PD-L1 expression induced by IFN- $\gamma$  was also suppressed by BET inhibition, which is known to be an adaptive immune evasion mechanism. Changes in MYC expression, which may regulate PD-L1 in particular cellular contexts, had no effect on BET inhibition's ability to suppress PD-L1 [106].

BET inhibitors have been shown to improve the efficacy of cancer immunotherapies in recent preclinical studies. In the context of MYC-driven B cell lymphoma, BET inhibitors exhibited improved activity when treated with anti-PD-1 and agonistic anti-CD137 (4-1BB) [107]. JQ1 increased the effectiveness of anti-PD-1 therapy in KRAS-driven NSCLC, which was linked to less CD4<sup>+</sup>FOXP3<sup>+</sup> regulatory T (Treg) cell infiltration [108]. These studies, taken together, suggest that BET bromodomain inhibitors activate the immune system of the host, which could be used to boost immune responses against tumors.

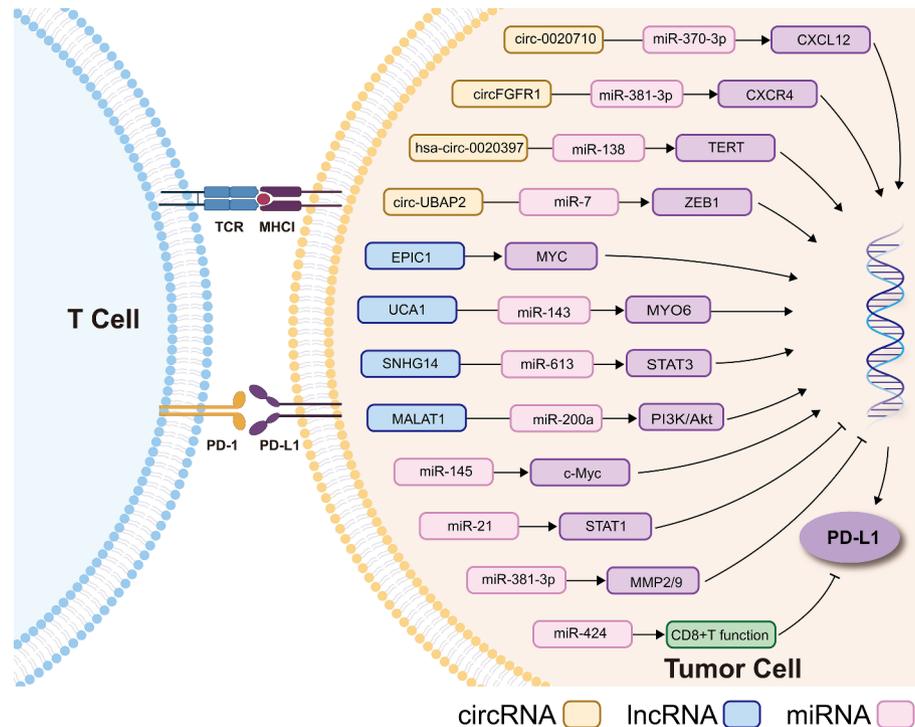
### Non-coding RNAs

Recently, non-coding RNAs have been observed to play important roles in tumor immunity [109, 110]. Non-coding RNAs are a special class of functional RNAs including microRNAs (miRNA) and long non-coding RNAs (lncRNA), which play critical roles in initiation and development of tumors [111]. miRNAs secreted by tumor cells results in immunosuppression during cancer development [112]. miR-21, miR-146, and miR-195 were all reported to promote immunity through toll-like receptor (TLR) signaling [113]. Some miRNAs such as miR-21 are

regulated by the CSF1-ETS2 (V-ets erythroblastosis virus E26 oncogene homolog 2) pathway, which promotes tumorigenesis and angiogenesis due to M2 reprogramming of the tumor infiltrating myeloid cells [114].

Cancer cells have multiple immune escape mechanisms to evade T-cell responses, with PD-1 pathway being a classical example. Bioinformatics analyses showed that several key lncRNAs contribute to immune escape. An important lncRNA, EPIC1, was shown to enhance methylation of the 27th amino acid in histone H3 (H3K27) by binding the enhancer of zeste homolog 2 (EZH2) protein, leading to low transcript level of IFN- $\gamma$  and inhibition of IFN-JAK-STAT1 signal pathway. Cancer cells with EPIC1 overexpression have a strong resistance to PD-1 blocking treatment [115]. According to the tight relationship between non-coding RNAs and resistance to PD-1 blockade, miR/PD-1 and lncRNA/PD-1 signaling pathways have been investigated as targets for potential cancer immunotherapy drugs. The compounds downregulating the miRNAs and lncRNAs targeting PD-1/PD-L1 axis may be candidates for new inhibitors [116].

Circular RNAs (circRNAs) were firstly found as single-stranded covalently closed circular RNA molecules structures of viroids [117]. The major functions of circRNAs include regulation of gene transcription, protein binding, serving as templates for protein translation, or miRNA sponge through which binding sites for miRNAs are supplied [118]. The expression of circRNAs was observed in different diseases and dysregulated expression may affect tumor occurrence and development [119, 120]. It has been shown that circRNAs play an important role in cancer immunology by increasing surface PD-L1 expression [121]. For example, in melanoma cells circ-0020710 binds more competitively to miR-370-3p than chemokine (C-X-C motif) ligand 12 (CXCL12) as miRNA sponge. Accumulation of CXCL12 can then recruit more immune suppressor cells which results in cytotoxic lymphocyte (CTL) exhaustion, and introduce the formation of immunosuppressive microenvironment which contributes to the resistance of PD-1/PD-L1 pathway blockade. Therefore, inhibition of circ-0020710 or CXCL12 increases efficacy of cancer treatment when combined with PD-1 inhibitors [122]. Furthermore, the expression of PD-1 in T cells and PD-L1 in cancer cells are found to be affected by multiple circRNAs, which also work on the development of resistance to PD-1/PD-L1 blockade. For PD-1 expression level in the T cells, circRNAs mostly act through miRNA sponging and influence downstream reaction of related mRNAs [123–125]. On the other hand, mainly through miRNA sponge function, alteration of circRNAs would affect the PD-L1 expression in cancer cells [126–131]. Figure 2 summarizes the relationship between non-coding RNAs and PD-1/PD-L1 axis in cancer cells.



**Fig. 2** The regulation of PD-1/PD-L1 axis via non-coding RNAs

### Future directions and conclusions

PD-1/PD-L1 immunotherapy has proven to be an exciting and productive area of research for treatment of malignancies; however, this type of therapy only shows long-term efficacy in a minority of patients. The technologies and drugs related to epigenetic regulation have great potential for improving the clinical efficacy of PD-1/PD-L1 immunotherapy. DNMTi, HDACi, and inhibitors of non-coding RNAs altogether are promising therapeutic agents to reverse resistance to PD-1/PD-L1 blockade in a wide variety of cancers. We eagerly await results of ongoing clinical trials summarized in Table 1, which will provide information on the safety, efficacy, and potential biomarkers for these combinatory treatments. Utilizing epigenetic therapies to eliminate PD-1/PD-L1 inhibitor resistance may prove to be a safe and effective way for treating multiple types of cancer. Although combination with epigenetic drugs and inhibitors of PD-1/PD-L1 showed promising results on cancer treatment, the mechanism for such increased efficacy remains elusive, and further clinical studies are needed for elucidation of the optimal treatment conditions for the patients. The resistance induced by PD-L1 expression was regulated by two different mechanisms in cancer cells: primary resistance (intrinsic resistance) and acquired resistance. In current studies, epigenetic regulatory drugs including DNA methyltransferase inhibitors, histone deacetylase inhibitors, histone methyltransferase inhibitors, BET inhibitors, and non-coding RNAs can affect the

oncogenic signaling in the cancer cells, and the resistance induced by those belongs to the primary resistance [80, 88, 104, 107]. On the other hand, some histone methyltransferase drugs, BET inhibitors and non-coding RNAs can also trigger TIL changes regulated by IFN- $\gamma$  that is responsible for PD-L1 expression on immune system cells and cancer cells which reflects the mechanisms of acquired resistance [107, 116].

### Abbreviations

PD-1	Programmed cell death protein 1
PD-L1	Programmed death ligand-1
NSCLC	Non-small cell lung cancer
TNBC	Triple negative breast cancer
HL	Hodgkin's lymphoma
HDAC6	Histone deacetylase 6
DNMTs	DNA methyltransferases
HDACs	Histone deacetylases
lncRNAs	Long noncoding RNAs
DNMTi	DNA methyltransferases inhibitors
CTCL	Cutaneous T-cell lymphoma
TILs	Tumor infiltrating lymphocytes
EZH2	Enhancer of zeste homolog 2
SAM	S-adenosylmethionine
CIK	Cytokine-induced killer
DCR	Disease control rate
ORR	Objective response rate
CR	complete rate
HATs	Histone acetyltransferases
MCC	Merkel cell carcinoma
HMT	Histone methyltransferase
SETDB1	SET domain bifurcated 1
MLL	Mixed-lineage leukemia
BET	Bromodomain and extra-terminal
miRNA	microRNAs
TLR	Toll-like receptor

TAM	Tumor-associated macrophage
CTA	Cancer testis antigens
dsRNA	Double-stranded RNA
ERVs	Endogenous retroviruses
IFN- $\gamma$	Interferon gamma
HDACi	Histone deacetylase inhibitors
HATI	Histone acetyltransferase inhibitors
BETi	BET inhibitors

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### Author contribution

Mengyuan Dai, Miao Liu, Can K uc k, Hua Yang and Hua You conceived the hypothesis, did the literature search, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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### Data availability

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### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

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#### Competing interests

The authors declare that they have no competing interests.

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