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Cancer Epigenetics, Tumor Immunity, and Immunotherapy

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Abstract

Epigenetic mechanisms, including DNA methylation, histone post-translational modifications, and chromatin structure regulation, are critical for the interactions between tumor and immune cells. Emerging evidence shows that tumors commonly hijack various epigenetic mechanisms to escape immune restriction. As a result, the pharmaceutical modulation of epigenetic regulators, including ‘writers’, ‘readers’, ‘erasers’, and ‘remodelers’, is able to normalize the impaired immunosurveillance and/or trigger antitumor immune responses. Thus, epigenetic targeting agents are attractive immunomodulatory drugs and will have major impacts on immuno-oncology. Here, we discuss epigenetic regulators of the cancer–immunity cycle and current advances in developing epigenetic therapies to boost anticancer immune responses, either alone or in combination with current immunotherapies.

Epigenetics and Cancer

Epigenetics is defined as the DNA sequence-independent inheritance of phenotype or gene expression [1]. By modulating which, when, and where genes are expressed, epigenetic machinery determines cell fates during differentiation and maintains cell identities during and after cell division [2]. There are four major mechanisms of epigenetic regulation: DNA methylation, histone post-translational modifications, chromatin structure regulation, and noncoding RNA regulation [1]. Here, we mainly focus on the first three mechanisms, which are chromatin-based mechanisms (Figure 1). These epigenetic mechanisms usually work in a coordinated manner to provide precise and durable gene regulation. DNA and histone marks and chromatin structure are dynamically regulated by four classes of epigenetic regulators. These regulators are commonly known as ‘writers’, which add the epigenetic marks; ‘erasers’, which remove the epigenetic marks; ‘readers’, which recognize specific epigenetic marks to mediate downstream effects; and ‘remodelers’, which modulate

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chromatin status (Figure 1). There are ~1000 epigenetic regulators in mammals, forming one of the largest protein groups (Table 1).

Epigenetic features are commonly dysregulated in cancer. Genome-wide DNA hypomethylation in cancer cells was first observed during the 1980s, while tumor suppressor genes are usually silenced by DNA hypermethylation at their promoters [3]. Similarly, loss of histone H4K16 acetylation and H4K20 trimethylation was reported to be a common hallmark of human cancer [4]. One of the surprising findings from cancer genome-sequencing studies is the high rate of alterations in many epigenetic regulator genes [5], such as loss-of-function mutations of genes encoding the SWI/SNF chromatin remodeling complex in ~20% of all cancers [6].

The accumulation of genetic and epigenetic alterations is a key characteristic of cancer cells [7]. Some genetic mutations create neoantigens, while epigenetic alterations may lead to the reactivation of genes, the expression of which is normally limited to immune-privileged stages or organs, such as cancer/testis antigens (CTAs) [8]. Both tumor neoantigens and autoantigens can be immunogenic [9].

Recent studies revealed that epigenetic regulation is critical for anticancer immune response and the evasion of immunosurveillance by tumor cells, nominating epigenetic targeting agents a new category of immune modulators. In this review, we will discuss the impact of epigenetic regulation on the interactions between tumor cells and immune cells and the emerging strategies to target the epigenetic machinery to boost anti-tumor immune responses.

Cancer–Immunity Cycle and Cancer Immunotherapy

The human immune system should theoretically be capable of eradicating cancer cells through an acquired immune response executed by T cells. A series of stepwise events, called the ‘cancer–immunity cycle’, is required for tumor cell clearance by the immune system [10] (Figure 2). This self-amplifying process supposedly will end with complete clearance of nascent tumors. However, clinically detectable tumors often develop due to failed immunosurveillance through various mechanisms. For example, dendritic cell (DC)-mediated T cell priming and activation can be prevented by the lack of DC cells, DC-suppressive mechanisms, or activation of an immune check-point, such as CTLA-4 [11,12]. Lack of proper chemokines and an immunosuppressive tumor microenvironment (TME) may block the migration or infiltration of T cells into tumor tissue [13,14]. Even if tumor antigen-specific T cells infiltrate tumor tissue, their tumor-killing activity can be blocked by regulatory cells in the TME, such as regulatory T cells, macrophages, myeloid-derived suppressor cells, and cancer-associated fibroblasts, or by activation of immune checkpoints, such as PD-L1, on tumor cells or macrophages [15].

An understanding of these immune escape mechanisms has provided therapeutic opportunities by lifting immune suppression and restoring antitumor immune responses. For example, the discovery of immune checkpoint-mediated immune suppression led to the development of immune checkpoint blockade therapies (ICBTs). Antibody-based therapies targeting CTLA-4, PD-1, or PD-L1 have achieved lasting responses in some patients against

a range of cancer types, especially those with considerable immunogenicity [16–18]. The success of ICBTs is, arguably, the most significant advance in cancer treatment over the past decade.

Despite the long-term efficacy of ICBTs for some patients, there are many patients who do not benefit from this advanced treatment for three major reasons. First, many cancers do not have strong immunogenicity, such as tumors from breast, prostate, glioblastoma, and pancreatic adenocarcinoma. The estimated percentage of patients with cancer who are eligible for any of the approved ICBTs is still <50% in the USA [19]. Second, not all patients with immunogenic tumor types respond to the treatment, due to tumor cell-intrinsic and extrinsic mechanisms [20]. A pan-cancer overall response rate is estimated to be ~25% [19]. Third, acquired resistance appears in some patients. This occurs through mechanisms that are induced or selected by the ICBTs, such as loss of antigens, inactivation of antigen-presenting machinery, desensitization to immune attack, and alternative immune suppressive pathways [20]. Intensive investigations have focused on expanding the application of current ICBTs and improve the response rate. Given that epigenetic regulation has important roles in antitumor immune responses, combining ICBTs with epigenetic drugs (epidrugs) could sensitize less-immunogenic tumors and prevent both primary and acquired resistance.

Impact of Epigenetics on the Cancer–Immunity Cycle

Epigenetic mechanisms are critical for many processes in the cancer–immunity cycle (Figure 3). Here, we discuss their impacts on these specific processes, either in tumor or immune cells.

In Tumor Cells: Generation of Tumor Antigens

Deamination of 5-methyl-cytosine, either spontaneous or mutagen triggered, results in CNT transitions. Signature analysis revealed that DNA methylation-associated mutagenesis is the single most important source of genetic alterations, leading to neoantigen formation in most cancers [21]. CTAs are encoded by a group of genes, the expression of which is limited to male germ cells in healthy conditions [9]. However, demethylation of CpGs associated with these genes, as well as other epigenetic dysregulation, can cause CTA-coding genes to escape epigenetic silencing and re-express in tumors [22]. As a regenerative organ, the testis has an immune-privileged status [9]. Thus, when the protein products of these gametogenic genes are reactivated in tumor tissues, which are not immune privileged, they are capable of inducing an acquired immune response [22]. Similarly, the dysregulated epigenetic program in tumors can result in the reactivation of developmentally restricted genes, providing tumor differentiation antigens [23].

In Tumor Cells: Cytokine Production

Proinflammatory cytokines are required by effector T cells to enter the TME and execute an immune attack on tumor cells. Recent studies showed a strong connection between epigenetics and cytokine production in tumor cells. One such example is ‘viral mimicry’ as the result of DNA methyltransferase (DNMT) inhibition. Endogenous retroviruses (ERVs) represent >8% of the human genome but are predominantly silenced. DNA methylation is

the major mechanism maintaining ERV silencing, and DNA demethylation in ERV promoters restores expression of ERV RNAs. ERV transcripts are mostly nonfunctional themselves. However, these transcripts can trigger the pattern-recognition receptor MDA5, which normally senses viral infection by recognizing viral double-strand (ds) RNAs. MDA5 induces signaling cascades that result in the secretion of type I interferon and eventually immune cell-induced killing. Thus, DNMT inhibitor (DNMTi) treatment tricks cancer cells into a 'viral mimicry' state, in which they behave as virus-infected cells, leading to activation of the interferon pathway. These changes were shown to enhance the effectiveness of immune checkpoint inhibitors [24,25]. Further studies revealed that histone deacetylases (HDACs) and KDM1A (LSD1), an 'eraser' of H3K4me1/2, also have similar roles in the suppression of ERVs and ERV-induced activation of the interferon pathway [26,27].

Similar to the dsRNA sensor MDA5, cyclic GMP-AMP synthase (cGAS) detects the abnormal presence of dsDNA in the cytosol, which signals infection or DNA damage [28]. cGAS then activates STING to trigger an innate immune response, especially the expression and secretion of cytokines [28]. Thus, STING agonists have been suggested as the next generation of immune-therapy agents [29]. However, robust activation of the STING pathway requires not only STING activation, but also sufficient STING protein to mediate the signaling cascade. The STING pathway is disrupted or epigenetically silenced in many tumors, enabling cancer cells to evade immunosurveillance [30]. It was found that the histone H3K4 demethylases KDM5B (JARID1B) and KDM5C (JARID1C) bind to the STING promoter and block the interferon response induced by cytosolic DNA in breast cancer cells [31]. Treatment with KDM5 inhibitors (KDM5i) induced STING expression and triggered a robust interferon response in a cytosolic DNA-dependent manner in breast cancer cells. These findings demonstrate that KDM5i act as STING inducers, representing a potential new class of cancer immunotherapeutic drugs, especially in tumors with low expression levels of STING.

Epigenetic enzymes also regulate interferons, cytokines, and chemokines through mechanisms other than through MDA5 or STING. For example, both DNMT and KMT6A (EZH2) directly suppress the expression of Th1-type chemokines, such as CXCL9 and CXCL10 [32], which are critical for T cell recruitment and infiltration. Counterintuitively, KDM6B (JMJD3), the methyltransferase with the counter role of KMT6A (EZH2), also suppresses chemokine expression [32]. Another methyltransferase, KMT3A, is required for the interferon pathway by catalyzing the methylation of STAT1, a key transcription factor of the interferon response [33].

In Tumor Cells: Tumor Antigen Presentation

Epigenetics contributes to the dysregulation of antigen-presenting machinery in tumor cells, which enables tumor cells to become invisible to T cells. To present self- and tumor-specific peptides to CD8 T cells, proteins in tumor cells need to be digested by the proteasome to generate short oligopeptides. Transporter associated with antigen processing 1 and 2 (TAP1 and TAP2) form a heterodimer to transport these peptides from the cytosol to the endoplasmic reticulum (ER). In the ER, antigen peptides are loaded onto nascent MHC-I molecules with the assistance of chaperone proteins. Antigen-loaded MHC-I, which consists

of two polypeptide chains, human leukocyte antigens (HLA) and β2-microglobulin (B2M), is then delivered to the cell surface for display [34].

DNMT and HDAC both suppress MHC-I expression in tumor cells, evidenced by the re-expression of MHC-I after treating cells with DNMTi and HDACi [35,36]. In some cases, loss of MHC-I is caused by the epigenetic silencing of other genes involved in the antigen-presenting machinery, such as B2M, TAP-1, and TAP-2 [37,38]. Treating tumor cells and patients with DNMTi led to the increased expression of genes required for antigen presentation [37]. Interestingly, the deacetylation of histones was also responsible for the downregulation of MHC-I in devil facial tumor, an unusual disease that can transmit between Tasmanian devils as an infectious cell line [39].

In Tumor Cells: PD-L1 Expression

PD-L1 binds to the immune checkpoint receptor PD-1 on T cells, leading to the suppression of T cell proliferation, cytokine production, and cytotoxic activity, a phenotype described as ‘T cell exhaustion’ [40]. The upregulation of PD-L1 in some tumors is likely a result of selection caused by T cell immune responses. Epigenetic mechanisms certainly contribute to the regulation of PD-L1 expression. For example, in multiple cancers, methylation of the PD-L1 promotor was found to be negatively correlated with PD-L1 expression and prognosis [41–43]. Additionally, the histone acetylation ‘eraser’ HDAC6, methylation ‘writer’ KMT2A, and acetylation ‘reader’ bromodomain and extraterminal (BET) protein BRD4 activate PD-L1 expression in melanoma, pancreatic cancer, and ovarian cancer, respectively [44–46]. Thus, small-molecule inhibitors of HDAC6, KMT2A, and BET proteins suppressed PD-L1 expression and promoted antitumor immunity [44–46]. By contrast, ARID1A, a SWI/SNF ‘remodeler’ subunit frequently mutated in ovarian cancer, was shown to repress PD-L1 expression [47].

In Tumor Cells: Response to T Cell Attack

Besides epigenetic ‘writers’, ‘erasers’, and ‘readers’, ‘remodelers’ also alter interactions between cancer cells and immune cells in the TME. Sequencing of tumors isolated from patients with clear cell renal cell carcinoma who had received anti-PD-1 ICBT revealed that the mutation status of the *PBRM1* gene was associated with clinical benefits [48]. PBRM1, ARID2, and BRD7 are signature components of the PBAF form of the SWI/SNF chromatin-remodeling complex [6]. An *in vitro* CRISPR/Cas9 screen identified that loss of PBRM1, ARID2, or BRD7 in melanoma cell lines sensitized cells to immune attack by CD8 T cells [49]. Increased response to IFNγ, a key cytotoxic cytokine secreted by NK and T cells, in PBAF-null cells appears to be responsible for the phenotype [49]. Thus, loss of the PBAF complex may serve as a biomarker for the response to ICBTs. However, utilization of this association to sensitize PBAF-intact tumors remains challenging, since PBRM1, ARID2, and BRD7 are not enzymes and there are no inhibitors of them available. Targeting their associated enzymes, such as BRG1, may serve as an alternative approach.

In Immune Cells: Lymphocyte Development

Epigenetic machinery has been implicated in cell-fate decisions during lymphocyte development [50]. Alterations in epigenetic regulators directly cause hematological

malignancies. Key examples include translocation of KMT2A (MLL1) driving acute leukemia [51,52], and KMT6A (EZH2) gain-of-function mutations driving non-Hodgkin's lymphoma [53].

The functional and phenotypic changes that occur during activation of the adaptive immune system also largely rely on the epigenetic machinery. Chromatin architecture and histone regulators are essential determinants of DC function [54]. For example, the histone H3K4 demethylase KDM5B negatively regulates the activation of bone marrow-derived DCs, leading to an incomplete T cell response [55]. By contrast, a 'reader' of methylated DNA, MBD2, is required for the phenotypic activation of DCs and their ability to initiate a T cell response [56]. Additionally, the DNA methylation 'eraser' TET2 and the histone acetylation 'eraser' HDAC2 coordinate to repress interleukin-6 expression by DCs, limiting the inflammatory response [57].

In Immune Cells: T Cell Activation

The primary T cell response in lymph nodes requires interactions between T cell receptors (TCR) on naïve T cells and MHC-peptides on antigen-presenting DCs. Upon the co-stimulation provided by mature DCs, the TCR–MHC-peptide interaction initiates an autonomous program of T cell differentiation and proliferation. This program not only increases the number of cells carrying the initial TCR sequence by clonal expansion, but also equips the lymphocytes with effector functions. During these processes, there is a global change in the epigenetic landscape in T cells, including DNA methylation [58,59], histone modifications [60,61], and genome accessibility [62], indicating the fundamental role of epigenetics in T cell activation.

The priming and activation of cytolytic T cells is accompanied by global DNA methylation remodeling [58]. Differentially methylated regions include *de novo* methylation on enhancers active in naïve T cells and promotor demethylation on effector genes, such as *Gzmk* and *Gzmb* [58]. Consistent with this, DNMT3A, a methyltransferase in charge of *de novo* DNA methylation, controls early effector $CD8^+$ T cell fate decisions. Loss of DNMT3A leads to fewer effector cells, due to the ineffective repression of genes that are supposed to be silenced in effector cells [59].

Bivalent chromatin with the active transcriptional mark H3K4me3 and the suppressive mark H3K27me3 was found at gene loci associated with T cell proliferation and differentiation in naïve T cells. During priming and activation, most of these loci lose H3K27me3 while retaining the permissive H3K4me3 modification [60,61]. Additional analysis of enhancer marks, such as H3K4me1 and H3K27Ac, revealed a highly dynamic repertoire of enhancers during T cell activation [63].

In Immune Cells: T Cell Exhaustion

Chromatin organization has a central role in T cell exhaustion, as highlighted by recent studies. T cell exhaustion was originally discovered in a study of lymphocytic choriomeningitis mammarenavirus (LCMV), a natural pathogen of mice [64]. ATAC-seq showed that persistent LCMV infection-induced exhausted T cells have ~6000 open

chromatin regions that are different from effector T cells [65]. This difference is comparable with the difference between hematopoietic lineages [66].

PD-1 blockade has the ability to reinvigorate exhausted T cells in both chronic infection and tumor settings, as shown by transcriptional, cellular, and functional changes [67–69]. However, the reinvigoration is usually not sustainable. After PD-1 blockade, reinvigorated effector T cells became re-exhausted, likely due to the failure of the blockade to reprogram the epigenetic landscape of exhausted T cells into effector T cells, shown by ATAC-seq analysis [70]. A *de novo* DNA methylation program in effector T cells is required for the development of fully exhausted T cells [71]. Interestingly, exhaustion-associated DNA methylation is preserved during ICBTs [71], consistent with the ATAC-seq analysis.

In addition to the chronic LCMV infection model, tumor models created by injecting antigen-carrying cancer cells into TCR transgenic mice have also been established to study the epigenetic contribution to T cell exhaustion. ATAC-seq showed that a consistent chromatin-remodeling program dominated the exhaustion of effector T cells, which was absent in the formation of memory T cells [72]. Additionally, there are two discrete chromatin states of T cell exhaustion. The first stage is plastic, because T cells can be rescued. The later stage is permanent, in which cells are resistant to reprogramming [72].

Targeting Epigenetic Regulators to Boost Antitumor Immune Responses

Targeting epigenetic aberrations is considered one of the most attractive cancer therapies for several reasons. First, recurrent mutations of epigenetic modulators and dysregulation of epigenetic features are widely observed in tumors. Second, in contrast to genetic changes, epigenetic alterations are largely reversible. Third, epigenetic features are regulated by enzymes or chromatin-binding proteins that are targetable. Thus, epidrugs can be developed to treat cancer by suppressing oncogenic epigenetic regulators and restoring normal epigenetic features.

Over the past two decades, major efforts from both academia and industry have been devoted to the development of epidrugs. Before 2020, there were only FDA-approved epidrugs for cancer treatment, including four pan-HDACi and two DNMTi (Table 2). These were approved to treat T cell lymphoma, multiple myeloma, or myelodysplastic syndromes, all of which are hematopoietic malignancies (clinical responses reviewed in [73,74]). Another HDACi, chidamide (also called HBI-8000), was approved by the Chinese Food and Drug Administration for treating peripheral T cell lymphoma (clinical responses reviewed in [75]). Several additional FDA-approved drugs, (hydralazine, procaine, and procainamide, for treating hypertension, local anesthetics, and cardiac arrhythmia, respectively) have also been shown to have DNMTi activity [76–78]. Similarly, valproic acid, an approved seizures drug, was also found to be a HDACi. These existing drugs with newly identified epigenetic modulating activities are still evaluated in the clinical trials for cancer treatment. In January 2020, tazemetostat, a KMT6A (EZH2) inhibitor, was approved for treatment of epithelioid sarcoma, making it the first approved histone ‘writer’ inhibitor and the first epidrug to treat solid tumors [79].

Interestingly, emerging evidence suggests that some epigenetics-targeting molecules, including drugs that are approved or under preclinical and clinical studies, modulate the tumor immune microenvironment and induce robust antitumor immune responses [24,25,27,31].

Treating tumor-bearing animals with DNMTi and/or HDACi altered the immunosuppressive TME and enhanced tumor-infiltrating lymphocytes [26,35,47,81]. These effects are the result of enhanced tumor antigen expression and/or presentation, ‘viral mimicry’ effects, activation of DC cells, suppression of T cell exhaustion, or combinations thereof. Additional epidrugs, such as inhibitors targeting KMT6A (EZH2) [82], KDM1A (LSD1) [83], PRMT5 [84], and BET proteins [85,86], were also capable of remodeling the TME in animal models. Similar changes in the TME have been observed in tumor tissues isolated from patients who received epidrugs [37,87]. Thus, epidrugs are attractive immunotherapy agents to boost antitumor immune responses, either as single agents or in combination with other anticancer agents, including ICBTs.

In addition to FDA-approved DNMTi and HDACi, new inhibitors of DNMTs and HDACs have been developed to improve efficiency and achieve stability, high specificity, and low toxicity. For example, guadecitabine (SGI-110), TdCyd, FdCyd, aza-TdCyd, and ASTX727 are cytidine analogs that inhibit DNMTs through a mechanism similar to azacitidine and decitabine. These drugs have already entered clinical trials. New categories of DNMTi, such as DNA-binding compounds, oligonucleotides, and *S*-adenosyl-I-methionine (SAM) competitors, are under preclinical investigation [88]. There are more than a dozen new HDACi, representing different specificities against 18 HDACs (including SIRTs, a subgroup of NAD+ dependent HDACs) in the human genome, also in clinical trials [89].

Furthermore, intensive efforts are also being dedicated to targeting other epigenetic regulators (Table 2). Many epigenetic targeting agents are under clinical investigation for the treatment of both hematological and solid tumors. For example, inhibitors that target the histone mark ‘writers’ KMT6A (EZH2), KMT4 (DOT1L), and PRMT5, ‘erasers’ KDM1A (LSD1), and ‘reader’ BET proteins, have entered clinical trials. Other inhibitors, such as KMT2, KDM4, and KDM5 inhibitors, are still at the preclinical stages for oncology indications. Since the combination of epidrugs with checkpoint inhibitors showed synergistic effects in animal experiments [71,81,85,86,90], some clinical-stage epidrugs are being evaluated in clinical trials in combination with CTLA-4, PD-1, and PD-L1 ICBTs (Table 3).

Concluding Remarks

Cancer epigenetics and cancer immunology are both fast-moving fields, attracting major investigational efforts. Recent studies have shown that epigenetic regulation affects all cancer hall-marks, including all aspects of the interaction between tumor cells and the immune system. As a result, epigenetic modulation can elicit robust antitumor immune responses. Although some changes are broad, others are more restricted to certain cells and/or tissues. Here, we suggest that epigenetic therapies are novel immunotherapies by themselves. These findings provide unique opportunities to combine epidrug-based therapies

with other cancer treatment strategies, including current and upcoming immunotherapies. These combinations could not only have synergistic effects, but could also reduce adverse effects and prevent drug resistance. Identifying the most effective epigenetic targeting strategies to boost anti-tumor immune responses, especially in solid tumors, and developing rationale-based combinational strategies will have major impacts on our practice of immuno-oncology in the future (see Outstanding Questions).

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Highlights

Epigenetic mechanisms affect all aspects of the cancer–immunity cycle.

Some epigenetic targeting strategies are novel immunotherapies.

Epigenetic drugs could have synergistic effects with current cancer therapies, including immunotherapies, and prevent resistance to current cancer therapies.

Outstanding Questions

Why are current epigenetic drugs ineffective against most solid tumors?

Which epigenetic targeting strategies can achieve specific immunomodulatory effects?

Which epidrugs are synergistic with current and upcoming cancer immunotherapies?

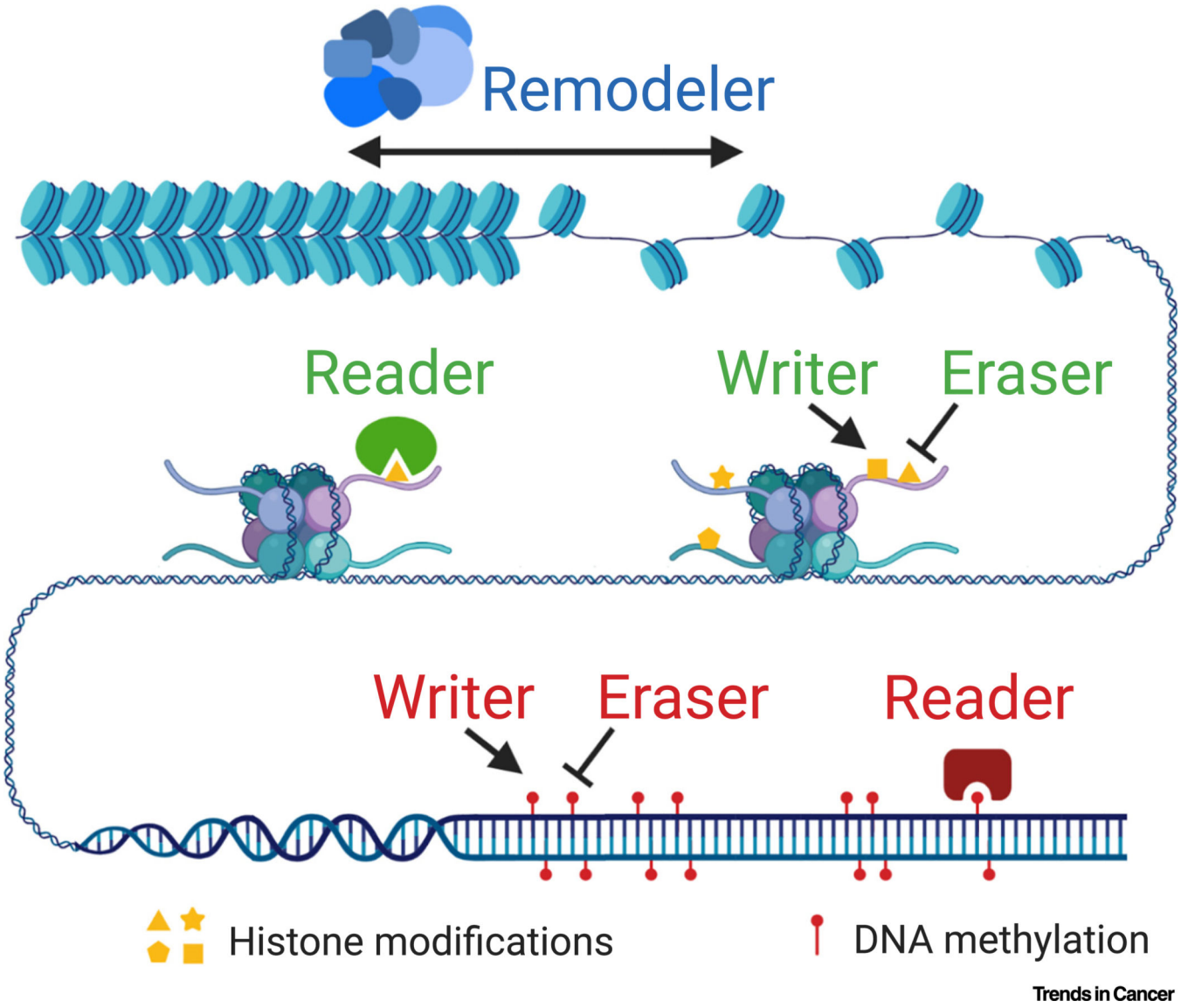
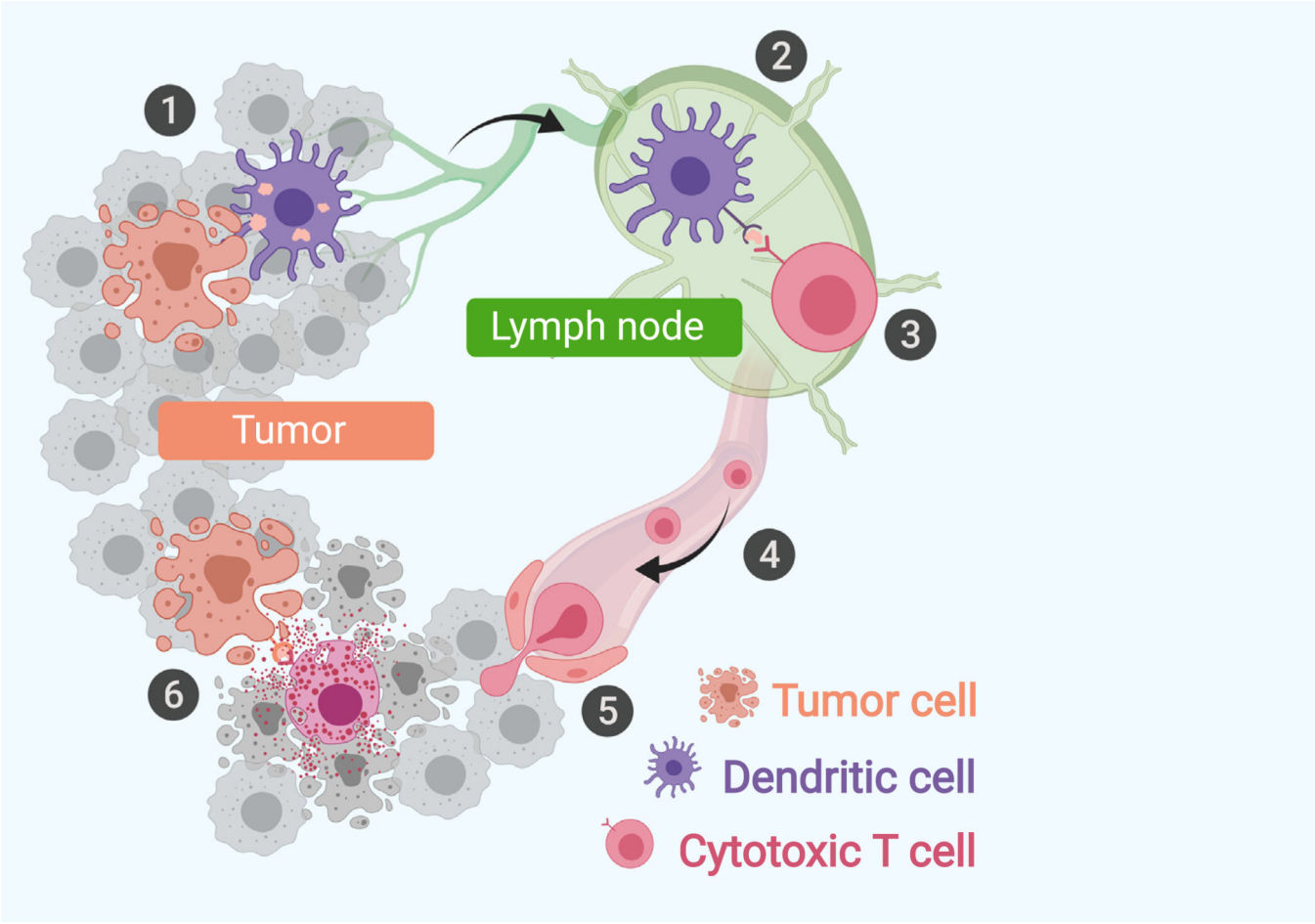


Figure 1. Molecular Basis of Chromatin-Based Epigenetic Mechanisms and their Regulators.

Genomic DNA is wrapped around histone octamers to form nucleosomes, which are further packaged into the human nucleus in a highly organized manner. The packaging states of chromatin are dynamically regulated by chromatin-remodeling complexes ('remodelers') to allow or deny access of selected *cis*-elements by their *trans*-factors. Core histones can be modified at multiple residues through covalent bonds by methylation, acetylation, phosphorylation, ubiquitination, and many other modifications. DNA can be methylated (or hydroxymethylated) at the 5th position on the pyrimidine ring in cytosines, and less commonly in mammals at the nitrogen in the 6th position on the adenine in adenosines. Histone post-translational modifications and DNA methylation are added or removed by specific enzymes ('writers' and 'erasers', respectively) and recognized by their binding proteins ('readers').



Trends in Cancer

Figure 2. The Cancer–Immunity Cycle.

The cancer–immunity cycle comprises six major steps: (1) releasing: tumor-associated antigens are released by tumor cells into the microenvironment, mostly due to cell death; (2) presenting: released antigens are captured by dendritic cells (DCs) in the tumor microenvironment (TME). Antigen-loaded DCs then process and present the antigens on the cell surface with major histocompatibility complex (MHC) complexes and travel to lymphoid organs; (3) priming: naïve T cells in lymphoid organs recognize selected peptide–MHC complexes through T cell receptors (TCRs), which triggers the priming and activation of effector T cells; (4) trafficking: differentiated effector T cells leave lymphoid organs, and travel along blood vessels to scan peripheral tissues until they find their antigens in tumors; (5) infiltrating: T cells enter the tumor bed and migrate into the TME to become tumor-infiltrating lymphocytes (TILs); and (6) attacking: T cells recognize cancer cells carrying the matched antigen through interaction between the TCR and peptide–MHC complex and kill cancer cells by direct or indirect immune attack. Immune attack leads to the release of additional antigens from the dying tumor cells, which triggers a new round of antitumor immune response.

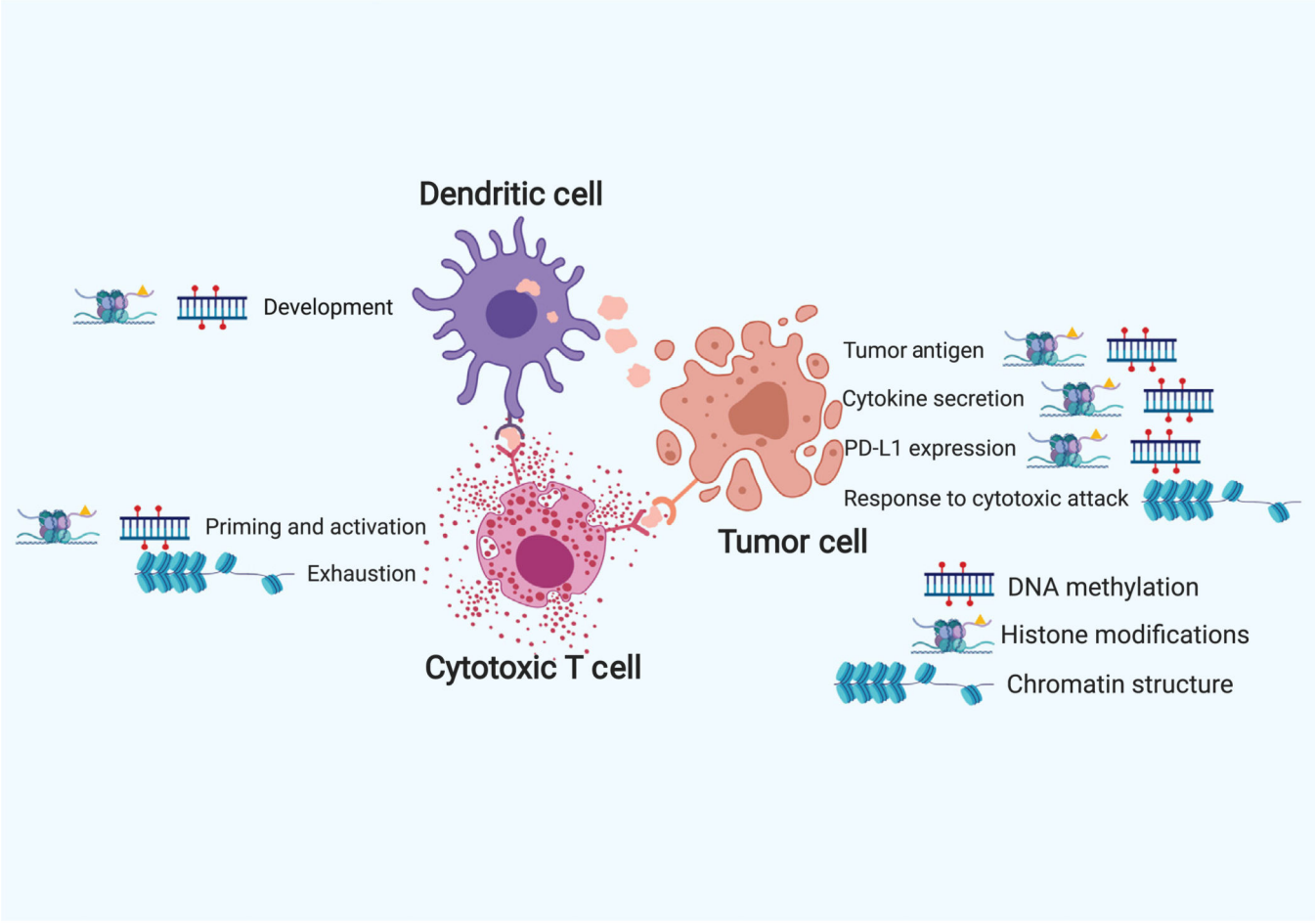


Figure 3. Major Epigenetic Regulation in Tumor Immunity.

Histone post-translational modifications and DNA methylation play key roles in adaptive immune response, including dendritic cell development and T cell priming and activation. In tumor cells, histone and DNA modifications affects production of tumor antigens, silencing of anti-tumor cytokines, and induction of the PD-L1 checkpoint. Recent studies revealed the contributions of chromatin remodeling responding to cytotoxic attack in tumor cells and exhaustion phenotype in tumor infiltrating CD8 T cells. Abbreviation: PD-L1, programmed death ligand 1.

Table 1.

Major Groups of Epigenetic Regulators

Epigenetic features	Regulator	Gene family
DNA methylation	Writer	DNA methyltransferases (DNMTs)
	Reader	5-methylcytosine-binding domain proteins (MeCP2 and MBDs)
	Eraser	Ten-eleven translocation dioxygenases (TETs), ALKBH1
Histone modifications	Writer	Lysine methyltransferases (KMTs), protein arginine methyltransferases (PRMTs), lysine acetyltransferases (KATs or HATs), histone ubiquitin ligases, histone kinases, and others
	Reader	Chromodomain, Tudor domain, MBT domain, PhD finger, bromodomain-containing proteins
	Eraser	Lysine demethylases (KDMs), histone deacetylases (HDACs and SIRTs), histone deubiquitinating enzymes, histone phosphatases, and others
Chromatin structure	Remodeler	SWI/SNF, ISWI, CHD, and INO80/SWR complexes

Table 2.

Epigenetic Drugs Approved or under Clinical Trials

Category	Target	Approved drug and indications	Drugs under clinical trials
DNMT inhibitor	DNA methylation writers	Azacitidine (myelodysplastic syndromes), decitabine (myelodysplastic syndromes), procainamide (cardiac arrhythmias), hydralazine (essential hypertension), procaine (local anesthetics)	Tioguanine, FdCyd, TdCyd, Aza-TdCyd, fluorocyclopentenylcytosine, guadecitabine
HDAC inhibitor	Histone acetylation erasers	Vorinostat (cutaneous T cell lymphoma), romidepsin (cutaneous T cell lymphoma), belinostat (peripheral T cell lymphoma), panobinostat (multiple myeloma), valproic acid (seizures), chidamide (peripheral T-cell lymphoma, by CFDA)	Tacedinaline, mocetinostat, abexinostat, entinostat, pracinostat, resminostat, givinostat, quisinostat, kevetrin, tefinostat, nanatinostat, domatinostat, ricolinostat, ME-344, CG200745, CUDC-101, AR42
KMT6A inhibitor	Histone methylation writer	Tazemetostat (epithelioid sarcoma)	SHR2554, CPI-1205, GSK2816126, PF-06821497, MAK683
SIRT activator	Histone acetylation erasers	None	SRT2104
BET inhibitor	Histone acetylation readers	None	Mivebresib, molibresib, birabresib, INCB057643, ZEN003694, FT-1101, GSK2820151, CC-90010, CPI-0610, PLX51107, ABBV-744, BAY1238097, BI 894999, BMS-986158, GS-5829
PRMT5 inhibitor	Histone methylation writer	None	JNJ-64619178, PF-06939999, GSK3326595
PRMT1 inhibitor	Histone methylation writer	None	GSK3368715
KDM1A inhibitor	Histone methylation eraser	None	Seclidemstat, IMG-7289, tranylcypromine, GSK2879552, INCB059872, phenelzine sulfate
KMT4 inhibitor	Histone methylation writer	None	Pinometostat

Table 3.

Clinical Trials with the Indicated Clinicaltrial.gov NCT IDs Combining Immune Checkpoint Inhibitors and Epigenetic Targeting Agents[a]

Epigenetic targeting agent	Immune checkpoint inhibitor					
	Ipilimumab (anti-CTLA4)	Nivolumab (anti-PD-1)	Pembrolizumab (anti-PD-1)	Atezolizumab (anti-PD-L1)	Avelumab (anti-PD-L1)	Durvalumab (anti-PD-L1)
DNMTi						
Azacitidine	02530463, 02397720	02530463, 02397720, 01928576	03264404, 03769532, 03094637, 02260440, 02816021, 02845297, 02546986, 02959437, 02900560, 02512172	02508870	03390296, 03699384, 02953561, 03390296, 02951156	02811497, 03019003, 02811497, 02775903, 03161223, 02117219, 02250326, 03019003
Decitabine	02890329	02664181, 03358719	03445858, 02996474, 03969446, 03240211, 02957968, 03233724		03395873	
Guadecitabine	02608437, 02890329	03576963	02901899, 02998567, 03220477	02892318, 02935361, 03179943, 03206047		03308396, 03257761, 03085849
HDACi						
Vorinostat			02619253, 03150329, 03426891, 02395627			
Romidepsin		02393794	03278782, 02512172			03161223
Panobinostat	02032810					
Chidamide	02718066					
Mocetinostat	03565406	03565406, 02954991	03220477			02805660, 02993991
Valproic acid		02648633			03357757	
Abexinostat			03590054			
Entinostat	03552380, 02453620	03838042, 03552380, 02453620, 03250273, 01928576	03179930, 02936752, 02909452, 03765229, 02437136, 02697630, 03978624	02708680, 03280563	02915523	
Domatinostat					03812796	
BETi						
INCB057643			02959437			
BMS-986158		024-19417				
KMT6Ai						
Tazemetostat			03854474	02220842		
CPI-1205	03525795					
KDM1Ai						
INCB059872		02712905	02959437			

[a]In addition, sintilimab (anti-PD-1) is being tested in combination with chidamide (HDACi) (NCT03820596), and tremelimumab (anti-CTLA-4) is being tested in combination with azacitidine (DNMTi) (NCT03019003) and guadecitabine (DNMTi) (NCT03085849).