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REVIEW

DNA Methyltransferase Inhibitors: Catalysts For Antitumour Immune Responses

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Abstract: Epigenetics is a kind of heritable change that involves the unaltered DNA sequence and can have effects on gene expression. The regulatory mechanism mainly includes DNA methylation, histone modification and non-coding RNA regulation. DNA methylation is currently the most studied aspect of epigenetics. It is widely present in eukaryotic cells and is the most important epigenetic mark in the regulation of gene expression in the cell. DNA methyltransferase inhibitors (DNMTi) have been increasingly recognized in the field of cancer immunotherapy, have been approved for the treatment of acute myeloid leukaemia (AML) and are widely being used in clinical trials of cancer immunotherapies. DNMTi promote the reactivation of tumour suppressor genes, enhance tumour immunogenicity, and stimulate a variety of immune cells to secrete cytokines that exert cytotoxic effects, promote tumour cell death, including macrophages, natural killer (NK) cells and CD8+ T cells, and upregulate major histocompatibility complex (MHC) class I expression levels. Here, we mainly summarize the epigenetics related to DNMTi and their regulation of the antitumour immune response and DNMTi combined with immuno-therapeutics or histone deacetylase inhibitors to demonstrate the great development potential and clinical application value of DNMTi.

Keywords: DNA methyltransferase inhibitors, histone deacetylase inhibitor, immunomodulation, immune cells, immunotherapy, DNA methylation, epigenetics

Introduction

In recent years, the epigenetic therapies used in cancer have made significant progress, mainly due to the rapid development of genome-wide high-throughput sequencing technology. Investigators can use sequencing technology to detect all changes in gene expression associated with epigenetic modifications, and these technologies are rapidly translating into tools for cancer treatment and prevention.[1] Epigenetic regulation of genes can modulate gene expression, the alterations of which can be used by tumour cells to disrupt immunogenic and immune recognition mechanisms, thereby acquiring an immune escape phenotype.[2–5]

Immune escape is an important factor in the development and evolution of tumours.[6] One of the most effective escape strategies adopted by cancer cells is disruption of the antigen presentation process. Epigenetic silencing affects almost all antigen processing and presentation processes.[7] The important role of epigenetics in tumour immune escape provides a solid theoretical foundation for the use of epigenetic-related drugs to improve the immune targeting of tumour cells.

Some studies have found that tumour epigenetic drugs can improve the antitumour immune response, and DNA methyltransferase inhibitors (DNMTi) can upregulate the

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expression level of MHC class I molecules, increase the presentation level of tumour-associated antigens, and ultimately enhance the immunogenicity of tumours by removing DNA hypermethylation modifications in the promoter region of MHC class I molecules.[8,9] DNMTi can induce the expression of tumour-associated antigens and regulate the activity of immune cells to improve the antitumour immune response. In this review, we introduce epigenetic and DNA methyltransferase inhibitors and summarize the effects of DNMTi on regulating antitumour immunity and improving the efficacy of immunotherapy.

Tumour Epigenetics And DNA Methylation

Epigenetics can regulate gene expression by abnormally modifying and controlling the spatial structure of genomic DNA sequence and participates in the process of tumorigenesis and development. These epigenetic abnormalities are reversible because they do not alter the properties of the genomic DNA sequence, thus providing a basis for epigenetic therapy in cancer. DNA methylation usually refers to the addition of a methyl group to the base of a DNA molecule by the action of DNA methyltransferases (DNMTs), most commonly provided by S-adenosyl methionine (SAM), and the hydrogen at the 5′ position of cytosine is replaced by a methyl group to become 5-methylcytosine.[10,11] DNA methylation, which exists widely in eukaryotic cells, is the most important epigenetic mark for regulating gene expression. DNA methylation plays a key role in gene silencing, X chromosome inactivation, genome stability, and imprinting, and it is a chromatin modification that has been extensively studied.[12–14] (Figure 1 summarizes the components of epigenetic modulation.)

Abnormalities in DNA methylation play an important role in processes such as cancer initiation, progression, invasion, and metastasis.[15–17] The relationship between DNA methylation and cancer was first discovered in 1983: DNA methylation levels in cancer cells have been found to be significantly reduced genome-wide.[18] Detection of genome-wide hypomethylation levels in peripheral blood has been reported in many tumorigenic diseases; for example, in patients with brain tumours, gastric cancer, liver cancer, and breast cancer, the genome-wide DNA in peripheral blood is hypomethylated.[19] The main cause of reduced methylation levels in cancer cells is demethylation of repetitive sequence regions of the

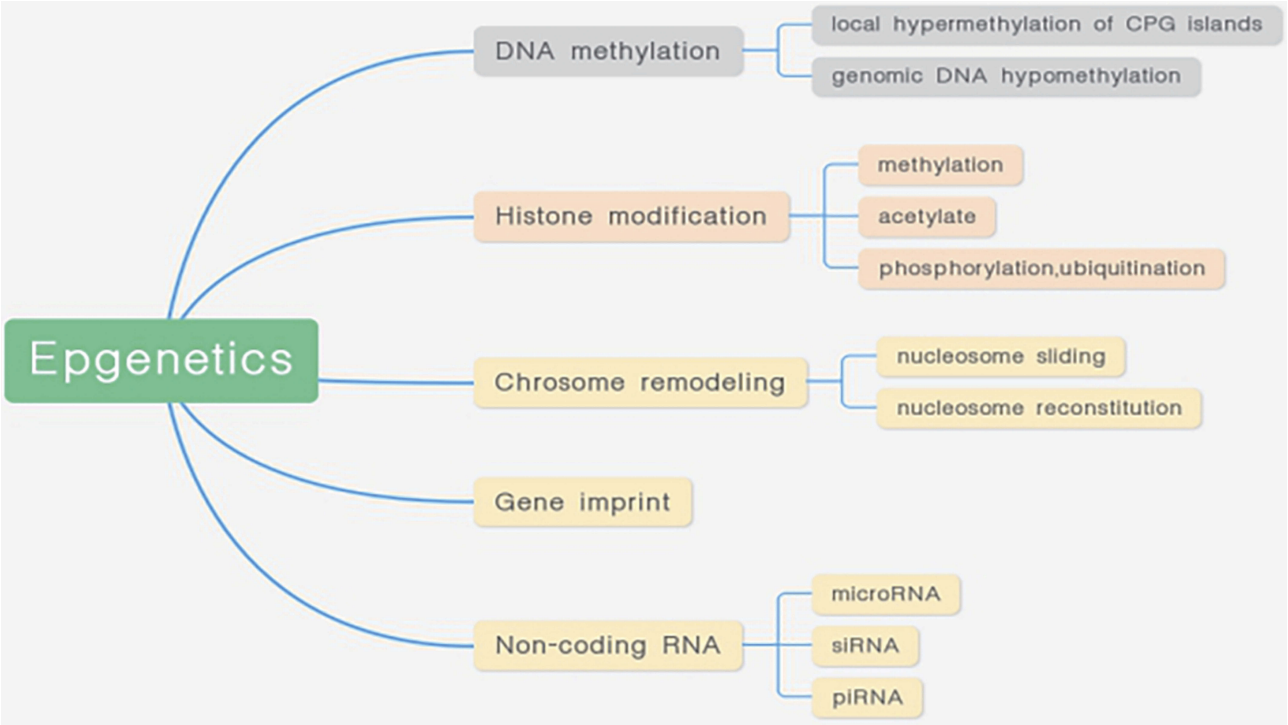

Figure 1 Basic composition of epigenetics.
Notes: Epgenetics includes: DNA methlation, histone modification, chrosome remodeling, gene imprint, non-coding RNA.The changes of DNA methylation in tumors are manifested in the decrease of global methylation level of the genome and the increase of methylation level of CpG islands in the promoter regions of some genes.

genome.[20] However, it has been shown that both low and high levels of DNA methylation coexist in cancer cells. Low levels of DNA methylation are associated with the activation of proto-oncogenes, which leads to genomic instability, while high levels of DNA methylation silence the promoters of tumour suppressor genes, which results in the inactivation of tumour suppressor genes.[21,22]

Studies have shown that the proportion of methylated CpG islands in clear cell renal cell carcinoma (ccRCC) and papillary renal cell carcinoma (pRCC) is as high as 31%[23] and 7%[24] respectively. The WNT pathway is one of the key pathways in cancer. Through this pathway, the expression of its downstream target, β-catenin, is suppressed, and the expression of some proto-oncogenes is inhibited.[25] Therefore, inhibiting the activity of DNMTs and blocking the hypermethylation of DNA in cancer cells can inhibit the growth of tumour cells or kill tumour cells, which may stimulate new ideas for cancer therapy.[26] The study of DNMTi has also become a hot topic in cancer drug development.

DNA Methyltransferase Inhibitors

The two most classic drug classes used in epigenetic therapy are DNMTi and histone deacetylase inhibitors (HDACi).[27] DNMTs are key enzymes that catalyse DNA methylation, mainly DNMT1, DNMT3A, and DNMT3B. These three enzymes catalyse the formation of 5mC from cysteines in DNA CpG islands and ultimately suppress gene expression.[28] DNMTi constitute a class of cytidine analogues that are divided into two classifications; in one class, a nucleotide analogue binds to DNA to form a covalent complex that promotes the degradation of DNMT.[29] In the other class is the non-nucleotide analogue DNMTi, which binds directly to the methylated region of the DNMT.[30] Representative nucleic acid analogues are decitabine (DAC) and azacitidine (AZA). They are currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of acute myeloid leukaemia (AML), chronic myelomonocytic leukaemia (CMML), and myelodysplastic syndromes (MDS).[31–33] Due the severe cytotoxicity induced by these drugs, several research teams have successively found that such drugs can exert their demethylation-related antitumour effects only at low doses, a finding that has pioneered epigenetic therapy.[34] Non-nucleoside analogues such as procainamide, SGI-110 and quinazoline, propiophenone, pyrrolopyridine derivatives, and other similar drugs are still under development.[35]

DNMTi can restore the expression activity and function of tumour suppressor genes by inhibiting the activation of DNA methylation, thereby inhibiting the growth of tumour cells and inducing their apoptosis; thus, DNMTi can be used as potential anticancer drugs in cancer therapy.[36–38] Further studies[39] found that, although DNMTi exhibit great clinical promise in blood-borne tumours, they are less effective as treatments for solid tumours. Compared with first-generation DNMTi, second-generation DNMTi such as SGI110 were confirmed to have greater stability and to induce less toxicity in normal tissues in vivo.[40,41] For example, SGI-1027 is a novel small molecule inhibitor of DNMT[42,43] that does not inhibit DNMT activity by binding to either RNA or DNA but rather achieves demethylation by inducing the degradation of DNMT. Targeting DNA hypermethylation using nucleoside analogues is an effective way to reprogramme the epigenome of cancer cells, thereby inhibiting cancer cell proliferation, promoting cancer cell differentiation, enhancing immune system recognition of cancer cells, and ultimately leading to cancer cell death, providing a new theoretical and experimental basis for the future application of demethylation drugs in the treatment of cancer. (Common DNA methyltransferase inhibitors and their mechanisms of action are summarized in Table 1.)

DNA Methyltransferase Inhibitors Regulate Tumour Immunity

The interaction of anticancer drugs with the host immune system has been implicated in therapeutic response.[44] The major histocompatibility complex (MHC) class I is at the core of antigen presentation, and the expression of MHC class I molecules in tumour cells is often inhibited by irreversible mutations or reversible hypermethylation, resulting in downregulation.[45] DNMTi can upregulate MHC class I levels in a variety of cancer tissues, as has been demonstrated in breast, lung, colon, and thyroid histotypes, as well as in human papilloma virus (HPV)-related cancers, sarcomas, and gliomas,[46–50] and they promote the release of interferon-γ from tumour-specific cytotoxic T lymphocytes (CTLs), which kill target cells.[51] In addition, similar results have been observed in ovarian cancer cells and xenograft melanoma models.[52] In addition to promoting MHC class I expression in tumour cells, DNMTi can also induce the expression of tumour-associated antigens. Experiments have shown that DNMTi can upregulate almost all antigen processing and presentation machinery components in

Table 1 Representative DNA Methyltransferase Inhibitors

Therapeutic Agent	Nth Generation	Drug Name	Mechanism Of Action
Nucleosides	First-generation	Azacitidine	It is involved in the synthesis of RNA or DNA at high concentrations Inhibition of DNMT blocks methylation.
		Decitabine	At high concentrations it can lead to blocked DNA synthesis and cytotoxicity; at low concentrations it leads to changes in gene expression profiles.
	Second-generation	Guanosine decitabine	It enhances stability in the aqueous phase, improves resistance to cytidine deaminase (CDA) degradation, and prolongs half-life
		Zebularine	It forms a covalent complex with DNMT to inhibit DNA methylation
Non-nucleoside	First-generation	Procainamideandprocaine	It binds tightly to CpG island dense regions of DNA,thereby interfering with the binding of DNMT to DNA.
		RG108	Non-covalent binding to the DNMT1 active site to achieve a block to DNA methylation.
		EGCG	It binds non-covalently to the catalytic active site of DNMT to inhibit the methylation catalytic activity of DNMT.
		MG-98	Acts on the mRNA of DNMT1 and radically inhibits the expression and synthesis of DNMT1.
		SGI110	The constitutive methylation level of the CTA promoter in cancer cells treated for treatment induction was significantly reduced
		SGI1027	Induction of Degraded DNMTs for Demethylation.

mouse and human tumour cells, including the expression level and intra-tumoural distribution of the tumour-associated antigens (TAA) and LMP2 and LMP7 proteasome subunits. In addition, DNMTi can also improve the costimulatory properties of tumour cells by upregulating the expression of surface molecules such as CD40, CD80, CD86, and ICAM1, as well as by restoring the sensitivity of tumour cells to the apoptosis triggered by immune cells using the enhanced expression of death-inducing receptors such as FAS.[53–56] After treatment with AZA, non-small cell lung carcinoma (NSCLC) cells showed significantly enhanced expression of antigen presentation-related genes and interferon signalling; additionally, the apoptosis rate and the viral defence protein and immune-related transcription factor expression levels were significantly increased.[57,58]

Cancer-testis antigens (CTAs) constitute a family of antigens that are closely related to tumour development. CTAs are expressed in testis, placenta and tumour tissues and are mainly regulated by DNA methylation levels. DNMTi are able to promote the overexpression of CTAs by tumour cells, thereby assisting host CTL in distinguishing tumour cells from healthy cells while being able to upregulate the levels of multiple oncogenes of various CTAs, including the extremely immunogenic oesophageal squamous epithelial tumour-testis antigen 1B (CTAG1B/NY-ESO1), thereby intensifying the antigen presentation process.[59] Elevation in CTA level can be found in most cancers,[47,48,60] such as mesothelioma,[61] renal, oesophageal, pleural, and liver cancers.[62] In addition, the hypomethylating agent SGI-110 was found to induce hypomethylation and CTA gene expression and enhance the expression of MHC I and intercellular cell adhesion molecule 1 (ICAM-1).[63]

Rouloisan et al found that DNMTi activates the classical IFN signaling pathway in ovarian cancer cell lines,which activates the cytosolic dsRNA sensors TLR3 and MDA5 through an increase in dsRNA, thereby inducing IFNB and JAK/STAT signalling. One RNA that triggers this response is transcribed from hypermethylated endogenous retroviruses (ERVs).[64] The involvement is similar for dsRNA and MDA5 sensors in colon cancer cells, and the canonical IFN response is critical for the suppression of colon cancer stem cells by DNMTi.[65] Decitabine can activate the NOTCH1 signalling pathway, which in turn inhibits cancer cell proliferation and affects the immune system in patients

with muscle-invasive bladder cancer.[66] DAC and AZA not only kill targeted cells directly through their cytotoxic effects but also affect antigen presentation in blood cells: on the one hand, DAC promotes the expression of MHC class I and II molecules for the treatment of chronic lymphocytic leukaemia (CLL);[67] on the other hand, AZA is used for the treatment of Hodgkin lymphoma (HL), which can generate more abundant antitumour T cells than are generated in patients treated with HDACi, indicating that AZA can effectively activate the antigen presentation process.[68]

DNA Methyltransferase Inhibitors Are Regulators Of Immune Cells

Maturation and activation of immune cells are regulated at the epigenetic level. From the onset of lineage formation, immune cells are regulated by DNA methylation.[69] (The regulation of immune cells by DNMTi is summarized in Figure 2.) For example, epigenetic changes are closely associated with lymphocytes, macrophage polarization, myeloid-derived suppressor cell function, and regulatory T cell (Treg cell) development and function.[70–73] The effects of DNMTi on multiple immune cell functions are addressed below.

CD8+ And CD4+ T Cells

The generation of memory T cells against cancer-specific neoantigens is a key factor in achieving sustainable responses to immunotherapy. Memory T cells are usually multipotent T cells that maintain long-term plasticity and survival. In contrast, effector T cells have limited survival times; they heavily depend on the presence of antigen but are prone to exhaustion after prolonged exposure to antigen. The lineage of effector

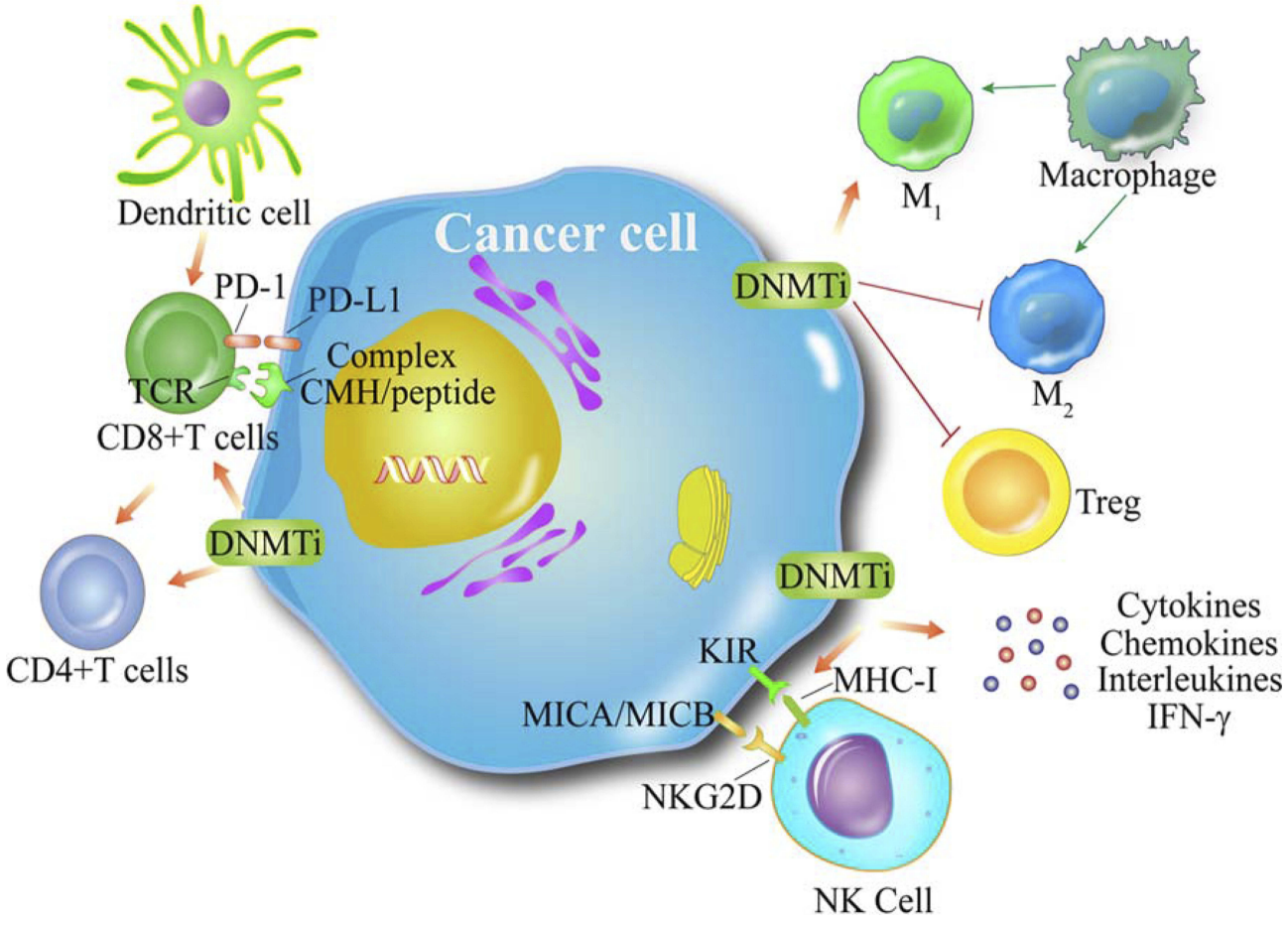

Figure 2 The regulation of DNMTi on immune cells.

Notes: While enhancing the cytotoxicity of CD8 + T cells, DNMTi can assist CD4 + T cells by inducing the expression of key immunostimulatory cytokines. DNMTi inhibits the expression of Treg cells; it can inhibit M1 and promote M2 to regulate macrophages. Promotes KIR expression on NK cell surface, binds to MHC class I molecules to recognize abnormal cells, and increases NKG2D-dependent NK cell-mediated killing of these cells in vitro.

or memory T cells is tightly regulated by histone modification-promoting memory gene silencing or DNA methylation.[74,75] The role of DNA methylation in T cell status is as follows: in terms of their developmental trajectory, CD8+ T cells can be broadly classified as "naive" prior to exposure to antigen and, following exposure to antigen, as "effectors" that mount a response against cells bearing the cognate antigen.[76] Sustained expression of some checkpoint inhibitors, such as PD-1 and TIM3, and exhaustion markers, which deplete T cells, is associated with specific epigenetic profiles.[77,78]

Interestingly, pretreatment with DNMTi rejuvenates tumour-infiltrating CD8+ T cells and reverses drug resistance in an ICB-resistant model.[79] DNMTi are capable of promoting CTL action by mediating the transcription of antitumour cytokines. In non-proliferating T lymphocytes, interleukin 2 (IL2) transcription has been closely linked to the enhanced effects of demethylation at the promoter, specifically in the enhancer region of IL2.[80] When CD8+ T cells are exposed to antigen, the IL2 locus is significantly demethylated, which results in the expression of large amounts of IL2.[81] In immature CD8+ T cells, there are three highly methylated CpG islands in the upstream regulatory sequence of IFN-γ; in effector T lymphocytes, these three CpG islands are demethylated, enabling T cells to produce large amounts of IFN-γ. In addition, in memory T cells, these sites are partially methylated and rapidly demethylated upon certain types of stimulation. These phenomena suggest that DNMTi enhance the function of CTLs by mediating demethylation to enhance and maintain the expression levels of IL2, IFN-γ, and other antitumour cytokines during tumour immunity. DNA methyltransferase 1 (DNMT1)-mediated DNA methylation inhibits tumour production of the T helper 1 (TH1)-type chemokines CXCL9 and CXCL10, which in turn affect the transport of effector T cells to the tumour microenvironment.[82] These processes further define the pathway by which epigenetic therapy may remodel the TME to induce an antitumour state.

Epigenetic therapy with HDACi and DNMTi has been demonstrated to regulate the expression of the chemokine CCL5 by reducing Myc levels,[83] possibly through the demethylation of gene bodies,[84] and a key requirement for an immune response driven by CD8+ T cells is antigen presentation via MHC class I. Without this step, binding between the TCR and the cognate antigen on the CD8+ T cells cannot occur. Differences in chromatin accessibility also distinguish dysfunctional T cells from functional memory T cells, suggesting that epigenetic programmes also mediate cellular exhaustion.[77] CD4+ T cells comprise a diverse family of helper T cells with opposing activities against tumour cells: Th1 CD4+ T cells have antitumour properties, whereas Th2 CD4+ T cells have pro-tumorigenic function.[85] The functional relevance of the epigenetic pathways involved in the differentiation and maturation of cell subsets remains unclear, and it has been found experimentally that CD4+ T cells isolated from 68 patients with MDS were able to secrete large amounts of IL17 after AZA treatment.[86] Furthermore, the number of IL17A-secreting CD4+ T cells in the peripheral blood of AML and MDS patients was significantly increased after AZA treatment.[87] These results suggest that DNMTi can enhance the cytotoxic effect of CD8+ T cells and help CD4+ T cells by inducing the expression of key immune-stimulatory cytokines.

Regulatory T Cells

Regulatory T cells (Treg cells) can be divided into natural regulatory T cells (nTerg cells) and inducible regulatory T cells (iTreg cells), according to different sources, and they can be divided into resting Tregs (rTreg cells), activated Tregs (aTreg cells) and cytokine-secreting Treg cells, according to different functions.[88–90] As regulatory T cells in cancer immunosuppression-implants for anticancer therapy, Treg cells are characterized by the expression of the FOXP3 transcription factor, which plays an essential role in immune suppression.[91,92] Epigenetic modification, as an important way to regulate FoxP3 gene expression, plays an important role in its stable expression, including through DNA methylation and histone phthalation.[93] It was found that the number of Tregs was significantly reduced in the AZA-treated group compared with the control group after AZA treatment using peripheral blood samples from 68 MDS patients containing regulatory T cells (Treg), indicating that DNMTi can inhibit the expression of Treg cells.[94]

Macrophages

As heterogeneous innate immune cells, macrophages have important theoretical research and clinical application prospects. M1 macrophages display antitumour phagocytic properties, whereas M2 macrophages have pro-tumorigenic properties.[95] These dual roles have also been described in tumours.[96] Experimentally, it has been found that DNMT3b is aberrantly expressed in obese mice, causing an increase in DNA methylation in the promoter region of peroxisome proliferator-activated receptor

gamma 1 (PPARγ1, a nuclear receptor and a key transcription factor involved in M2 polarization whose promoter region is rich in CpG and prone to epigenetic regulation), which suppresses its expression, leading to restricted macrophage polarization to M2 and chronic inflammation in adipose tissue; knocking down DNMT3b shows the opposite trend.[97] It has also been shown that DNMT1 causes an increase in the methylation level of the suppressor of the cytokine signalling 1 (SOCS1) promoter, enabling its continual suppression. After DNMT1 silencing using the DNMT inhibitor 5-azadC, the degree of methylation in the promoter region of the SOCS1 gene was reduced, thereby blocking the LPS-induced activation of the JAK2/START3 pathway in macrophages and reducing the pro-inflammatory phenotype.[98] Thus, the use of DNMTi targeting DNMT can be used to control the pro-inflammatory M1 phenotype and promote an anti-inflammatory M2 response. In summary, the effects of reducing M1 and promoting M2 can be achieved by applying HDACi and DNMTi alone or in combination. This finding lays the foundation for the future discovery and application of new epigenetic modifying drugs. These examples support epigenetic strategies that may allow modulation of the state of macrophages and thus antitumour immunity.

Myeloid-Derived Suppressor Cells (MDSCs) And Dendritic Cells (DCs)

The important function of various myeloid cells, including MDSCs and DCs, is antigen presentation, and their antitumour potential is largely determined by their ability to activate T cells. Maturation of DCs is also controlled by chromatin regulators, such as special AT-rich binding protein 1 (SATB1), which regulates MHC class II expression and modulates its antitumour potential.[99] Epigenetic modifiers, such as HDACi and DNMTi, have been shown to directly increase MHC II and costimulatory molecule (CD40 and CD86) expression in peripheral MDSCs from breast and lung cancer patients.[100–102] The percentage of MDSCs in the tumour microenvironment and spleens of mice bearing TRAMP-C2 prostate cancer cells or TC1/A9 primary lung epithelial tumour cells was significantly reduced after the mice were subcutaneously injected with AZA, and the number of cyclophosphamide-induced MDSCs in the mice accumulated with increasing doses of AZA. The percentage of CD11b+/Gr1+ MDSCs was significantly reduced and accompanied by an increase in the percentage of CD11c+ and CD86+/CD8+ DCs after AZA treatment in vitro cultured tumor-infiltrated CD11b myeloid cells. indicating that DNMTi could partially induce MDSCs to differentiate into dendritic cells (DCs);[100] thus, DNMTi can inhibit the negative regulatory cells of tumour immunity.

NK Cells

Killer cell immunoglobulin-like receptors (KIR) on the surface of NK cells recognize abnormal cells by binding to MHC class I molecules. In the tumour microenvironment, modification of the DNA regulatory sequences of KIR by hypermethylation is a common tumour escape mechanism. Therefore, promoting the expression of KIR using DNMTi would be an effective approach for cancer immunotherapy.[103] Some studies[104] have shown that the development and function of immune cells are regulated by DNA methylation. Different concentrations of the DNMT inhibitor decitabine on NK cells affect cell viability, proliferation, cytotoxicity and activation performance. Demethylation agents can be used to treat acute myeloid leukaemia (AML) by modulating NK cell activity. NK cells directly kill tumour cells, and in the presence of IFNγ, NK cells are usually activated and are relatively more cytotoxic.[105] Although DNA methylation is an epigenetic mechanism regulating KIR expression in NK cells, the effects of hypomethylating agents on NK cell function have not been well characterized. Decitabine has been shown to increase cell surface expression of recombinant UL16 binding protein (ULBP)[106] and MHC class I-related molecule B (MICB)[107] in AML cells, increasing natural-killer group 2 member D (NKG2D)-dependent sensitivity of these cells to NK-mediated killing in vitro. When applied to NK cells under non-proliferative conditions, 5-azacytidine increases KIR expression, which results in reduced NK cytolytic activity,[108,109] whereas decitabine was shown to improve the responsiveness of human NK cells in vitro. However, it has been found that low-dose decitabine in tumour-bearing mice reduced the antitumour response of NK cells.[110] How DNMTi affect NK cell activity requires further basic experimental studies in the future.

DNMTi: Contributor To Cancer Immunotherapy

Epigenetic therapies show advantages when used in concert with novel immunotherapies. In a phase I dose-escalation experiment of 5-AZA-CdR in 12 patients with recurrent epithelial ovarian cancer, Odunsi et al observed

increased T cell responses in most patients.[111] The mRNA expression of testicular cancer antigens involved in NK and T cell signalling and recruitment, immune checkpoint blocking molecules, immunostimulatory cytokines, and genes involved in the interferon pathway was higher after treatment with guadecitabine(SGI-110) and DAC compared with the immunomodulatory effects of AZA treatment.[112] In addition, this combination reduced the number of MDSCs, indicating that the immunomodulatory effects of DNMTi may be useful for immunotherapy.[113] In melanoma, 5-azacytidine can induce specific double-stranded RNA production for host viral defence mechanisms, upregulate the transcription of interferon-β and elevate malignant cell sensitivity to CTLA-4 inhibitors.[114] However, in the melanoma B16 mouse model, low-dose 5-azacytidine with anti-CTLA4 showed the same effect in controlling tumour growth in vitro and in vivo.[64] 5-AZA-CdR has been reported to regulate the expression of CTA and class I human leukocyte antigen (HLA), thereby improving tumour cell immunogenicity.[115] 5-AZA has been found to upregulate PD-L1 in EOC and NSCLC cell lines and can activate cytosolic dsRNA sensing in colorectal cancer, ultimately activating the viral/IFN response,[65,116] which demonstrates that DNMTi can induce cancer cells to behave as virus-infected cells and trigger dsRNA sensing. Importantly, improved viral defence pathway signalling levels correlate with improved immune checkpoint inhibitor treatment response and long-term survival of cancer patients.[117] SGI-110 has been found to reactivate ERVs to stimulate cancer cell immune response pathways, which provides the rationale for combinatorial therapy with immune checkpoint therapies.[118]

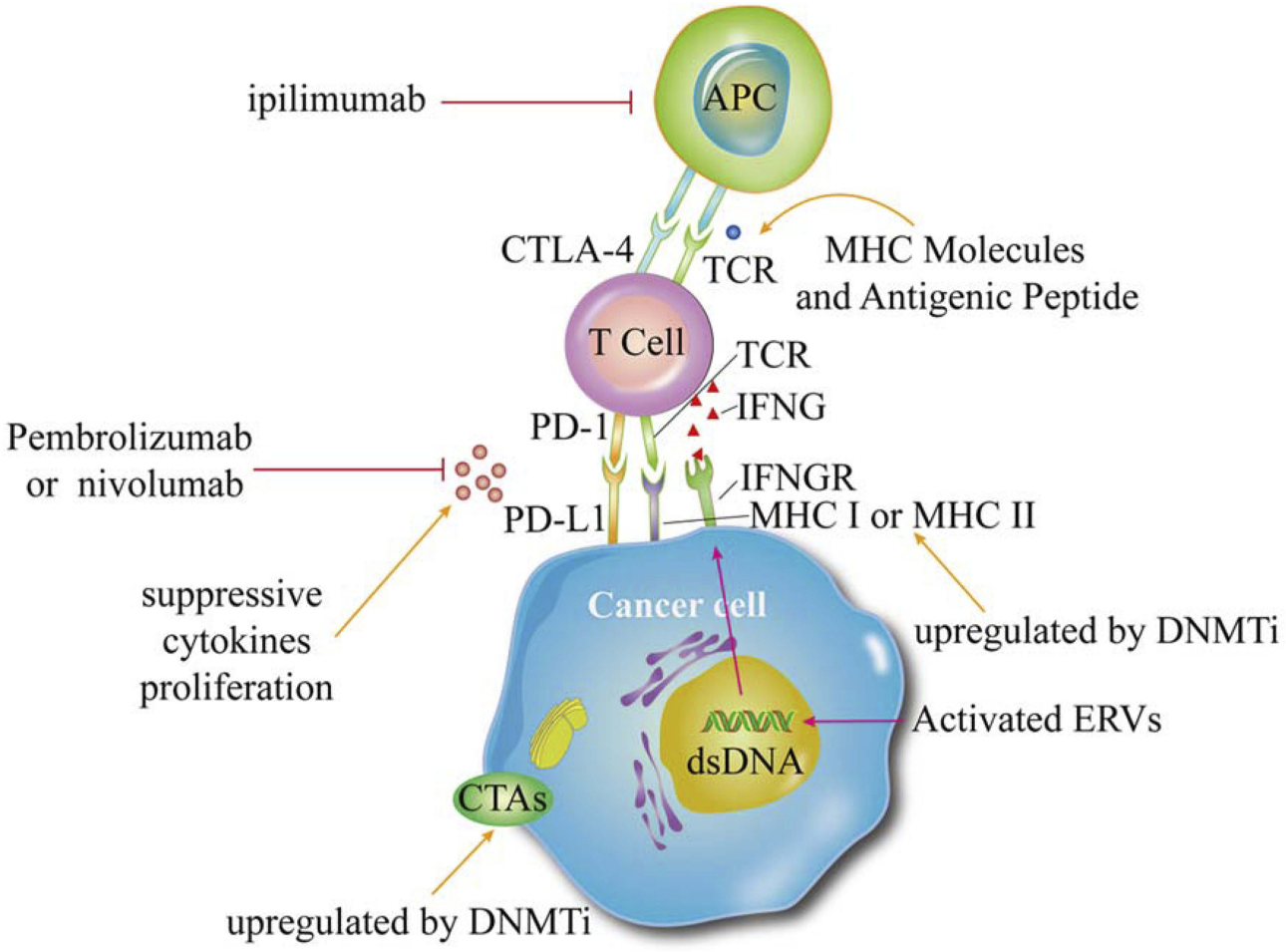

Figure 3 Advantages of Combining DNMTi with immune checkpoint inhibitors.
Notes: T cell stimulation is driven by antigen and requires the coordinated engagement of several other receptors and molecules expressed on the T cell surface as well as antigen-presenting cells (APCs) or tumor cells. DNMTi can inhibit different signaling pathways involved in adaptive immune responses and enhance antitumor effects by combining with immune checkpoint inhibitors.

(The advantages of combining DNMTi with immunotherapy are summarized in Figure 3).

Notably, DNMTi-resistant patients were found to have elevated levels of PD-L1, PD-L2, and CTLA-4. The combination of DNMTi and PD-1/PD-L1 inhibition may solve the problem of resistance to AZA or DAC. Regarding the addition of immune checkpoint therapy to a small number of patients with advanced NSCLC who progressed after low-dose DNMTi therapy, approximately 20% of patients responded to immune checkpoint therapy, did not progress at 24 weeks, and, in general, achieved a standard response,[113] which was a surprising result. In a single-centre trial of azacitidine in combination with nivolumab in relapsed/refractory acute myeloid leukaemia (AML), the overall response rate (ORR) to treatment was 33%, including 15 (22%) complete responses, 1 partial response, 7 haematologic improvements maintained for > 6 months, and 6 patients (9%) with stable disease for > 6 months, and the response rate and OS results of the azacitidine and nivolumab regimens were also encouraging.[119] In addition, a randomized clinical trial compared pembrolizumab plus azacitidine with pembrolizumab plus placebo in patients with advanced non-small cell lung cancer, but no significant difference in PFS was observed.[120] When idarubicin, cytarabine and nivolumab are used to treat newly diagnosed AML or high-risk MDS, the median relapse-free survival time of the responders was 18.54 months, and the median overall survival time was 18.54 months. The rationality of the combined medication has been shown, and the study is still in progress (NCT02464657). We expect positive clinical results.[121] At present, clinical experimental data from the use of DNMTi combined with immunotherapy are limited, and such studies are still in progress. The advantages of combination therapy have been initially shown, but there are still some uncertainties. This makes us wonder whether the sample size is too small to show experimental deviation. What is the best time to use combination drugs? Are there any other potential therapeutic molecular biomarkers? DNMTi combined with immunotherapy still presents many challenges, and a large number of preclinical or clinical experiments are required.

DNMTi Combination HDACi Therapy

Histone deacetylase inhibitors (HDACi) induce cell cycle arrest, differentiation and cell death in cancer cells, reduce angiogenesis, and modulate the immune response.[122] The activity of HDACs can affect the expression of MHC (major histocompatibility complex) and co-stimulatory molecules.[123,124] Histone acetylation may play an important role in regulating T cell development, differentiation, and cell function[125] and, in combination with DNMTi, can also increase the response of antitumour CD8+ T cells.[126] Class I/IIa HDACi combination enhances class I MHC cell surface expression and the expression of co-stimulatory molecules CD40 and CD86 in tumour cells.[127,128] In addition, we found that class II HDACi enhance Treg cell number and function, and class I HDAC inhibitors enhance the function of NK cells and CD8 T cells.[129] However, the molecular mechanisms by which HDACi regulate genes involved in immune recognition are not fully understood. Upregulation of MAGE-A gene in cancer cells by 5-AZA-CdR/TSA combination has been reported.[130] While the use of a combination of decitabine/HDAC inhibitors can induce an increase in CTA and PD-L1 expression, the results suggest that the CTA expression and the epigenetic regulation of PD-L1 may be correlated. In the future, anti-PD-1/PD-L1 combination therapy with decitabine and HDACi will be considered to overcome the possible induction of PD-L1 expression. DAC and HDACi (panobinostat or valproic acid) downregulate the expression of epigenetic modifiers (e.g., KDM2B and SUV39H1) when used in combination to treat acute myeloid leukaemia cells.[131] These findings are beneficial for understanding the mechanism of action in combined epigenetic drug therapy.

In a randomized clinical study in which 184 patients with HR-MDS or CMML were randomly assigned to AZA ± vorinostat or AZA monotherapy with a median follow-up of 23 months, the ORR was 38% in patients treated with AZA monotherapy compared with 27% in the AZA plus vorinostat arm,[132] showing no advantage. Panobinostat in combination with AZA was used for previously untreated AML or high-risk MDS, and 27.5% of patients treated with PAN + AZA were in CR, compared with 14.3% of patients receiving AZA, but there was no significant difference in the 1-year OS rate.[133] AZA plus pracinostat improved OS compared with AZA monotherapy. In contrast, AZA monotherapy in HR-MDS patients showed no improvement in overall patient survival after treatment with VS AZA plus pracinostat.[134] It remains unclear whether the combination of HDACi and HMA is beneficial in patients with MDS and AML. In addition,

studies have demonstrated that the addition of vorinostat does not improve the efficacy of azacitidine in the treatment of acute myeloid leukaemia; when 217 adults with AML were randomly selected to receive AZA monotherapy or AZA plus vorinostat (VOR), there was no improvement in overall response rate or overall survival.[135] There is still some uncertainty regarding HDACi combined with DNMTi, which we intend to further explore in the future.

Summary

Research on DNMT inhibitors has become a hot topic in the field of anticancer drug research. Recently, some DNMT inhibitors were in the preclinical and clinical research evaluation stage, and their inherent cellular toxic side effects limited the clinical application of demethylating drugs. Big data show that DNMTi can effectively stimulate the expression of the major histocompatibility complex (MHC), significantly improve the immunogenicity of tumours, and enhance the killing of tumours by effector T cells. Preclinical studies have confirmed that both decitabine and azacitidine promote the expression of genes involved in the immune system,[9,136] and DNMTi can regulate a variety of immune cells, such as lymphocytes, NK cells, macrophages, dendritic cells and so on. DNA methyltransferase inhibitors are expected to play an important role in cancer immunotherapy. However, DNMTi modulation of immune cells is closely related to the state of cell activity, and the drug dose and regulatory mechanism need to be further elaborated in basic experiments.

In addition, the combination of methylase inhibitor and immune checkpoint inhibitor has initially shown advantages, and the combination of these with HDACi is uncertain. Currently, the experimental clinical data on combined drugs are lacking, and the clinical sample sizes are small; therefore, the findings cannot be generalized. Further research is needed. We found that DNMTi are also synergized with other classes of epigenetic drugs. For example, dual inhibition of DNMT and LSD1 was shown to synergistically reactivate epigenetically silenced genes in cancer cells.[137] DNMTi are also used in combination with isocitrate dehydrogenase (IDH) inhibitors, lenalidomide (LEN), nucleoside analogues sapacitabine, venetoclax, and other combinations to treat tumours.[138] Do these novel therapeutic strategies also have an impact on the body's antitumour immunity? Ways they may be applied more widely in the clinical treatment of cancer are directions for follow-up research. More epigenetic drugs will be approved for marketing in the next few years. Such emerging drugs are expected to inject new vitality into the treatment of cancer and have bright prospects for development.

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Disclosure

The authors report no conflicts of interest in this work.

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