

## Review

# Epigenetic drugs for cancer treatment and prevention: mechanisms of action

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## Abstract

This review provides a brief overview of the basic principles of epigenetic gene regulation and then focuses on recent development of epigenetic drugs for cancer treatment and prevention with an emphasis on the molecular mechanisms of action. The approved epigenetic drugs are either inhibitors of DNA methyltransferases or histone deacetylases (HDACs). Future epigenetic drugs could include inhibitors for histone methyltransferases and histone demethylases and other epigenetic enzymes. Epigenetic drugs often function in two separate yet interrelated ways. First, as epigenetic drugs *per se*, they modulate the epigenomes of premalignant and malignant cells to reverse deregulated epigenetic mechanisms, leading to an effective therapeutic strategy (epigenetic therapy). Second, HDACs and other epigenetic enzymes also target non-histone proteins that have regulatory roles in cell proliferation, migration and cell death. Through these processes, these drugs induce cancer cell growth arrest, cell differentiation, inhibition of tumor angiogenesis, or cell death via apoptosis, necrosis, autophagy or mitotic catastrophe (chemotherapy). As they modulate genes which lead to enhanced chemosensitivity, immunogenicity or dampened innate antiviral response of cancer cells, epigenetic drugs often show better efficacy when combined with chemotherapy, immunotherapy or oncolytic virotherapy. In chemoprevention, dietary phytochemicals such as epigallocatechin-3-gallate and sulforaphane act as epigenetic agents and show efficacy by targeting both cancer cells and the tumor microenvironment. Further understanding of how epigenetic mechanisms function in carcinogenesis and cancer progression as well as in normal physiology will enable us to establish a new paradigm for intelligent drug design in the treatment and prevention of cancer.

**Keywords:** cell death; DNA methylation; drug; epigenetic therapy; gene expression; histone acetylation; histone methylation.

## Introduction

Epigenetics investigates heritable changes in gene expression caused by mechanisms that do not involve alterations in DNA sequence. These changes can last for multiple generations, contributing to the non-Mendelian inheritance of phenotypic alterations. Epigenetic mechanisms play essential roles in physiological and pathological processes. Increasing evidence supports the notion that cancer is not only a genetic disease but also an epigenetic disease (1–5). It is well established that epigenetic alterations can be used as therapeutic targets in many diseases including cancer (6–10). Epigenetic alterations contribute to carcinogenesis by the basic mechanisms of epigenetic activation of oncogenes (11, 12) or well-documented epigenetic inactivation of tumor suppressor genes (TSGs) (1–5). Some epigenetic changes occur early in development, preceding the onset of tumor (13–15). Feinberg et al. have proposed an epigenetic progenitor origin for human cancer (16). These provide strong rationales for utilization of epigenetic drugs not only for cancer therapeutics but also for cancer prevention.

Epigenetic drugs are chemicals that act on the epigenome of cells to either alter certain gene expression or counteract aberrant epigenetic changes which lead to cancer initiation and progression or other diseases. These drugs differ from standard chemotherapeutic drugs. In a broad sense, most chemotherapeutic drugs work by impairing mitosis, and thus they effectively target and kill fast growing cells. Certain chemotherapeutic drugs aim to prevent angiogenesis and block blood vessel growth and thus slow tumor growth. In contrast, epigenetic drugs are intended to restore normal states of gene expression by reactivating aberrantly silenced genes through modulation of the epigenome. These epigenetic drugs can be cytotoxic to cancer cells and even normal cells when applied at sufficiently high doses.

In this review, we briefly discuss the basic concepts underlying the process of epigenetic gene regulation. We then provide an overview of molecular mechanisms of action for several representative epigenetic drugs on cancer cells and premalignant cells. We also discuss recent discoveries that can have a significant impact on further development of epigenetic drugs for cancer therapeutics and prevention.

## Basic concepts of epigenetic gene regulation

The epigenetic control of gene expression in mammalian cells depends on three distinct yet related mechanisms: DNA methylation, histone modifications and the action of non-

coding RNAs (ncRNA). In addition, emerging evidence suggests that the replication timing and subnuclear repositioning of chromatin domains are two important epigenetic regulators for certain promoters (17–20). DNA methylation and histone modifications act on transcription, whereas ncRNA affects the levels of gene product at the post-transcriptional steps. Several outstanding reviews on histone modifications, DNA methylation, chromatin remodeling and gene expression have been published recently (4, 5, 21, 22). Three pairs of enzymes are involved in the reversible modifications of DNA and histones, resulting in changes to the chromatin structure. DNA methyltransferases (DNMTs) and demethylases dictate DNA methylation. Histone acetyltransferases (HATs) and deacetylases (HDACs) modulate acetylation of histones, whereas histone methyltransferases (HMTs) and demethylases (HDMs) determine methylation at lysine or arginine residues of histones.

DNA methylation in the context of the sequence of 5'-cytosine-guanosine (CpG) controls genome stability, gene imprinting and gene transcription. This process is regulated by three DNMT enzymes. These enzymes can be further classified as maintenance methyltransferases (DNMT1), which copy pre-existing methylation marks onto new DNA strands during DNA replication, or *de novo* methyltransferases (DNMT3A, 3B), which methylate previously unmethylated CpG sequences.

DNMTs play key roles in initiation and progression of cancer, in addition to their essential roles in normal development and cell physiology. Several early studies have shown that both *Dnmt1* and *Dnmt3b* play important roles in carcinogenesis and survival of cancer cells. A recent study showed that *Dnmt3a* also plays an essential role in tumorigenesis of melanoma (23). Cancer cells have aberrant patterns of DNA methylation including DNA hypermethylation of certain gene promoters and global demethylation of the genome. One or more DNMTs are often overexpressed in a variety of cancers. Some tumors exhibit aberrant concurrent hypermethylation of numerous genes, a phenomenon known as the CpG island methylation phenotype (CIMP), first described in a distinct subset of human colorectal carcinomas (24), and then in a variety of other human neoplasms. The molecular causes of CIMP are not well understood. However, increased expression and aberrant targeting of DNMTs and SIRT1 expression could contribute to the occurrence (25, 26). DNA hypermethylation is one of the major silencing mechanisms for many TSGs in cancer cells (1–5).

Although the enzymology of DNMTs is well understood, the enzymes that remove methylated cytosines from DNA have remained enigmatic. Two recent reports suggest that DNA demethylation is initiated by the same enzymes that establish the methylation mark in the first place, i.e., DNMT3A and DNMT3B (27–29).

All known acetylations of histones 3 and 4 are correlated with an active promoter and gene transcription (30). HATs and HDACs are the enzymes that catalyze the reversible acetylation and deacetylation of histones as well as other proteins. HDACs are considered to be among the most promising targets in drug development for cancer therapy. HDAC

proteins comprise a family of 18 members in humans and are separated into four classes based on their homology to yeast proteins (31, 32). Class I includes HDACs 1, 2, 3 and 8. Class II consists of six HDAC proteins: HDACs 4, 5, 6, 7, 9 and 10. Class III consists of seven members that are homologs of Sir2 proteins in yeast: SIR1 to SIR7. HDAC11 is the sole member of class IV based on phylogenetic analysis. Class I, II and IV HDAC proteins operate by a metal ion-dependent mechanism. In contrast, class III HDAC proteins operate by a NAD<sup>+</sup>-dependent mechanism unrelated to the other HDAC proteins.

Histone methylation not only plays a key role in establishing and maintaining stable gene expression patterns during cellular differentiation and embryonic development but also in cancer (33). The methylation of histones can be an activating or repressive epigenetic mark depending on which lysine or arginine residue is methylated and how many methyl groups on the residue are added (34). Histone lysine/arginine methylation is a dynamic process in which sequence-specific *N*-methyltransferases and demethylases function to add and remove methyl groups. Among the HDMs, the largest family is the JmjC-domain-containing family, whose members are Fe (II) and 2-oxoglutarate-dependent oxygenases. The human JMJD2 HDM subfamily has six members, of which JMJD2A–C have been shown to catalyze demethylation of the methylated forms of histone 3 lysine 9 (H3K9) and histone 3 lysine 36 (H3K36), whereas JMJD2D is selective for the demethylation of H3K9. The dynamic interactions between the two classes of enzymes and their biological functions have been well reviewed (35).

It is important to bear in mind that DNA methylation and histone modification are both independent yet interrelated (36, 37). There is crosstalk among these two modifications at multiple levels. Recent studies further support this notion. Several HDAC inhibitors can reduce one or more DNMT proteins via mechanisms such as ubiquitin-dependent proteasomal degradation of DNMT1 or decreased DNMT3B mRNA stability (38–41). The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation probably due to the fact that LSD1 demethylates and stabilizes *Dnmt1* (42). The multiple interactions of the multidomain protein Np95 with hemimethylated DNA and repressive histone marks as well as DNMTs and HMTs integrate the two major epigenetic silencing pathways (43).

### Approved epigenetic drugs

Only three epigenetic drugs have been approved by the US Food and Drug Administration (FDA). Two are DNMT inhibitors: 5-azacytidine (azacitidine; Vidaza<sup>®</sup>) and 5-aza-2'-deoxycytidine (decitabine; Dacogen<sup>®</sup>), and one is an HDAC inhibitor: suberoylanilide hydroxamic acid (SAHA or vorinostat; Zolinza<sup>®</sup>). 5-Azacytidine was the first epigenetic drug approved by FDA in 2004, and gained approval in Europe in 2008. 5-Azacytidine and decitabine are used to treat patients with a bone marrow disorder known as myelodysplastic syndromes (MDS). MDS are characterized by the pro-

duction of abnormal, immature blood cells that often progress into terminal blood cancers. MDS are difficult to treat and higher risk patients have a median survival rate of less than one year. In a recent phase III clinical study, Vidaza<sup>®</sup> was able to double the survival time for patients with higher risk MDS (44). The HDAC inhibitor vorinostat has been approved for treating refractory cutaneous T cell lymphoma (CTCL) (45).

Additional FDA-approved drugs for other indications can also possess properties of epigenetic drugs. Valproic acid (VPA; Stavzor<sup>®</sup>) is a commonly prescribed drug for the treatment of epilepsy and is also effective as a mood stabilizer and in migraine therapy. VPA has also been determined to be a potent HDAC inhibitor (46). In addition, VPA could induce active DNA demethylation (47). Arsenic trioxide (Trisenox<sup>®</sup>) is a chemotherapeutic drug approved to treat patients with acute promyelocytic leukemia. It was subsequently discovered that arsenic trioxide is also a DNMT inhibitor (41, 48). In fact, researchers have hypothesized that many commonly used FDA-approved pharmaceutical drugs can cause persistent changes in the epigenomes of cells (49), and thus potent epigenetic drugs could be found by carefully screening these drugs.

It has become increasingly clear that other key epigenetic enzymes exist (50). They could be targeted by small molecule inhibitors and offer greater specificity of action. These potential targets include HMTs, HDMs and ubiquitin-related enzymes. Some currently approved and emerging epigenetic drugs are listed in Table 1. It is worth mentioning that epigenetic drugs are intended for not only malignant disease but also other diseases such as HIV and syndromes involving chromosomal instabilities and mental retardation (2, 51).

## Molecular mechanisms of action

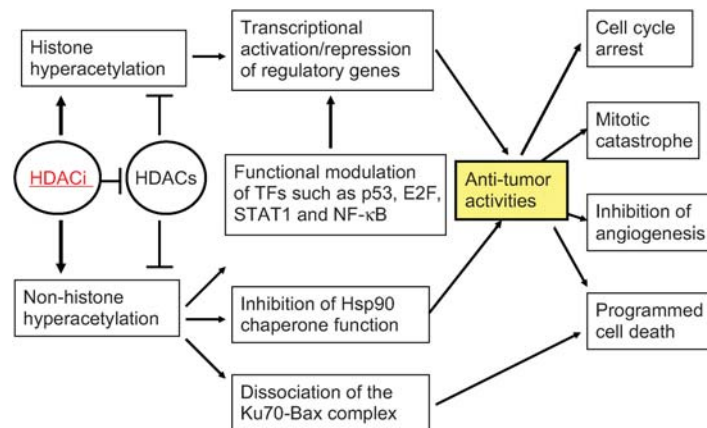
Epigenetic drugs are multifunctional agents that exert their effects on target cells via multiple mechanisms. They can modulate the epigenome of cells via epigenetic mechanisms. They can also induce cancer cell growth arrest, differentiation or cell death via non-epigenetic and/or epigenetic mechanisms. The following is an overview of the molecular mechanisms of action of some representative epigenetic drugs.

### DNMT inhibitors (DNMTi)

DNMTi display diversity in gene modulation and functional mechanisms in cancer cells (41, 52, 53). Decitabine is the second FDA-approved drug from the nucleoside analog family (54). It is a prodrug that requires activation via phosphorylation by deoxycytidine kinase. During DNA synthesis in the S phase, the nucleotide analog is incorporated into the nascent DNA chain where it produces an irreversible inactivation of DNMTs. In addition, decitabine induces proteasomal degradation of free DNMT1 enzyme through a mechanism that is dependent on DNA synthesis and the targeting of incorporated decitabine residues by DNMT1 itself (55). The demethylation of DNA in cancer cells by this and

**Table 1** Selected epigenetic drugs in clinical use or development.

Enzyme class	Inhibitor name	Compound class	Enzyme specificity	<i>In vitro</i> potency	Targeted cancer types	Developmental stage
DNMT	5-Azacytidine (Vidaza <sup>®</sup> )	Nucleoside analog	DNMT1, 3A, 3B	μM	Many	Launched for MDS
DNMT	RG108	Synthetic molecule (rationally designed)	DNMT	μM	?	Preclinical
HDAC	Vorinostat (Zolinza <sup>®</sup> )	Hydroxamic acid	HDAC class I, II, IV	μM	Many	Launched for CTCL
HDAC	Depeptide (Romidepsin, FK228)	Cyclic tetrapeptide	HDAC class I (1, 2)	nM	Many	Phases I–II
HDAC	Entinostat (SNDX-275/MS-275)	Benzamide	HDAC class I (1, 2, 3)	μM	Many	Phases I–II
Sirtuin	Resveratrol	Polyphenol	Sirtuins (and DNMT1, 3B)	μM	Colorectal; lymphoma; multiple myeloma	Phase I
HMT	Chaetocin	Thiodioxopiperazine	SUV39 family	μM	Multiple myeloma	Preclinical
HMT	BIX-01294	Diazepin-quinazolin-amine	G9A	μM	?	Preclinical
HDM	Pargyline	Benzylamine	LSD1 (low selectivity)	mM	?	Preclinical
HDM	Polyamine analogs	Polyamine	LSD1	μM	?	Preclinical



**Figure 1** Molecular mechanisms of anticancer activities by HDACi.

HDACi induce histone hyperacetylation, which in turn modulates gene activity at the transcriptional level. Alternatively, HDACi modulate non-histone protein hyperacetylation that leads to functional modulation of a number of TFs that also regulate transcription of other genes. The other major effects are the inhibition of hsp90 chaperone function and dissociation of the Ku70-Bax complex. All these events lead to antitumoral effects including cell cycle arrest, mitotic catastrophe, programmed cell death and inhibition of angiogenesis.

other analogs of cytosine can lead to the reactivation of silent TSGs, induction of differentiation or senescence, growth inhibition and loss of clonogenicity (52, 56). More specifically, decitabine at lower doses activates the p53/p21(Waf1/Cip1) pathway to inhibit cell proliferation (57). The drug induced apoptosis via p53 and gadd45-dependent mechanisms in colon cancer cells (58). A mechanistic association was established between induction of 15-lipoxygenase-1 by decitabine (at low dose) and apoptosis in colon cancer cells (59).

The DNA damage induced by decitabine also plays important roles in gene induction as well as in growth inhibition and cell cycle arrest. Decitabine treatment results in growth inhibition and G<sub>2</sub> arrest, both hallmarks of a DNA damage response. Decitabine led to formation of DNA double-strand breaks in an ATM (ataxia-telangiectasia mutated)-dependent manner, and this damage was repaired following drug removal (60). With low nanomolar concentrations of the drug, decreased proliferation and survival is associated with ATM activation, H2AX phosphorylation, increased p21(Waf1/Cip1) expression and induction of the genes known to be methylated in testicular germ cell tumors (61). DNA damage induced by DAC or 5-azacytidine upregulates p21(Waf1/Cip1) in a DNMT-independent manner via the DNA damage/ATM/p53 axis (62). DAC activates the ATM- and RAD3-related (ATR) signaling pathway and thus elicits a specific p53 phosphorylation-acetylation cascade to induce expression of p21(Waf1/Cip1) (63). The elevated p21(Waf1/Cip1) in turn triggers Emi1 downregulation that maintains G<sub>2</sub> arrest (64).

### HDAC inhibitors (HDACi)

Cancer cells often express high levels of HDAC isoenzymes, especially class I HDACs, and thus display hypoacetylation

of histones (65). HDACs remove the acetyl group from histones and induce hypoacetylation of histones; this causes chromosomal DNA to tightly wrap around histones and prevents access to transcription factors (TFs), leading to transcriptional repression (32). In general, HDACi can restrain HDAC activity and induce hyperacetylation of histones, promoting the binding of TFs to DNA and activating transcription. By contrast, HDACi can also repress the expression of other genes. Various studies showed that HDACi can upregulate or downregulate transcription of a large common set of genes that control important molecular pathways, including cell survival, proliferation, mitosis and angiogenesis (66, 67) (Figure 1).

HDACi can modulate gene expression and function via non-epigenetic mechanisms. HDACi have been reported to enhance acetylation of TFs, such as p53 (68), STAT1 (69), NF-κB (70), or affect transcription complexes containing HDACs with subsequent modulation of gene transcription (71). HDACi also indirectly affect many crucial proteins by hyperacetylating heat shock protein Hsp90 (58) and DNA end-joining protein Ku70 (72). Hsp90 is one of the most prominent chaperone proteins, functioning to facilitate the stability and activities of client proteins, many of which are cellular signal transducers such as protein kinases and TFs (73). Hypoacetylation is important for Hsp90 to maintain its chaperone function and HDAC6 was identified as the deacetylase of Hsp90 (64). HDACi, such as FK228, LBH589, LAQ824 and vorinostat, induce acetylation and inhibit the ATP binding and chaperone function of Hsp90. This promotes the polyubiquitylation and degradation of progrowth and prosurvival client proteins. These client proteins include EGFR, ErbB2, Src, Raf-1, Bcr-Abl, mutant FLT-3, AKT, Kit, androgen receptor, estrogen receptor and hypoxia-inducible factor-1α (HIF-1α) (68, 74–77). Ku70 functions to suppress apoptosis by sequestering Bax from mitochondria, whereas HDACi-induced hyperacetylation of Ku70 disrupts the

Ku70-Bax interaction and promotes Bax translocation to the mitochondria and thus induces apoptosis (72, 78–80).

The anticancer mechanisms of HDACi include cell cycle arrest, cell differentiation, mitotic catastrophe, and programmed apoptosis and necrosis. These mechanisms have been reviewed extensively (7, 10, 81, 82) (Figure 1). Studies illustrating how HDACi exert their anticancer activities via acetylation of non-histone proteins have been well reviewed by Spange et al. (83). The effects of HDACi on tumor angiogenesis will be discussed in the following section.

### HDACi target tumor angiogenesis

Tumor angiogenesis is necessary for tumor growth and metastasis; therefore, the inhibition of tumor angiogenesis offers a new strategy in anticancer therapy. HIF-1 $\alpha$  plays crucial roles in mediating tumor angiogenesis during hypoxia, a common environment for tumor cells. One of the key target genes of HIF-1 $\alpha$  is vascular endothelial growth factor (VEGF), which induces tumor blood vessel formation via activating VEGF-VEGFR signaling (84).

HDACi show great promise at inhibiting angiogenesis in preclinical animal models and early phase clinical trials (85). HDACi can efficiently repress HIF-1 $\alpha$  levels and its transcriptional potential by directly targeting HIF- $\alpha$  and p300 complex (86). HDACi TSA decreases expression of HIF-1 $\alpha$  and VEGF by increasing expression of its negative regulators of p53 and pVHL and thus inhibits angiogenesis (87). Hsp90 plays an important role in protecting HIF-1 $\alpha$  from p53 and pVHL-independent degradation through proteasome pathway. Recently, several studies showed that HDACi can repress HDAC6 and induce Hsp90 hyperacetylation, which results in the increased interaction and degradation of HIF-1 $\alpha$  by Hsp70 (88, 89). The class I HDACi might disrupt the function of Hsp90 indirectly via acetylation of Hsp70 and thus inhibition of its function (90). The efficient repression of HIF-1 $\alpha$  by HDACi provides a rationale for combining HDACi with other antiangiogenesis agents such as inhibitors of VEGF receptor which can offer great benefits by targeting multiple pathways in tumor progression and angiogenesis (91).

### HMT and HDM inhibitors

Greiner et al. reported the first inhibitor of a lysine-specific HMT in 2005 (92). This inhibitor, the fungal metabolite chaetocin, is specific for the methyltransferase SU(VAR)3–9. Other inhibitors have been discovered through screening chemical libraries. One inhibitor, termed BIX-01294, selectively impairs the G9a HMT and the generation of dimethylated lysine 9 of histone 3 (H3K9me2) *in vitro*. This is a biologically active HMT inhibitor that allows for the transient modulation of H3K9me2 marks in mammalian chromatin (93).

Inhibitors of HDM have been discovered and explored for cancer therapy. Inhibitors of monoamine oxidase such as *trans*-2-phenylcyclopropylamine and pargyline have been shown to inhibit lysine-specific demethylase 1 (LSD1), although their inhibitory activity and selectivity for LSD1

are very low (94). Recently, Ueda et al. have identified the first cell-active LSD1-selective inhibitors 1 and 2 which should be useful as lead structures in further drug development (95). Inhibition of LSD1 by polyamine analogs results in re-expression of aberrantly silenced genes in human colon cancer cells (96, 97). In summary, the inhibitors of HMTs and HDMs possess therapeutic potential for cancer and other diseases (98).

### Combination therapy with epigenetic drugs for leukemia and solid cancer

It has been shown that epigenetic drugs alone achieved only modest antitumor activity. Epigenetic drugs in combination with other modalities have often led to better therapeutic effects for leukemia and solid cancer in preclinical studies.

#### Combination with chemotherapy

A large number of studies have demonstrated better antitumoral effects of HDACi when combined with other chemotherapeutic drugs. These drugs include proteasome inhibitors (99, 100), death inducing ligand TRAIL (101), receptor tyrosine kinase inhibitor AEE788 (102), mammalian target of rapamycin (mTOR) inhibitor rapamycin (103), paclitaxel (104), retinoids (105) and Hsp90 inhibitor (75).

Proteasome inhibitor bortezomib combined with vorinostat, MS275 or VPA, showed synergistic induction of apoptosis in human multiple myeloma cells as well as other types of cancer cells via induction of oxidative injury, inhibition of aggresome formation and reduction of TRAIL protein degradation (106–108). HDACi have been demonstrated to effectively sensitize resistant cells to TRAIL-induced apoptosis in various types of cancer cells (109, 110). Importantly, TRAIL in conjunction with HDACi did not increase any cytotoxicity to non-malignant cells, such as normal prostate epithelial cells and hepatocytes (110, 111). The combination of vorinostat with murine DR5-specific monoclonal antibody MD5-1 synergistically induced apoptosis in various cancer cells *in vitro* and caused regression of established tumors *in vivo*, whereas single agent treatment had little or no effect in a mouse breast cancer model (99, 112). In summary, the combination of HDACi with chemotherapeutic drugs could be an effective treatment strategy for various tumor types.

#### Combination with immunotherapy

Metastatic cancer utilizes several immune escape mechanisms to go undetected. One key mechanism is to down-regulate a cassette of genes involved in antigen processing and presentation (113–115). Several studies have revealed that these genes are under epigenetic control in malignant carcinomas. The epigenetic silencing of these genes could be reversed by epigenetic drugs such as HDACi, resulting in enhanced immune recognition and improved immunotherapy (113, 116–118). Many investigators including us have revealed that cancer-germline (CG) antigens could be induced by DNMTi and/or HDACi, and that these tumor

antigens provide excellent targets for cancer immunotherapy (119–121). It is noteworthy that CG antigens are expressed constitutively and can be further upregulated by epigenetic agents in cancer stem cells, offering a key immunological target for therapeutic strategies targeting this critically important type of cancer cell (122). In summary, the MHC antigens, tumor antigens, and proteins in the antigen processing and presentation machinery could be upregulated by epigenetic drugs if they are aberrantly downregulated by epigenetic mechanisms in cancer cells. The upregulation of these proteins and restoration of the immunological functions sensitize cancer cells to subsequent cancer immunotherapy. A model for enhanced therapeutic efficacy by combining epigenetic drugs and other modalities such as immunotherapy is proposed (Figure 2).

Different classes of HDACi can exert their functions via different mechanisms. Some HDACi can enhance the production and suppressive functions of FoxP3(+) regulatory T cells (124). In the context of immunotherapy, it might be necessary to avoid the use of this particular type of HDACi. However, they could be useful in the settings where immunosuppression is desired.

### Combination with oncolytic virotherapy

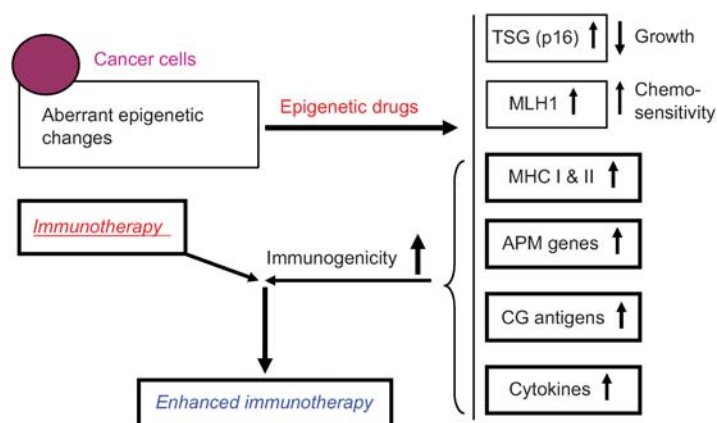
Oncolytic viral therapy represents a promising novel approach for cancer treatment. However, it is likely that some combination therapy will be necessary to have a meaningful impact on this disease (125). Several studies have shown that HDACi could enhance antitumoral effects by oncolytic viruses. This has been demonstrated for adenovirus (Ad) (126, 127), herpes simplex virus (128, 129), vesicular stomatitis virus and vaccinia virus (130). There could be multiple mechanisms involved in such effects. For example, FR228 (romidepsin) enhanced the expression of coxsackie

and adenovirus receptor, the receptor for Ad subgroup C, resulting in increased infection efficiency of Ad5 in lung cancer cells (127). It is interesting to note that HDACi inhibit cellular innate antiviral responses such as production of interferons, thus enhancing replication of oncolytic herpes simplex virus, vesicular stomatitis virus and vaccinia virus (128, 130). This particular mechanism of drug action can create a friendly tumor microenvironment to promote replication of a variety of oncolytic viruses in cancer cells (131).

### Epigenetic agents for cancer chemoprevention

Cancer is a growing health problem around the world owing to aging populations, increasing urbanization and global environmental changes. In the United States, approximately 1.5 million new cases and 562 000 deaths from cancer were projected to occur in 2009 (132). Cancer caused approximately 7.6 million deaths worldwide in 2005. At least one-third of all cancer cases are preventable through changes in lifestyle and improved prevention and screening policies, according to the World Health Organization (133).

Chemoprevention is a method to prevent or delay the development of cancer by taking medicines, vitamins or other agents. Tamoxifen, a selective estrogen receptor modulator, is the first FDA-approved chemoprevention drug. In women at high risk of developing breast cancer, tamoxifen reduces this risk by as much as one-half (134). Much more research needs to be done before effective and safe chemoprevention drugs are available for various types of cancer (135). Epigenetic events play an important role in carcinogenesis, thus investigators have focused on epigenetic events as targets for chemoprevention (16, 136–139). Several dietary phytochemicals, such as epigallocatechin-3-gallate (EGCG), sulforaphane (SFN), curcumin, genistein and quer-



**Figure 2** A model for enhanced therapeutic efficacy by combining epigenetic therapy with other therapies.

Cancer cells possess aberrant epigenetic modifications. Treatment with epigenetic drugs (such as inhibitors of DNMT, HDAC and HDM) leads to corrections in the epigenome of the cancer cells. The key effects include the reactivation of TSGs (such as p16) that inhibit tumor growth and DNA repair genes (such as MLH1) that increase sensitivity to chemotherapy. The treatment in many cases also enhances tumor immunogenicity by restoring expression of MHC class I and II antigens, other antigen processing and presentation machinery (APM) components (e.g., TAP proteins), inflammatory cytokines and CG antigens, as well as antigens expressed from activated transposons and endogenous retroviruses (123). These effects result in increased sensitization of cancer cells to subsequent immunotherapy.

etin, can function as epigenetic modulators. These and other epigenetic agents are promising agents for cancer chemoprevention (137–141).

### Epigallocatechin-3-gallate (EGCG)

EGCG is the most active and the major polyphenolic compound from green tea. A cell surface receptor for EGCG is the 67-kDa laminin receptor that confers EGCG responsiveness to many cells at physiological concentrations (141). The cancer preventive mechanisms of EGCG include the inhibition of metabolic activation of carcinogens and/or stimulation of their detoxification, scavenging of reactive oxygen species (ROS), induction of apoptosis or differentiation of malignant or transformed cells, and inhibition of angiogenesis or metastasis.

The receptor tyrosine kinases are one of the most critical targets of EGCG in cancer cells (142). Earlier research by teams of Weinstein and Rorke demonstrated that EGCG inhibits EGFR signaling pathway in various types of cancer cells (143, 144). The mechanisms of inactivation of EGFR can include alterations in lipid organization in the plasma membrane and sequestering of inactivated EGFR into endosomes (145, 146). EGCG-mediated downregulation of EGFR could also be achieved via phosphorylation at Ser1046/1047 by p38 MAPK in colon cancer cells (147). Through this and other signaling pathways, EGCG can negatively regulate protein serine/threonine phosphatase-2A to positively regulate p53-dependent apoptosis (148). NF- $\kappa$ B and AP-1 appear to be two downstream potential targets in exerting chemopreventive activities of EGCG (141, 149). EGCG also suppresses lung cancer cell growth through Ras-GTPase-activating protein SH3 domain-binding protein 1 (150).

As a DNMTi, EGCG can reactivate DNA methylation-silenced genes in cancer cells (52, 151). *RECK* is a novel tumor suppressor gene that negatively regulates matrix metalloproteinases and inhibits tumor invasion, angiogenesis and metastasis. EGCG partially reversed the hypermethylation status of the *RECK* gene and significantly enhanced *RECK* mRNA expression, resulting in reduced invasiveness of carcinoma cells (152). EGCG can reactivate glutathione-S-transferase pi by promoter demethylation and chromatin remodeling in prostate cancer cells (153) and it inhibited telomerase via both epigenetic and genetic pathways, leading to cancer cell death (154).

Interestingly, EGCG can trigger apoptosis or necrosis of breast cancer cells depending on the dosage (155). Low dose of EGCG (50  $\mu$ M) induced apoptosis, whereas high doses ( $\geq 100$   $\mu$ M) triggered necrosis in MCF-7 human breast cancer cells. EGCG exerted a dose-dependent effect on ROS generation and intracellular ATP levels in cancer cells, leading to either apoptosis or necrosis. The apoptotic cascade involves c-Jun N-terminal kinase activation, Bax expression, loss in mitochondrial membrane potential and activation of caspase-9 and caspase-3 (155). EGCG or polyphenon E from green tea have been examined in multiple clinical trials (156, 157).

### Sulforaphane (SFN)

People who consume higher levels of cruciferous vegetables, such as broccoli, Brussels sprouts and cabbage, reduce their susceptibility to cancer at a variety of organ sites. SFN, an isothiocyanate, is one of the key chemopreventive molecules in these vegetables. Induction of cell growth arrest and apoptosis, and induction of phase 2 enzymes represent two important mechanisms for chemoprotection often shared by SFN and other phytochemicals (158, 159). Intriguingly, SFN causes autophagy and inhibits apoptosis in human prostate cancer cells (160). Autophagy is another potential avenue for cancer prevention as it was concluded in a recent study that autophagy suppresses tumorigenesis through elimination of the signaling adaptor protein p62 (161).

One novel mechanism of chemoprotection by SFN is to function as an HDACi (162). SFN inhibited HDAC activity and suppressed tumorigenesis of colorectal cancer in *Apc<sup>min</sup>* mice (163). In a chemoprevention model for prostate cancer, SFN inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice (164). Interestingly, another mechanism of chemoprevention could be attributed to positive immunological consequences. SFN treatment is associated with enhanced cytotoxicity of natural killer cells in the TRAMP mice (164) and it can restore the age-related decrease of Th1 immunity (165). SFN has been undergoing clinical trials for its chemoprevention properties (166).

New data indicate that primary dysfunction in the tumor microenvironment can be crucial for carcinogenesis; therefore, effective chemoprevention should not only target pre-malignant and malignant cells but also the tumor microenvironment (167).

### Expert opinion and outlook

The first generation of FDA-approved epigenetic drugs has firmly established the notion that epigenetic modulation is a viable treatment option for cancer. However, this innovative and rapidly developing area of pharmacology is still in its infancy. New and substantial improvements are needed and expected in the coming years. According to a news report, approximately 30 epigenetic drugs are under development by more than a dozen biopharmaceutical companies and most of these drugs are indicated for cancer therapy (168). A Web search for clinical trials (via ClinicalTrials.gov) with a key word 'epigenetic drug' returned 29 studies most of which are related to cancer (169).

The second generation will most certainly possess higher specificity owing to better understanding of the roles of epigenetics in cancer, leading to intelligent designs of epigenetic drugs. As pointed out by Best and Carey, the future challenge for the biopharmaceutical industry exists in three related areas: biology, chemistry and development (50). Future drugs could include inhibitors targeting other epigenetic enzymes. Some endogenous molecules regulate the activity of class I HDACs *in vivo* (170). These molecules should be explored as potential targets as the field moves forward. MicroRNAs (mi-

RNAs) are important molecules in gene regulation in both cancer and normal cells. A specific subgroup of miRNAs called 'epi-miRNAs' directly and indirectly modulate the activity of the epigenetic machinery (171). Some miRNAs that are regulated by epigenetic mechanisms (172) could serve as important epigenetic targets.

In summary, we envision that, within decades, epigenetic drugs will become a standard class of pharmaceutical drugs for treatment of not only leukemia but also of many solid cancers. Additionally, some epigenetic drugs will be utilized for cancer chemoprevention.

## Highlights

- Three epigenetic drugs have been approved by the FDA to treat hematologic malignancies and a few dozen are under development for treating diseases including solid tumors.
- Owing to their ability to modulate gene expression to either enhance chemosensitivity, immunogenicity or dampen innate antiviral response of cancer cells, epigenetic drugs often lead to better efficacy when combined with chemotherapy, immunotherapy or oncolytic virotherapy.
- Cancer cells resistant to a particular inhibitor have been observed. Combination therapies can be employed to overcome this resistance.
- HDACs affect not only acetylation of histones but also acetylation of many other nuclear and cytoplasmic proteins. Thus, HDACi, especially pan-HDACi, appear to possess global effects on target cells.
- Lack of specificity is a common pitfall for first-generation epigenetic drugs.
- Intelligent design will foster the next generation of epigenetic drugs possessing higher specificities by targeting one specific enzyme or one subclass of enzymes.
- A better understanding of aberrant epigenetic changes in premalignant cells and malignant cells will lead us to more targeted epigenetic drugs.
- Identification of new targets (epigenetic enzymes) and development of new inhibitors can offer greater specificity and potency of next-generation epigenetic drugs.

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