Clin Oncol Cancer Res (2011) 8: 191–201
DOI 10.1007/s11805-011-0580-x

From Bench to Bedside: Targeting Epigenetics for Cancer Therapy

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Received November 1, 2011; accepted November 16, 2011

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ABSTRACT The initiation and progression of cancer not only involves genetic abnormalities, but also epigenetic alterations, such as DNA methylation and histone modifications. Epigenetics refers to the heritable changes that do not involve any structural changes in the target gene, i.e., DNA sequence and protein sequence. Thus, these epigenetic aberrations are potentially reversible, allowing the malignant cells to revert to a state with more normal characteristics. The use of epigenetics is emerging as an effective and promising approach to treat cancer. Epigenetic drugs, which target two well-known epigenetic pathways, namely, DNA methyltransferases and histone deacetylases, are already being applied for the cancer treatment. In the current study, an overview regarding the understanding of epigenetic alterations in the development of cancer and the current state of epigenetic drug discovery is provided.

KEY WORDS: cancer epigenetics, DNA methylation, histone modifications, epigenetic drugs.

Abbreviations: (DNMT), DNA methyltransferase; (HDAC), histone deacetylase; (HATs), histone acetyltransferases.

Cancer Epigenetics

Epigenetic alteration patterns in cancer cells

Epigenetic is a term originally defined by C.H. Waddington to describe the heritable changes in gene expression that are not related to changes in DNA sequence[1–3]. Two major levels of epigenetic modification have been indentified over the last five decades, namely, DNA methylation and histone modification. These epigenetic events play important roles in all aspects of biology, including replication, transcription, recombination, and DNA repair. In eukaryotic cells, DNA wrap around histones to form nucleosomes, which further associate to form the condensed structure of chromatin. Changes in chromatin structure through covalent modifications of histone proteins or DNA lead to the profound regulation of gene expression. The unexpected deactivating genes that are supposed to be active or activating genes that are supposed to be inactive by certain physical marks on histones or DNA over time would lead to failure in the control of cell cycle, programmed cell death, and eventually result in uncontrolled proliferation, namely, tumors.

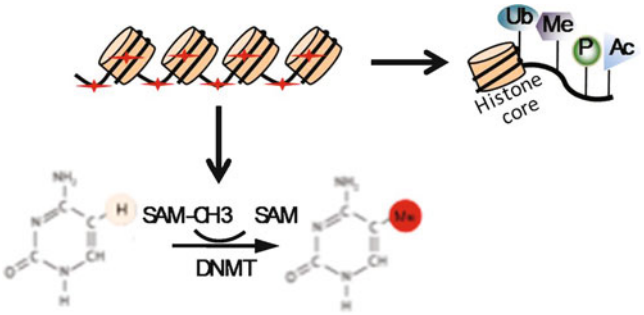

Fig.1. Epigenetic modifications. DNA is wrapped around histones, whose tails can be covalently marked by various types of modifications, such as acetylation, methylation, phosphorylation, and ubiqutination. DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group (CH3) from S-adenosylmethionine (SAM) to the 5-carbon position of cytosine to converse cytosine to 5-methylctosine.

DNA Methylation in cancer

The only known epigenetic modification of DNA in mammals is the DNA methylation, which involved the enzymatic addition of a methyl group to the 5-position (C5) cytosine of the pyrimidine ring. The reaction was carried out by DNA methyltransferases, using S-adenosylmethionine (SAM) as methyl donor (Fig.1). A study using high-performance liquid chromatography found that cytosines (1%) in a normal genome are methylated[4]. Initiation and progression of cancer were associated with the profound changes in DNA methylation patterns (Fig.2), which were the first identified as cancer-associated epigenetic alteration[5,6]. Promoter CpG-island hypermethylation and global hypomethylation occur frequently in tumor tissues. Low levels of DNA methylation in the promoter region were correlated with gene activation. Approximately half of the human genes, including housekeeping genes and tissues-specific genes, contain CpG-islands in their promoter regions that are usually low in methylation. Methylation of DNA near the transcriptional initiation site abolishes gene activation either by recruiting transcription repressors or by blocking the binding of transcription activators. Genome-wide hypomethylation contribute to chromosomal instability. Aberrant transcription initiations result in abnormal expression of genes that would normally be silenced by methylation[7–9]. Thus, the importance of DNA methylation in cancer had become a flourishing realm of investigation recently.

DNA hypermethylation in cancer

Abnormal DNA hypermethylation patterns are present more frequently in cancer cells compared to normal cells. Hypermethylation on chromosomes 3p, 11p, and 17p CpG-island containing regions that are normally unmethylated, was found in various human tumors by Baylin's group[10–14]. Another recent study showed that aberrant hypermethylation of CpG-island was wide-spread in the genome of different types of tumor cells[15]. This was an early event of transformation and would serve as an excellent biomarker for early cancer diagnosis. The methylation of CpG-island in the promoter region resulted in transcriptional silencing either by promoting or preventing the recruitment of regulatory proteins to DNA. For example, methylated DNA was recognized by histone deacetylases (HDACs), which mediate gene silencing[16,17]. Alternatively, recruitment of transcriptional c-myc to the targeting-binding site was impaired by DNA methylation[18]. To date, cancer-associated hypermethylation have been found in numerous tumor-susceptible genes. Inactivation of the tumor-suppressor Rb gene through hypermethylation, in its promoter region was found both in breast cancer and sporadic retinoblastoma[19–22]. Hypermethylation also occured in many other susceptible genes that were known or likely to play a role

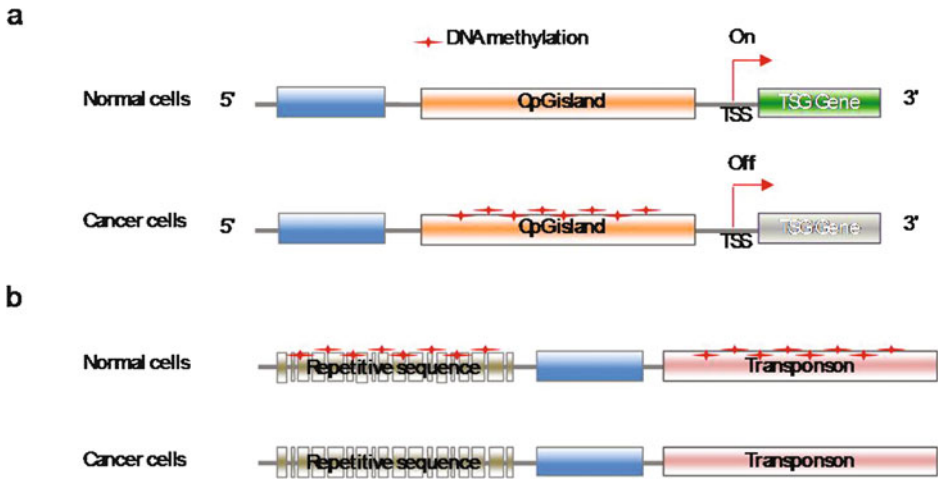

Fig.2. DNA methylation patterns in normal and cancer cells. A, Methylation of CpG islands. In normal cells, actively transcribed tumour suppressor genes (TSG) are associated with unmethylated CpG islands. During tumorigenesis, many CpG islands in tumor suppressor gene promoters become hypermethylated, leading to aberrant transcriptional silencing TSG genes. **B,** Methylation of repetitive elements and transponsons. Most repetitive elements and transponsons are hypermethylated in normal cells. In contrast, in cancer cells repeat-rich sequences and transponsons become hypomethylated which contributes to genomic instability, a hallmark of tumour cells, and tumorigenesis.

in carcinogenesis, i.e., p16INK4a, cell cycle regulation (p15INK4a), DNA repair (BRCA1) and apoptosis (DAPK). The abnormal hypermethylation in these susceptible genes were also associated with unfavorable clinical outcome.

DNA hypomethylation in cancer

Hypomethylation is the other type of methylation defect that was found in various malignancies. Although hypomethylation was first demonstrated to have a link with cancer in 1983, few details are known about the biological significance of aberrant DNA hypomethylation compared to hypermethylation in gene silencing. Hypomethylation of highly repeated DNA sequences[23–26], which resulted in the genomic instability and gene-specific hypomethylation events that lead to aberrant gene expression, are mainly responsible for the global DNA hypomethylation found frequently in different types of cancers such as breast, lung, prostate, cervical, and brain cancer, and show tight correlation with the grade of malignancy[27–31]. Hypomethylation in tumor cells was primarily due to the loss of methylation from interspersed repeats and tandem repetitive regions of the genome, including heterochromatic DNA repeats, dispersed retrotransposons, and endogenous retroviral element, which might promote tumor formation or progression by fostering DNA rearrangement[32–38]. Satellite DNAs were paired with array repeats, which composed of various oligonucleotide sequences and usually observed in constitutively heterochromatic chromosome regions. Hypomethylation of satellite 2 DNA from chromosomes 1 and 16, and satellite 3 DNA from chromosome 9 are usually found in human tumors ($> 50\%$), including human hepatocellular carcinomas, breast adenocarcinomas, and ovarian epithelial carcinomas[32,39,40]. Hypomethylation of endogenous retrotransposons and retroviral element were also associated with carcinogenesis. DNA demethylation activated the transcription of retrotransponsons retroviral-derived elements and promotes cancer-predisposing, or tumor progression-linked genomic rearrangement. Frequent activation of the expression of full-length transcripts from retrotransposons was found in certain types of murine cancer[41]. Several studies demonstrated that hypomethylation in Line-1 (Long interspersed nucleotide elements) and Alu-repetitive elements were higher in tumors than in normal tissues, such as lymphocytic leukemia[42], hepatocellular carcinomas[43], prostate carcinomas and neuroendocrine tumors. Proto-oncogenes were also found to be associated with hypomethylation of cancer-linked gene region, in addition to the highly repeated sequences. Hypomethylation of the gene-regulatory regions involved in the regulation of gene expression contributed to the carcinogenesis by altering the recruitment of transcriptional factors. Recently, the increasing hypomethylation of proto-oncogenes have been reported. Numerous proto-oncogenes that were upregulated in tumors (c-fos, c-myc, Ha-ras, and Ki-ras) had shown reduced levels of DNA methylation compared to the normal cells[44–46].

DNA methylation is catalyzed by a group of DNA methyltransferase enzymes (DNMTs). The DNMTs family contains DNMT1, DNMT1b, DNMT1o, DNMT1p, DNMT2, DNMT3a, DNMT3b with its isoforms, and DNMT3L. Among the DNMTs, DNMT1 is the most abundant DNA methyltransferase in mammalian cells and functions both as a maintenance methyltransferase and a de novo DNA methyltransferase[47–49]. Mice carrying the hypomorphic DNMT1 mutation resulted in genome-wide hypomethylation, having an increased risk of lymophoma[33]. Global loss of DNA methylation may act in concert to relieve gene repression in genomic regions that are usually silent in normal cells. These may directly or indirectly lead to the reexpression of proto-oncogenes or reactivation of transposons, followed by increased genomic instability, and eventual tumor formation and progression.

Histone modification in cancer

DNA is wrapped around a core of histones to form nucleosomes, which is the smallest structural unit of chromatin. Lysine, arginine, and serine residues in the N-terminal tails of histones were marked by varieties of post-translational modifications (Fig.1), including acetylation, methylation, phosphorylation, sumolyation, and ubiqutination[50]. The modification of histone tails had direct effects on various nuclear processes, including gene transcription, recombination, replication, DNA repair, and the organization of chromosomes. Genome-wide studies revealed that the combinatorial histone modification patterns in a specific genomic region can lead to an 'open' or 'closed' chromatin state; thus, resulting in the activation or repression of gene expression. For instance, histone marks, such as acetylation of histone H3 lysine 9 and 14 (H3K9ac and H3K14ac), trimethylation of histone H3 lysine 4 (H3K4me3), and monomethylation of histone H3 lysine 20 and histone H2B lysine 5 (H4K20me and H2BK5me), led to transcriptional activation. On the other hand, di- or trimethylated histone H3 lysine 9 (H3K9me2 and H3K9me3) and trimethylated histone H3 lysine 27 (H3K27me3) were associated with gene repression[51–53]. In addition to modulating the accessibility of chromatin, combinatorial histone modification can also serve as a marker to recruit subsequent protein interaction effectors, which are also involved in meditation of specific gene expression profiles. The combinatorial histone modifications, also called epigenetic code, determined the manifestation of a single eukaryotic genome in different developmental stages and that, if aberrant, will give initiate cancer and other diseases.

Similar to DNA methylation, the loss and gain of both acetylation and methylation of specific residues in histones were also observed in cancer cells, implying the alteration of general abnormal histone modification pattern across the genome (Fig.3). DNA hypermethylation in the promoter CpG-islands of tumor-suppressor genes of cancer were associated with a combinatorial histone modification pattern, such as losing histone H3/H4 acetylation and H3K4 trimethylation and gaining H3K9 methylation and H3K27 trimethylation, which

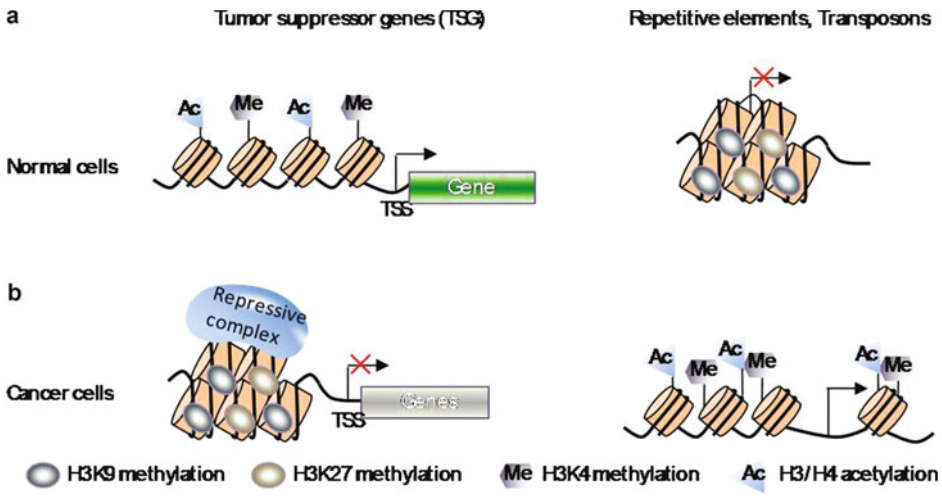

Fig.3. Combinatorial histone modification patterns in normal and cancer cells. A, In normal cells, the promoters of active tumor-suppressor genes (TSG) are enriched with combinatorial histone modification markers including acetylation of histone tails, methylation of lysine 4 on histone H3 (H3K4), and DNA repeats and transposons are associated with repressive markers including methylation of K27 and K9 of H3. **B,** In transformed cells, the promoters of TSG genes lose the 'active' histone marks and gain repressive methylation marks, such as lysine 9 or 27 on histone H3. On the other hand, DNA repeats and transposons are characterized by active marks, thus initiate unexpected transcription.

resulted in silencing certain tumor-suppressor gene (p21WAF1 and Rb)[7, 54].

Losing lysine acetylation, rather than gaining histone methylation, have been proposed to be the first event which leads to gene silencing. Histone acetylation is involved in various histone acetyltransferases (HATs), such as E1A-binding protein p300 (EP300), CREB-binding protein (CBP), GNAT (Gcn5-related N-acetyl-transferase), MYST3, and MYST4. The current study found that the binding of E1A and SV40T to p300 or CBP inhibited their acetyltransferase activity, which led to the loss of the global acetylation of H3K18, and consequent cellular hyper-proliferation and transformation[55,56]. Furthermore, the alterations of some HAT genes were found in various types of cancers. For instance, missense mutations of p300 increased the potential for malignant transformation of gastrointestinal, colorectal, gastric and breast tumors[57, 58]. In addtion, two CBP truncating mutations were indentified in ovarian cancer[59].

Abnormal HDAC activity has also been found to be associated with the development of different types of cancer, such as acute promyelocytic leukemia, acute myelogenous leukemia, gastric, and colorectal carcinomas. HDACs responsible for removing acetyl groups from histones were organized into three different classes based on their similarity to yeast HDAC proteins[60,61]. HDAC1-3 and 8 of the class I HDACs were localized in the nucleus. HDAC4-7, 9 and 10 of the class II HDACs were localized both in the nucleus and cytoplasm. SIRT1-7 of the Sir2-like class III HDACs, show sequence similarity to yeast transcriptional repressor Sir2[62]. HDACs bind to and deacetylate various target genes that play an important role in the control of cell growth, differentiation, and apoptosis. Alteration of HDAC genes in cancer seems to be common. The current study found that prostate cancer cells over express HDAC1 compared with their normal counterparts[63,64], with corresponding decrease in p21 expression. HDAC2 were over expressed in gastric carcinomas, colorectal carcinomas, cervical dysplasias, and endometrial stromal sarcomas[65,66]. HDAC3 was over expressed in colon cancer and also inhibited p21 expression[67]. The upregulation of HDACs resulted in chromatin compaction and inactivation of some growth suppressive genes, leading to the rapid proliferation of cancer cells.

Although losing histone acetylation was the primary event in gene silencing, the increased level of histone H3 methylation also played a critical role. Widespread changes in H3K9 methylation patterns were associated with aberrant gene silencing in various types of cancer[68,69]. G9a, a specific histone H3 lysine 9 methyl-transferase, had been found over expressed in liver and lung cancer and was implicated in uncontrolled proliferation possibly through the modulation of chromatin structure and transcriptional silencing[70–72]. Down-regulation of G9a results in chromosome instability and centrosome disruption in cancer cells. Furthermore, G9a knockout mice showed widespread genomic instability and increased incidence of lymphomas, suggesting the importance of H3K9me3 in maintaining chromosomal environments[70, 73].

The deregulation of H3K4me3 is also associated with cancer development. Both overexpression and abnormal mutations of chromosomal translocations of the MLL genes, which encoded the most thoroughly studied H3K4 methyltransferases, have also been reported in cancer. Rearrangement of the methyltransferase MLL1 led to the down-regulation of HOX genes and was responsible for the various forms of acute leukaemia by blocking hematopoietic differentiation[74]. MLL4 (KMT2D), which is involved in hepatitis B virus is dependent on liver carcinogenesis[75]. In addition, SMYD3, another H3K4 methyltransferase, was frequently found to be upregulated in colorectal, hepatocellular, and breast cancer, enhancing cell growth and promoting transformation[76,77]. Losing the responsiveness to PRMT1-mediated signals

contributed more broadly to leukemogenesis because PRMT1 is a key component of the Mixed Lineage Leukaemia (MLL) oncogenic transcriptional complex[78]. The activity of PRMT1 was usually found to be dysregulated in numerous types of cancer, including bladder, prostate, gastric, colon, colorectal, and breast cancer.

In addition to HMTs, histone demethylases (HDMs) that work together with HMTs to coordinate the global histone methylation patterns were also believed to contribute to tumorigenesis in various cancer types[79]. HDMs can be grouped in two families: the LSD family and the JMJC family. The LSD family can effectively remove mono- or di-methylated histone marker, such as H3K4 and H3K9 methylation; thus, acting as a co-repressor or a co-activator in gene regulation and involving in the cancer progression. The JMJC family proteins can also perform both activating and repressive functions by demethylating H3K27 or H3K4, respectively. Anomalous expression or activity of various members of the JARID1 family, specifically H3K4 demethylases, was found to have implications in cancer progression. Furthermore, certain mutations that inactivated histone H3K27 demethylase UTX were linked to different types of human cancer, whereas, low UTX activity tends to be a poor indicator of prognosis[80].

Epigenetic therapy of cancer

Tumorigenesis is associated with genetic and epigenetic alterations. Unlike mutations, the unique reversible property of epigenetic states provided exciting opportunities to develop novel epigenetic target drug to reactivate the expression of the epigenetically silenced cancer-related genes, such as tumor suppression genes, DNA mismatch repair genes, and cell cycle-related genes during tumorigenesis[81–84]. Five decades after covalent chromatin modifications were first discovered, two classes of epigenetic drugs that targets cellular epigenetic machinery, namely, DNMT inhibitors and HDAC inhibitors, which can effectively reverse aberrant DNA methylation and histone modification in cancer, respectively, have been discovered recently.

DNA methylation inhibitors

Among the epigenetic drugs, DNA methylation inhibitors were first proposed for cancer therapy. Two types of DNA methylation inhibitors, including nucleoside analogues and non-nucleoside analogues (Table 1), were utilized and characterized. Nucleoside analogues required DNA incorporation to function, whereas non-nucleoside analogues can directly block the DNA methyltransferase activity without DNA incorporation. 5-azacytidine (5-Aza-CR or azacitidine) and 5-aza-2′-deoxycytidine (5-Aza-CdR or decitabine), were the most widely studied nucleoside analogue DNMT inhibitors that were incorporated into the DNA in place of natural base cytosine during DNA replication in the S phase. Binding with the active sites of DNMTs, these drugs covalently trap these enzymes, which resulted in global DNA demethylation. 5-azacytidine was the first FDA-approved epigenetics-based drug for clinical use in the treatment of myelodysplastic syndromes and other hematological malignancies (Fig.4). Another cytidine analog that is stable in aqueous solution, 5-fluoro-2-deoxycytidine, is currently undergoing phase II study combined with other agents, such as cytindine deaminase inhibitor tetrahydrouridine, for the treatment of various tumors[85]. Zebularine, a novel DNMT inhibitor, is currently being investigated as an epigenetic therapy for cancer[86,87]. Unlike 5-azacytidine and 5-aza-2′-deoxycytidine, zebularine is more stable, which enables the drug to be delivered orally[88]. Although these properties make zebularine a promising candidate for cancer treatment, the requirement of higher dosage (1 g/1 kg body weight in mice) compared with 5-azacytidine and 5-aza-2′-deoxycytidine kept it from being used for clinical trials. Although these agents show promising anti-cancer efficacy, the toxicity associated with the incorporation of these nucleoside analogs into DNA resulted in the search for non-nucleoside DNMT inhibitors[2].

In the recent years, an increasing number of putative non-nucleoside inhibitors of DNMTs have been examined for their demethylating activity; and some of them were evaluated in pre-clinical models and in clinical trials[89,90]. These non-nucleoside inhibitors might be more promising

Table 1. DNMTs inhibitors in clinical development.

	DNA methyltransferase inhibitors	Alias	Target	Development stage
Nucleoside analogues	5-Azacytidine	Vidaza,Azacitidine	Celgene	FDA approved 05-2004
	5-Aza-2′-deoxycytidine	Decitabine, dacogen	Johnson & Johnson	FDA approved 05-2006
	5-Fluoro-2′-deoxycytidine	Fazarabine		Phase I
	5,6-Dihydro-5-azacytidine	DHAC		Phase I, II
	Zebularine			Preclinical
Non-nucleoside analogues	EGX30P	Oligonucleotide		NA
	Epigallocatechin-3-Gallate	EGCG		Preclinical
	MG98	DNMT1 antisense		Phase I
	RG108	NA		Preclinical
	Procainamide	Pronestyl		Preclinical
	Hydralazine	Apresoline		Phase I
	Psammaplin A	NA		Preclinical

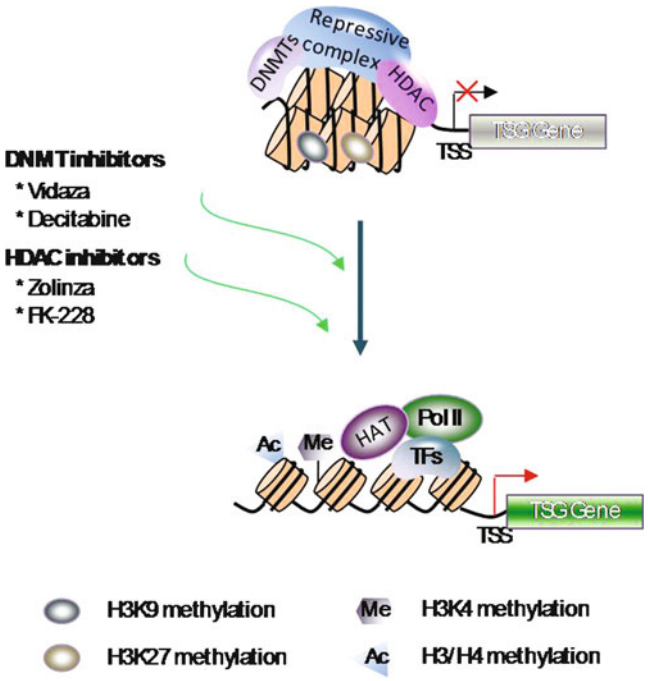

Fig.4. Epigenetic drug for cancer treatment. DNA methylation and histone deacetylation-mediated aberrant gene silencing in cancer involves recruitment of transcriptional repressive complexes in the gene promoter region. Four FDA approved DMNT inhibitors and HDAC inhibitors are effectively against cancer cells by inhibiting components of the epigenetic machineries leading to reactivation of critical genes. HAT: histone acetylase. Pol II: RNA polymerase II.

than the nucleoside analogues for clinical use because they inhibit DNA methylation by binding directly to the catalytic region of the enzyme without being incorporated into the DNA[91,92]. For example, procainamide inhibited the DNMTs by disrupting the interaction between DNMTs and its target sites[93,94]. Epigallocatechin-3-Gallate, a natural product from green tea, and RG108 specifically binded to the catalytic pocket of the human DNMT1 and rendered the enzyme inactive[91,95]. MG98, a phosphorothioate antisense oligonucleotide of the human DNMT1, which prevented the translation of DNMT1 mRNA, is currently being tested for clinical trials[96]. Other non-nucleoside analogue DNA-methylation inhibitors such as hydralazine, psammaplin A, and procaine are still undergoing studies[93, 97–100].

Histone deacetylase inhibitor

Aberrant tumor-suppressor gene silencing was tightly coupled with the loss of histone acetylation in cancer. Various compounds that inhibit HDACs and re-establish normal histone acetylation patterns, had demonstrated anti-tumor growth, proapoptotic, and prodifferentiation properties[101]. According to their chemical nature and mechanism of inhibition, HDAC inhibitors were classified into the following four groups: short-chain fatty acids, hydroxamic acids, cyclic tetrapeptides, and benzamides[81,102–104] (Table 2). Currently, two HDAC inhibitors (Fig.4), have been approved by the US Food and Drug Administration (FDA) for clinical use in the

Table 2. HDAC inhibitors in clinical development.

	Histone deacetylase inhibitors	Alias	Company	Development stage	Target specificity
Short-chain fatty acids	Valproic acid	Depakote, Depakene		Phase I, II FDA approved Anti-Seizure Drug	HDAC classes I, IIa
	Butyrates			Phase I, II	HDAC classes I, IIa
Hydroxamic acids	m-Carboxycinnamic acid bis-Hydroxamide	CBHA		NA	N/A
	PXD101	Belinostat	Topotarget	Phase I, II	pan-HDAC
	Oxamflatin			Phase I	N/A
	LAQ824			Phase I	HDAC 1 & 2
	Suberoylanilide Hydroxamic Acid	SAHA, Zolinza	Merck	FDA approved 10-2006	pan-HDAC
	Trichostatin A	TSA		NA	pan-HDAC
	LBH589	Panobinostat	Novartis	Phase II, III	pan-HDAC
	NVP-LAQ824			Phase II	N/A
	Pyroxamide			Phase I, II	HDAC Class I
Cyclic tetrapeptides	Apicidin			Preclinical	HDAC 1 & 3
	Depsipeptide	FK228	Celgene	FDA approved 11-2009	HDAC 1 & 2
	Cyclic hydroxamic-acid-containing peptide 1	CHAP1		NA	HDAC Class I
	Depudecin	Epoxide		NA	HDAC Class I
	Trapoxin A			NA	HDAC classes I, IIa
Benzamides	MS-275	Entinostat, Benzamidine	Syndax Pharmaceuticals	Phase II	HDAC 1 & 3
	CI-994 (N-acetyl dinaline)	Tacedinaline, Acetyldinaline	Pfizer	Phase II, III	HDAC 1 & 3
	MGCD0103	Mocetinostat	MethylGene	Phase II	HDAC 1 &3

treatment of T cell cutaneous lymphoma: vorinostat and romidepsin (SAHA and FK-228 formerly, respectively). The first generation of HDAC inhibitors was the small chain fatty acids, including sodium butyrate, arginine butyrate, sodium pheylbutyrate, and valproic acid. Short-chain fatty acids generally are not very potent in HDAC inhibition due to their short side chains, which limited their capability of binding with the catalytic pocket of HDACs[105–108]. However, short-chain fatty acids became a useful tool in studying the epigenetic regulation in tumorigenesis. Butyrate, the first known HDAC inhibitor, was synthesized in 1949. Trybutyrin, a stable and rapidly absorbed prodrug of natural bytyrate, was reported to be a potential preventive agent against cancer, including colon and gastric cancers[109]. Valproic acid, an anti-epileptic drug, was shown to selectively inhibit the HDAC1 catalytic activity in vitro at millimolar concentrations, and induce the degradation of HDAC2 through proteasomal pathway[110]. Valproic acid can be used in combination with other anticancer agents, and showed promise in combination therapy for cancer. Combination of valproic acid with oral hydroxyurea or 6-mercaptopurin was proven to be safe and effective in patients with advanced acute myeloid leukaemia[111], and has also been successfully used in combination with all-trans retinoic acid or decitabine in elderly patients with acute myelogenous leukaemia[112].

TSA, initially derived from Streptomyces hygroscopicus, was the first natural product that has been discovered to possess the HDAC inhibitor activity in 1990 and was widely used in research. The hydroxamic acid function group of TSA chelated a zinc ion in the active-site pocket of HDACs, thus, blocking their catalytic activity. TSA was shown to synergize with 5-Aza-CdR to inhibit tumor growth in a mouse cancer model[113]. However, its toxicity and low efficiency prevented it from being used for clinical trial and motivated the search for better molecules. Through quantitative structure-activity relationship and high-throughput transcriptional screening of a compound library, several novel and potent HDAC inhibitors, such as LAQ824, LBH589, and PXD101, were indentified and are currently under clinical trials[114–118]. The other member of the hydroxamic acid group, suberoyl anilide hydroxamic acid (SAHA), is the first HDAC inhibitor to get an FDA approval for clinical use in the treatment of T cell cutaneous lymphoma given orally or intravenously[119].

Cyclic tetrapeptides, one of the most structurally complex groups of HDAC inhibitor, were initially isolated from microorganisms. This group of HDAC inhibitors contained trifluoromethyl and pentafluoroethyl ketone, which served as a zinc binding functional group to inhibit HDAC activity. The natural product FK228, also known as depsipeptide, was derived from chromobacterium and displayed potent anti-tumor effects[120]. FK228 is the second FDA-approved drug from the nucleoside analog family. Trapoxin (a fungal product), binds covalently with HDACs via the epoxides, and irreversibly inhibited HDACs activity[121]. However, due to its instability and toxicity, it has not been used clinically. Apicidin, a novel cyclic tetrapeptide, is a potent fungal metabolite with broad spectrum of antiprotozoal activity against apicomplexan parasites via the inhibition of histone deacetylase (HDAC)[122]. Its clinical utility is currently being evaluated in anti-cancer clinical trials.

The benzamide-based HDAC inhibitors, which in general are less active than hydroxamic acids or cyclic peptides type of inhibitors, also inhibited HDAC activity selectively. MS-275 is a novel orally-active synthetic benzamide derivative that showed HDAC inhibition activity. It inhibited HDAC in vitro at micromolar concentrations by binding with the catalytic zinc ion[123]. A phase I study has been completed in patients with advanced acute leukemia[124]. MGCD0103 is an isotype-selective orally available, benzamide HDAC inhibitor, which primarily targets the human HDAC 1, 2, 3, and 11 but not other class of HDACs[125–127]. It has been evaluated in multiple phase I/II clinical trials for patients with advanced solid tumor or hematological cancers[128–130]. CI-994 (Tacedinaline) is another orally active HDAC inhibitor that has been tested in phase I/II studies in advanced solid tumors, including renal cell carcinoma and nonsmall cell[131]. However, the structurally detailed mechanism of its action in HDAC inhibition was not yet clearly understood. Additionally, CI-994 had demonstrated combination activity with gemcitabine, capecitabine, paclitaxel, and carboplatin, in a phase I trial for solid tumors[132–134].

Conclusion and Perspectives

Over the past twenty to thirty years, epigenetic drugs have attracted a great deal of interests owing to their potential as anti-cancer agents. These epigenetic drugs can regulate the expression of certain cancer-related genes, either by inhibiting the DNA methylation or by increasing the histone acetylation level; thus, inhibiting the proliferation of cancer cells and inducing apoptosis. A number of specific DNA methylation and HDAC inhibitors have been indentified and investigated; and some of them have been applied for the clinical treatment of various cancers. These inhibitors have also been applied to combinational therapy with other drugs to enhance the efficacy of existing anti-cancer therapies and reduce side effects, thereby improving the therapeutic index. The ultimate goal of the translational research was to develop the best anti-cancer epigenetic drug that will achieve high efficacy with minimal toxicity. However, the challenge for drug development, including epigenetic therapy, is the off-target effects of these drugs that would associate with toxicities. Whether HDACs or other chromatin-altering enzymes would provide these epigenetic drugs target specificity for particular DNMTs, remains a question that needs further research for an answer. The similarity of different catalytic site of the enzymes was the main reason of non-specific targeting of inhibitors. The current study found that the main difference among the enzymes was the shallow grooves around the rim of enzyme pocket[135]. This discovery has aroused hopes to search new epigenetic inhibitors that target the non-catalytic binding pocket of the enzymes that may affect the conformation

of the enzyme and inhibit the enzyme in a noncompetitive manner. Clearly, a well-established understanding of the underlying mechanisms of epigenetic regulation is extremely vital for the development of efficient and specific epigenetic inhibitors for cancer treatment. In addition to DNA methylation inhibitors and histone acetylation inhibitors, histone methylation-based epigenetic drugs are to be expected in the near future, which may open a completely new field of epigenetic therapeutics. The increasing understanding of epigenetic mechanisms will provide a rational approach to optimize drug efficacy, minimize toxicity, and further develop new classes of epigenetic drugs. Overall, the development of epigenetic drug is still in its early stage; thus, the continued progress of research and new breakthroughs in this field of study is expected in the future.

Acknowledgements

The authors would like to apologize to the other contributors whose work could not be thoroughly discussed due to the limited space. The authors would like to thank Daniel Tran for proofreading the manuscript.

Conflict of Interest Statement

No potential conflicts of interest were disclosed.

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