Epigenetic tools in potential anticancer therapy

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Human cancer represents a heterogeneous group of diseases that are driven by progressive genetic and epigenetic abnormalities. The latter alterations involve hypermethylation and hypomethylation of DNA, and changed patterns of histone modification, with resultant remodeling of the chromatin structure that cause deregulation of the transcription activity of many genes. Unlike the remarkable progress in understanding the processes by which DNA methyltransferases can regulate gene expression and histone deacetylases can induce alteration of chromatin structure, the roles of epigenetic events in tumors remain insufficiently explained. In contrast to genetic changes, the epigenetic alterations in cancer cells can be reversed by the inhibition of DNA methylation and histone deacetylation. Therefore, many inhibition agents for re-expression, predominantly of tumor-suppressor genes, have been identified and tested in laboratory models and numerous clinical trials. Despite in-vitro evidence that a single drug can lead to reactivation of methylated genes, inhibitors of DNA methyltransferases and histone deacetylases have been investigated in combination, or together with cytotoxic chemotherapy, radiotherapy, immunotherapy, or hormonal therapy to improve the therapeutic effect. Ongoing trials are recognizing that the identification of a target group of patients who are more likely to respond to the epigenetic therapy, defining of an optimal dose and schedule of treatment, and the development of more specific inhibitors with minimal unwanted side effects are necessary. Thus, new combinations of anticancer agents, including epigenetic modulators, may lead to a more effective control of cancer. ***Anti-Cancer Drugs*** **21:565–577 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Epigenetic alterations are heritable changes in gene expression without any accompanying changes in primary DNA sequences. They are caused by two main events: DNA methylation and histone/chromatin modifications. These changes, despite their heritability, could be reversible, raising the possibility of a new therapeutic target. Epigenetic regulation of gene expression is essential for the diversity of cell types during the development of the individual and for the maintenance of tissue-specific expression profiles in different cell types, despite the presence of identical DNA sequences in every cell. During the development of cancer, epigenetic regulation is disrupted, leading to the abnormal expression of many essential genes. Therefore, oncogenes, which are hypermethylated earlier, can be activated by the sequence-specific decrease of DNA methylation in tumors. On the other hand, tumor suppressor genes (TSGs), which are active earlier, are inhibited after the hypermethylation of their promoter sequences. Similarly, in cancer cells, altered histone posttranslational modifications with resultant remodeling of the chromatin structure are observed (Fig. 1).

Here we review the epigenetic processes in normal and tumor cells, and the potential contribution of epigenetic agents, including inhibitors of DNA methyltransferases (DNMTs) and histone deacetylases (HDACs), in anticancer therapy.

DNA methylation in normal and cancer cells

DNA methylation is the transfer of a methyl group (CH_3) from the universal methyl donor S-adenosylmethionine to the carbon in the fifth position of cytosine in the CpG dinucleotide sequence [1]. This transfer is mediated by any of three main DNA methyltransferases DNMT1, DNMT3A, or DNMT3B [2]. All DNMTs have multiple domains and are able to form variable complexes with other co-repressors. DNMT1 is known as a maintenance enzyme that preferentially binds to hemimethylated DNA and copies the pre-existing methylation patterns to the newly synthesized strand during DNA replication [2,3]. The DNMT3A and DNMT3B are generally accepted as de-novo methyltransferases because they can methylate completely unmethylated DNA sequences [3,4]. The other two methyltransferases, DNMT2 and DNMT3L, manifest very little or no DNMT activities, respectively [2,3]. The DNMT2 does not possess a large N-terminal recognition domain, and the DNMT3L protein may be catalytically inactive because of the absence of critical amino acid residues, but binds to DNMT3A and DNMT3B, thereby enhancing their DNA methylation activities [3,5,6].

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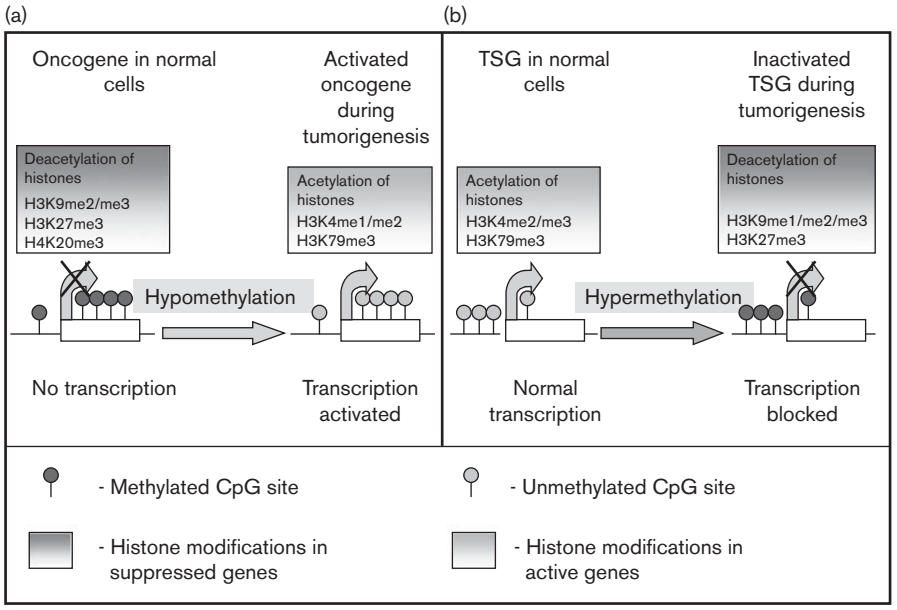
Fig. 1



Epigenetic regulation of cancer-associated genes. In cancer cells, oncogenes (a) are activated through DNA hypomethylation, histone acetylation, and methylation of lysines K4 and K79 in histone H3. In contrast, tumor suppressor genes (b) are inactivated during tumorigenesis by increasing the DNA methylation, histone deacetylation, and methylation of lysines K9 and K27 in histone H3. CpG, cytosine-guanine dinucleotide; Kme1/me2/me3, monomethylated, dimethylated, and trimethylated lysine; TSG, tumor suppressor gene.

Table 1 Locations and functions of DNA methylation in normal and cancer cells

	Normal cells		Cancer cells	
DNA methylation	Hypermethylation	Hypomethylation	Hypomethylation	Hypermethylation
Location	LINE, SINE, ALU, pericentromeric repeats Oncogenes Second X-chromosome Imprinted genes	TSGs Cell cycle controlling genes Housekeeping genes	Global: LINE, SINE, ALU, pericentromeric repeats Intergenic regions Oncogenes	Local: Promoters of TSGs DNA repair genes Cell cycle controlling genes
Function	Embryogenesis Imprinting X-chromosome inactivation Genome stability Regulation of gene transcription		Activation of viral sequences Activation of oncogenes Chromosomal instability	Inactivation of TSGs and cell cycle controlling genes Chromatin condensation

ALU, SINE sequences originally characterized by Alu endonuclease restriction; LINE, long interspersed nuclear elements; SINE, short interspersed nuclear elements; TSG, tumor suppressor genes.

In normal cells, DNMT functions are strictly regulated, but the mechanisms of this regulation have not been identified yet. DNA methylation patterns that are formed during early embryonic development remain relatively stable. They play an important role in processes such as cell differentiation, X chromosome inactivation, imprinting, protection against the expression of intragenomic parasitic elements (for example, *ALU* and *LINE* sequences), and maintaining genome stability by regulating gene transcription [3,7]. The locations and functions of the two methylation states in the normal cells are summarized in Table 1. The mammalian CpG dinucleotides have become strongly underrepresented during evolution as a result of spontaneous hydrolytic deamination of the relatively unstable 5-methylcytosine to thymine. These transitions occurring in the coding regions of genes can lead to pathogenic point mutations [7,8]. In human DNA, approximately 50–70% of CpG dinucleotides, mainly those located in the repetitive genomic sequences, are methylated [9,10]. The regions longer than 500 bp with a high density of CpG dinucleotides, where the G + C content equals or is greater than 55%, and the CpG to expected CpG ratio is greater than 0.65, are known as CpG islands (CGIs) [11]. They typically span the 5′ end of the gene region, including the promoter and exon 1 in many genes that are usually unmethylated in normal cells [12]. Approximately 60% of the human promoters contain CGIs [10]. Epigenetic modifications can be

transmitted into the next generation, similar to genetic alterations. They can regulate the expression of many genes, thereby affecting the functions of many pathways.

Recent developments in cancer research have shown that epigenome modifications are associated significantly with the development and progression of tumors. Reversibility of epigenetic alterations brings a new possibility of epigenetic therapy. Cancers, when compared with normal tissues, show widespread epigenetic alterations in DNA methylation, such as global DNA hypomethylation and hypermethylation of promoters in many cancer-related genes [13], as is schematically summarized in Table 1. A high variability of these processes in different types of cancers has been observed; therefore, intensive research is focused on the determination of the specific epigenetic profiles of these cancers.

Widespread DNA hypomethylation was found mainly in the repetitive sequences (*LINE, SINE, ALU*, pericentromeric regions), intergenic regions, and also in several oncogenes [1,14]. This may result in the upregulation of some oncogenes, loss of normal imprinting patterns, and genomic instability (Table 1) through alterations depicted in Fig. 1a. DNA hypomethylation occurs during the different stages of oncogenesis in a cancer type-specific manner [1]. Several studies have shown that DNA hypomethylation is already seen in early stages of urothelial, breast, and colorectal cancer development [15–17]. However, the hypomethylation could expand during tumorigenesis, as was shown in ovarian cancers and leukemias. Some investigators observed a correlation between the increasing DNA hypomethylation and the aggressiveness level of the cancer [18,19]. Other investigators have documented that DNA hypomethylation occurs during the later stages, and that it is typical for metastasizing prostate cancer [20].

Cancer-associated DNA hypermethylation is characterized by de-novo methylation of CGIs in the 5′ flanking region of TSGs, and in the DNA repair and cell cycle regulation genes [1,12]. Hypermethylation in the promoter sequences of these genes results in gene silencing. The scheme of TSG inactivation in tumor is depicted in Fig. 1b. The high variability of CpG island methylation levels and spectrum of inactivated genes was found in different types of tumors. Several examples of hypomethylated or hypermethylated genes related to cancer are summarized in Table 2. Several studies on hepatocellular, breast, and gastric carcinomas have documented that DNA hypermethylation in tumors is associated with the overexpression of DNMT1, DNMT3A, or DNMT3B [37–39].

However, the knockout of DNMT1 in the colorectal carcinoma cell line, HCT116, leads to only a 20% reduction in the overall genomic methylation content, mainly in the repetitive sequences, probably because of partial compensation by other DNMTs [40]. In accordance with this idea, the genetic disruption of both DNMT1 and DNMT3B caused very weak methyltransferase activity and a 95% reduction of genomic DNA methylation content [41]. It was shown that DNMT1 is necessary for the maintenance of aberrant CGIs methylation in human cancer cells; therefore, this enzyme has become one of the possible targets for the development of anticancer drugs [42]. Robertson *et al.* [43] showed that in the tumor tissues of the bladder, colon, and kidney,

Table 2 Examples of hypo- and hypermethylated genes in different cancers

Gene	Function	Methylation status	Cancers	Relation to cancer	References
MMP2	Degradation of extracellular matrix in embryogenesis	Hypo	Breast, prostate	Tumor invasion and metastasis	[21]
MAGEB2	Tumor-specific antigen recognized on melanoma cells by autologous cytolytic T lymphocytes	Hypo	Multiple types	Invasivity	[21]
MDR1	Energy-dependent drug efflux transporter	Hypo	Leukemia	Drug resistance	[7,22]
uPA	Protease	Hypo	Breast, prostate cancer	Metastasis	[21]
BRCA1, 2	DNA repair, maintenance of genome integrity	Hyper	Breast, ovary	Double-stranded breaks	[12,22]
CDH1	Cell adhesion	Hyper	Gastrointestinal, esophagus, breast, leukemia	Tumor invasion and metastasis	[7,12,23]
CDX1	Homeobox gene, colonic epithelium differentiation	Hyper	Colorectal	Impact on the differentiation of colonocytes	[24]
ER	Estrogen receptor	Hyper	Breast, ovary cancer	Hormone insensitivity	[12,25–27]
GSTP1	Detoxification	Hyper	Prostate, breast, kidney, hematological malignancies	Cancer chemoprevention	[12,28]
MGMT	DNA repair of 06–alkyl-guanine	Hyper	Multiple types	Genomic instability	[7,12]
MLH1	DNA mismatch repair gene	Hyper	Colon, gastric, endometrial tumors	Frameshift mutations	[7,12,22,29,30]
p15	Inhibitor of cyclin D/CDK4	Hyper	AML, MDS leukemia, multiple types	Insensitivity to anti-growth signals	[7,12,31,32]
p16	Inhibitor of cyclin D/CDK6	Hyper	Multiple types	Insensitivity to anti-growth signals, disrupt cell-cycle control	[7,12,13,22,33,34]
p21	Inhibitor of of cyclin E/CDK2 and cyclin D/CDK4	Hyper	Hematological malignancies	Cancer proliferation	[35,36]
RARβ2	Retinoic acid receptor-β2	Hyper	Colon, lung, head and neck	Insensitivity to retinoid signals	[12,29]
RASSF1A	Ras effector homologue	Hyper	Multiple types	Growth signal autonomy	[7,12]

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

and adjacent normal tissues, DNMT3B was overexpressed, whereas DNMT1 and DNMT3A increased only moderately.

Histone modifications in normal and cancer cells

During the regulation of gene expression, changes in DNA methylation are associated with numerous alterations in histones, namely H3 and H4. The histone modifications are very dynamic and rapidly changing processes, and their posttranslational modifications include lysine (K) acetylation, methylation of lysine and arginine residues, phosphorylation of serines and threonines, and ubiquitylation, ADP ribosylation and lysine sumoylation [44]. Variable combinations of histone modifications form the histone code, which influences gene expression in particular regions of the chromatin structure [45]. At present, the most widely investigated histone modifications are the acetylation and methylation of lysines. Acetylation of the ε-amino group in specific lysines of H3 and H4, such as H3K9, 14, 18 and H4K5, 8, 13, 16, makes the chromatin structure accessible for transcriptional factors, and is thereby associated with active transcription in euchromatin. These modifications are performed by histone acetyltransferases [44,46]. On the other hand, the deacetylation processes in transcriptionally silenced DNA sequences, together with hypermethylation of DNA were observed. Deacetylation is performed by HDACs that effectively remove the acetyl groups from histones, resulting in compact and inactive chromatin [47]. The HDAC family contains four classes of proteins, class I (HDAC1, 2, 3, and 8), class II (HDAC4, 5, 6, 7, 9, 10), class III (SIRT1-7), and class IV (HDAC11) [7,31]. Enzymes of classes I, II, and IV exhibit high homology in both structure and sequence, and they require Zn^{2+} ions for their catalytic activities. In contrast, enzymes of class III, sirtuins, have no similarities with the other classes of HDACs and they require NAD^{+} ions for their catalytic activities [48]. Further histone modifications, namely methylation and demethylation, are performed by histone methyltransferases and histone demethylases, respectively. The different histone methylation patterns are characteristic for the active genes in euchromatin (H3K4me2/me3, H3K79me3) and for the silenced genes in the heterochromatin structure (H3K9me2/me3, H3K27me3, H4K20me3) [44,49]. The level of methylation in particular amino acid residues is important for the interaction of histones with cofactors. Therefore, histone methylation seems to be essential for the regulation of transcriptional activity [49].

In cancer cells, histone-modifying enzymes may be disrupted by mutations, or they may be overexpressed, usually leading to an improper histone code. Variable levels of HDACs were found in various types of tumor [50]. For example, patients with renal cell cancer overexpressed HDAC isoforms 1 and 2 [51]. On the other hand, the knockdown of HDAC8 results in the inhibition of proliferation, reduction of clonogenic growth, cell cycle arrest, and differentiation in cultured neuroblastoma cells [52]. The protein complexes of HDACs with methyl-CpG binding proteins (MBDs), DNMTs, or some transcriptional repressors are able to inactivate many genes [28,53,54].

In more detailed studies of cancer cells, the promoters of silenced TSGs can be subjected to DNA methylation and aberrant histone code, occurring as a result of the decrease in lysine acetylation, H3K4 dimethylation and trimethylation, together with an increase in monomethylation, dimethylation, and trimethylation of H3K9, and H3K27 trimethylation [55]. These changes are summarized in Fig. 1b. Furthermore, the loss of H4K16 monoacetylation and H4K20 trimethylation was observed in the early stages of cancers, and these alterations were frequently associated with the DNA hypomethylation of repetitive sequences [56].

Interestingly, genes of the TGFβ pathway can be inhibited in human mammary epithelial cells through H3K9 dimethylation and deacetylation, before promoter DNA methylation [57]. Similarly, another study has shown that epigenetic silencing of the *p16* gene in DKO cells is caused by H3K9 methylation, before DNA methylation and H4 deacetylation. The authors suggest that the alterations in DNA and H4 might serve to lock the chromatin structure in a specific repressed state, which was originally initiated by the methylation of histone H3K9 [33]. In contrast, the HCT-116 colon cancer cells lacking DNMT1 had a disorganized nuclear architecture and altered H3 patterns, including an increase of acetylation and a decrease of K9 dimethylation and trimethylation, compared with the wild type cells [58]. Furthermore, Schulte *et al.* [59] observed that lysine-specific demethylase 1 (LSD1), which is one of the histone demethylase enzymes, was strongly expressed in undifferentiated neuroblastomas. Inhibition of LSD1 resulted in an increase in global H3K4 methylation and growth inhibition of neuroblastoma cells *in vitro*. These observations thus provide the first evidence that overexpressed LSD1 is involved in maintaining the undifferentiated, malignant phenotype of neuroblastoma cells. Therefore, targeting histone demethylases may provide a novel option for cancer therapy.

Other components of epigenetic silencing

In addition to the enzymes mentioned above involved in DNA methylation and histone modification, MBD proteins and noncoding RNA sequences are important components of epigenetic mechanisms of the regulation of gene expression. MBD proteins, namely MeCP2, MBD1, MBD2, and MBD3, operate as adaptors between methylated DNA and histone-modifying enzymes, including HDACs and histone methyltransferases [54,60]. These proteins are unable to bind to unmethylated

promoter sequences, but specific protein complexes of MBDs were found in methylated CGIs. In a study with breast cancer cell lines, only single or several MBD proteins bound specifically to hypermethylated TSG sequences [61]. For example, promoters of *RASSF1A* and *RARB* genes bound only to MeCP2, whereas promoters of *BRCA1* and *MGMT* genes were associated with MBD2. A combination of MeCP2 and MBD2 was found to be associated with the methylated *GSTP1* promoter sequence [28].

In the last two decades, several epigenetic studies have focused on microRNA (miRNA), which are short, noncoding, single-stranded RNAs with lengths of about 21–23 nucleotides. These sequences operate as endogenous silencers of numerous target genes. Moreover, several miRNAs act as tumor suppressors or oncogenes [62]. For example, the miRNA-29 family interacts directly with the untranslated 3′UTRs regions of *DNMT3A* and *DNMT3B* genes. The miRNA-29s were downregulated in lung cancer cells and acute myeloid leukemia cells. Conversely, higher expression of these miRNAs leads to the reduction of both DNMT3A and 3B enzymes, resulting in normal patterns of DNA methylation and the re-expression of some methylation-silenced TSGs, in both in-vitro and in-vivo experiments. These results indicate a potentially therapeutic usage of miRNA-29 as an effective demethylating agent [63,64].

Epigenetic regulation of gene expression seems to be a very complex and multifactorial process. A detailed understanding of the aberrant epigenetic events in cancer cells can help the development of a more effective therapeutic intervention by agents that have the potential to restore the earlier epigenetic profile of normal cells.

Inhibitors of DNMT

DNMTs are overexpressed in almost all types of tumors; thus, the promoter regions of numerous silenced TSGs are hypermethylated. The most widely investigated drugs with the ability to reverse aberrant DNA hypermethylation in inactive TSGs include 5-azacytidine (5AC, Vidaza), 5-aza-2-deoxycytidine (DAC, Dacogen, 5-aza-CdR), and 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one (ZEB, Zebularine). These compounds are able to incorporate into DNA (5AC, DAC), or RNA (5AC), sequences as cytidine analogues in the process of DNA replication or RNA synthesis, respectively [65]. DNA methylation is inhibited by covalent binding between the cytidine analogs incorporated in the DNA chain and DNMTs [66]. The US Food and Drug Administration (FDA) has approved 5AC in 2004 and DAC in 2006, for the therapy of myelodysplastic syndrome [67–69]. However, cytidine analogues can be deactivated by cytidine deaminase (CD); this seems to be one of the resistance mechanisms to these drugs [70,71]. ZEB, a cytidine deaminase inhibitor and a chemically stable DNMTi, seems to be the most potent drug because of its low toxicity and stability in aqueous solution [72,73]. Its inhibitory activity was confirmed in experiments in murine and human leukemia cell lines treated with a combination of DAC and ZEB, in which ZEB potentiated DAC by inhibiting CD, thereby enhancing the antileukemic activity of DAC [74,75]. Moreover, ZEB preferentially targeted cancer cells. Continuous treatment with this agent substantially inhibited the growth of analyzed human cancer cell lines. It was associated with a two to seven-fold induction of the *p21* mRNA level as compared with the normal fibroblast cell lines. Growth of the normal fibroblast cell lines was less affected without any changes in *p21* mRNA [76]. Consistent with the experiments conducted earlier, demethylation of *p16*, *p15INK4B*, and other genes was observed in the bladder tumor cell lines after ZEB treatment [31,34]. Furthermore, ZEB, in contrast to the AC, did not exhibit any influence on lytic or latent Epstein–Barr virus (*EBV*) gene expression in EBV-harboring Burkitt's lymphoma Akata cells. Therefore, ZEB might be safer than AC for the treatment of EBV-harboring tumors [23]. In contrast to these results, a recent study evaluating the 5AC, DAC, and ZEB treatments in acute myeloid leukemia blasts indicated a higher demethylating activity of DAC compared with 5AC, whereas ZEB caused no hypomethylation in the genes associated with leukemia pathogenesis [77]. The apparently contradictory DNMTi activities of ZEB could be explained by the different concentrations of the drugs used, 500 and 50 μmol/l, in experiments with and without a demethylation effect, respectively [31,77].

Recently, non-nucleoside DNMTis have been developed as additional effective compounds for epigenetic anticancer therapy. These small molecule inhibitors are able to reduce DNA methylation without incorporation into the DNA molecule. They can bind either directly to the catalytic site of the DNMT enzyme or to the CpG-rich sequences that prevent the binding between DNMTs and their target sequences [48]. One of these inhibitors, RG 108, directly blocks the DMNT active sites that cause the demethylation and reactivation of TSGs, but it does not affect the methylation of the centromeric satellite sequences [78]. Furthermore, procaine, which is also used as a local anesthetic, can bind to CpG-enriched DNA and thereby mask the DNMT target sequence. In breast cancer cells, procaine caused a 40% reduction of 5-methylcytosine in hypermethylated CGI and restored the expression of epigenetically silenced genes. Furthermore, it had a growth-inhibitory effect by causing mitotic arrest [29]. The derivate of procaine, procainamide, has been used for the treatment of cardiac arrhythmias. Moreover, in micromolar doses it can act as a partial competitive inhibitor of DNMT1. Procainamide inhibits the affinity of the enzyme to hemimethylated DNA and *S*-adenosyl-L-methionine, probably through its binding to GC-rich DNA sequences [29,79]. The polyphenol from green tea, (−)-epigallocatechin-3-gallate (EGCG), directly

inhibits DNMT activity [80]. Moreover, EGCG can inhibit angiogenesis and metastasis, and induces growth arrest and apoptosis through the regulation of multiple signaling pathways [81]. In addition, compound MG98, acts as an antisense oligonucleotide directed against the 3′ untranslated region of DNMT1 mRNA [22]. In a phase I study of MG98, the inhibition of DNMT1 expression was observed in 26 of the 32 patients with advanced solid tumors. Some clinical activity of this drug was seen, which was documented in one patient with partial response and further shown with prolonged disease stabilization [82]. However, in another study involving 23 patients with high-risk myelodysplasia or acute myeloid leukemia, no objective clinical response was noted [83]. Similarly, in a phase II clinical trial, no detectable effect of MG98 on DNMT1 decreasing activity in patients with metastatic renal cell cancer was observed [84]. Moreover, selected DNMT inhibitors with status of testing in preclinical studies and clinical trials are summarized in Table 3.

In contrast to the promising results, Chuang *et al.* [88] observed that non-nucleoside drugs including EGCG, hydralazine, and procainamide had weak inhibitory activities compared with the nucleoside analogues in different cancer cell lines. This discrepancy could be explained by the use of different methods, genes, or cell lines.

In general, both cytidine analogues and non-nucleoside inhibitors, because of their pleiotropic activities, seem to be effective modifiers of the cancer phenotype, and therefore hold promise as cancer therapeutic agents.

Inhibitors of HDAC

In addition to DNMTs, HDACs are also essential components in the regulation of gene expression. Recent studies have shown that the inhibition of HDACs could be a further prospective strategy for the reversion of aberrant epigenetic changes associated with cancer. Therefore, numerous preclinical studies have tested the anticancer activities of several classes of HDAC inhibitors (HDACis). Most of them, except the inhibitors of class III, act specifically against particular HDACs. Biological effects induced by the HDACi treatment range from cell growth arrest, to apoptosis and induction of terminal differentiation, but the effects of HDACis could be considerably broader and more complicated than was originally expected from their other pleiotropic effects [48,89].

HDACis are divided into several groups according to their chemical structure: short-chain fatty acids, hydroxamic acids, cyclic peptides, and benzamides [48]. The short-chain fatty acids include sodium n-butyrate (NaB), phenyl butyrate, and valproic acid (VPA, valproate, 2-n-propylpentanoic acid). Other compounds including trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA, Vorinostat, Zolinza), PXD101 (belinostat), LAQ-824, and scriptaid (SCR) belong to the hydroxamic acids group. Among the cyclic peptides is the depsipeptide (FK228, romidepsin). The group of benzamides contains MS-275 (SNDX-275), MGCD0103, and CI-994 [90,91]. Isothiocyanates with chemoprotective anticancer effects were identified in cruciferous vegetables [92]. Several studies indicate that one of them, sulforaphane, can act as an HDACi [93,94].

Mechanisms of HDAC inhibition were well documented in the studies on the activity of TSA. This compound binds to the HDAC active sites after chelation of Zn^{2+} ions, causing enzyme inactivation [90,95]. It was also found that TSA decreases the half-life of *DNMT3B* mRNA, thereby lowering the expression of relevant proteins in human endometrial cancer cell lines, and resulting in significant reduction of de-novo methylation activities of this DNMT [96]. Despite the fact that TSA is considered as one of the most potent HDACi

Table 3 Status of testing in selected DNMT inhibitors

Class	Inhibitor	Status of testing	Type of cancer	References
Nucleoside DNMTis	5-azacytidine (5AC), Vidaza	Phase I	Refractory solid tumors, head and neck carcinoma	[85]
		Phase II	AML	[67,86]
		Approved	MDS	[68]
	5-aza-2-deoxycytidin, Dacogen (DAC)	Phase I	AML	[86]
		Phase II	Renal cell carcinoma	[86]
		Approved	MDS	[69]
	Zebularine (ZEB), 1-(β-D-ribofuranosyl)-1, 2-dihydropyrimidin-2-one	Preclinical	Leukemia cell lines	[31,34,77]
Non-nucleoside DNMTis	RG 108	Preclinical	Leukemia, colon cancer cell lines	[78]
	Procaine	Preclinical	Breast cancer cell lines	[29]
	Procainamide	Preclinical	Colorectal cancer cell lines	[79]
	(−)-Epigallocatechin-3-gallate (EGCG)	Phase I	Breast cancer	[86]
		Phase II	Prostate cancer	[87]
	MG98	Phase I	Advanced solid tumors, high-risk myelodysplasia and AML	[82,83]
		Phase II	Metastatic renal carcinoma	[84]

All listed agents, except RG 108 and procainamide, were tested in variable combinations with inhibitors of histone deacetylases or together with chemotherapy, radiotherapy, immunotherapy, or hormonal therapy.
AML, acute myeloid leukemia; DNMTis, inhibitors of DNA methyltransferases; MDS, myelodysplastic syndrome.

according to results from in-vitro experiments, it has never been used in any clinical trials because of its high cytotoxicity.

Another hydroxamic acid, SCR, manifested relatively low toxicity and robust HDAC inhibition activity in pancreatic and breast cancer cell lines [97]. This compound induces the modifications of core histone tails by increasing H3K9 acetylation and H3K4 dimethylation, and by decreasing H3K9 dimethylation. In addition, after the treatment with both SCR and DAC, the demethylation and re-expression of the earlier hypermethylated *p16* gene in RKO colorectal cancer cells was observed [98].

Furthermore, SAHA inhibits HDACs through direct interaction with the catalytic sites of these enzymes, leading to the accumulation of the acetylated histones H2A, H2B, H3, and H4 [99]. In bladder carcinoma cells and endometrial stromal sarcomas, SAHA also increased the $p21^{WAF1}$ expression and caused accumulation of acetylated H3 and H4 [35,36].

VPA has been used for the past four decades in epilepsy and migraine therapy, and as an effective mood stabilizer [21]. This compound increases the level of H3 and H4 histone acetylation, which can cause the demethylation of some genes, especially in nondividing cells [21,100]. It is important to note that in the HEK293 cell line only small subsets of genes were induced by VPA. However, some of the activated genes were associated with metastasis (e.g. *MMP2*), or they were specifically expressed in different tumors as tumor-specific antigens (e.g. *MAGEB2*). For comparison, several of these genes were also activated by DAC, although less effectively than by VPA [21].

Benzamid MGCD0103, an isotype-selective HDACi, targets HDAC1, HDAC2, HDAC3, and HDAC11 under in-vitro conditions. In experiments *in vivo* with human tumor xenografts in nude mice, significant dose-dependent inhibition of tumor growth was found. In addition, the antitumor activity of MGCD0103 correlated with the induction of histone acetylation in tumor tissues [101].

In clinical trials, several HDACis, including phenylbutyrate, VPA, SAHA, PXD101, LAQ-824, depsipeptide, MS-275, MGCD0103, and CI-994, have been tested in various solid tumors and hematological malignancies; however, only SAHA has been approved by the FDA for therapy of advanced cutaneous T-cell lymphoma [102–108]. Detailed information about these clinical trials can be found on online databases [86,109]. In phase I, MGCD0103 was orally administered in patients with leukemia or myelodysplastic syndrome, in which the antileukemic activity was shown in three of the 29 patients who achieved a complete bone marrow response [110]. However, in most of the patients with advanced solid tumors of the colon, rectum, kidney or lung, no objective clinical responses were observed. Only five of the 32 patients with previously progressive colorectal, renal cells and lung cancers showed stable disease [111]. In patients with refractory solid tumors and lymphomas, MS-275 treatment was well tolerated in phase I clinical trials in which a partial drug response or prolonged disease stabilization were observed [108,112,113].

A recently synthesized HDACi, SB639, exhibited an excellent antitumor effect in the HCT116 xenograft model. This agent inhibits HDAC isoenzymes of classes I, II, and IV that are associated with the acetylation of histone H3 [114]. Several classes of HDAC inhibitors tested in different stages of preclinical or clinical studies are summarized in Table 4.

Although the FDA has approved SAHA for the treatment of cutaneous T-cell lymphoma, the treatment of solid tumors with HDACis treatment resulted in only modest antitumor activities. However, more effective combinations of HDACis with DNMTis or other chemotherapeutics may overcome the problem of high doses and low or no response to treatment in different cancers. In addition, intensive consumption of vegetables containing isothiocyanates and organosulfur compounds can be helpful in the inhibition of improper HDAC activities in human cancers as was observed in the colon, prostate, and breast cancer cell lines [120,121].

The cooperation of DNMTi and HDACi in the treatment of cancer cells

It is generally accepted that DNA hypermethylation is the dominant event in cancer-related gene silencing as compared with histone modification [122,123]. Therefore, DNMTis were supposed to be more potent reactivators of gene expression than HDACis. However, further studies have shown that histone modification, predominantly H3K9 methylation, could inactivate some genes before promoter hypermethylation [33,57]. Regardless of these results, no or moderate effects in gene reactivation after a single HDACi treatment were observed in a set of different genes [119,124]. Therefore, for the reversion of the epigenetic profiles of cancer cells, the simultaneous inhibition of DNMTs and HDACs was investigated in preclinical in-vitro experiments on cancer cell lines and *in vivo* in human xenografts in mouse models.

Breast cancer is one of the most prevalent cancers in women. The serious therapeutic problem of breast tumors with low levels of epigenetically inhibited estrogen receptor-α (ERα) is that they do not respond to the selective estrogen receptor modulator, tamoxifen [25]. Several in-vitro studies have shown that DAC and TSA treatment of ER-negative breast cancer cell lines results in the re-expression of functional *ERα* mRNA, and therefore sensitivity to tamoxifen is restored [116,117]. Similarly, co-treatment with DAC and scriptaid leads to the higher re-expression of *ER*, compared with treatment with only DAC or scriptaid. Moreover, the treatment of a xenograft

Table 4 Status of testing in selected HDAC inhibitors

Class	Inhibitor	Status of testing	Type of cancer	References
Short-chain fatty acids	Valproic acid (VPA), Valproate,	Phase I	Leukemia, MDS, brain cancer	[67,86]
	2-n-propylpentanoic acid	Phase II	Breast cancer, melanoma, MDS, AML	[67,86,106]
	Sodium n-butyrate (NaB)	Preclinical	Leukemia cell lines	[115]
	Phenyl butyrate	Phase I	Different solid tumors	[105]
Hydroxamic acids	Trichostatin A	Preclinical	Breast, prostate, lung cancer cell lines	[26,27,95,116–118]
	Suberoylanilide hydroxamic acid,	Phase I	NSCLC, leukemia, different solid tumors	[86]
	Vorinostat, Zolinza	Phase II	Breast cancer	[86]
		Approved	CTCL	[103]
	PXD101, Belinostat	Phase I	Solid tumors	[86]
		Phase II	Hematological malignancies, myeloma, ovarian cancer	[86]
	LAQ-824	Phase I	Different solid tumors	[107]
	Scriptaid (SCR)	Preclinical	Breast and colon cancer cell lines	[97,98,119]
	SB639	Preclinical	Colon cancer cell lines	[114]
Cyclic peptides	Depsipeptide, romidepsin, FK228	Phase II	CTCL, myeloma, different solid tumors	[48,86,108]
Benzamides	MS-275, SNDX-275	Phase I	Different solid tumors, lymphoma	[48,108,112,113]
		Phase II	MDS, NSCLC, breast cancer	[86]
	MGCD0103	Phase I	Leukemia	[86,110,111,108]
		Phase II	MDS, AML, different solid tumors, lymphoma	[86,108]
	CI-994	Phase II	Myeloma, pancreatic cancer	[86]
		Phase III	Lung cancer	[86]

All listed agents, except LAQ-824 and SB639, were tested in variable combinations with inhibitors of DNA methyltransferases or together with chemotherapy, radiotherapy, immunotherapy, or hormonal therapy.
AML, acute myeloid leukemia; CTCL, cutaneous T-cell lymphoma; MDS, myelodysplastic syndrome; NSCL, nonsmall cell lung cancer.

mouse model bearing MDA-MB-231 tumors with scriptaid or TSA alone caused a reduction of tumor size [119]. In addition, the epigenetic silencing of *ER* genes could play an important role in the tumorigenesis of prostate cancer because in several in-vitro studies on LNCaP, DU-145, and PC-3 prostate cancer cells, the *ER-α* and *ER-β* genes were re-expressed after combination treatment with TSA and DAC [26,27].

Lung cancer is one of the leading causes of cancer death in both men and women; therefore, several epigenetic studies *in vitro* were initiated in this type of tumor. A recent study using human lung cancer cell lines investigated the combination treatment with DAC and TSA or depsipeptide. In this experiment, HDACis impeded the removal of the DAC nucleotide analogs from the DNA strands, and therefore, together with DAC, synergistically inhibited cell proliferation [118]. Another example of the DAC and VPA combination treatment resulting in improved anticancer activity is an experiment with heterozygous $Ptch^{tm1Zim/+}$ mice. Epigenetic inactivation of the *Ptch* allele contributes to medulloblastoma or rhabdomyosarcoma formation. Combination of epigenetic inhibitors, but not the single agent, efficiently prevents this type of tumorigenesis. However, such treatment was successful only in the early-stage tumors in mice [124].

In addition to the anticancer activity of the simultaneous use of two inhibitors, several combinations of DNMTis and/or HDACis together with agents usually used in therapeutic modalities, such as cytostatics, hormones, or ionizing irradiation, also indicate some benefit for cancer patients [125,126]. The effect of VPA together with bosutinib (Src/ABL inhibitor) was analyzed in colorectal cancer cells *in vitro* and *in vivo*. VPA applied in low doses was able to enhance the cytotoxicity of bosutinib [127]. Furthermore, head-and-neck squamous cell carcinoma lines were pretreated by DNMTis with or without HDACis before ionizing irradiation. This combination leads to a significant radiosensitization effect [128].

As mentioned above, AC, DAC, and SAHA are frequently used in hematological anticancer therapy with considerable effect. Clinical trials in solid tumors are ongoing with more or less success. At present, phase I and II combination therapy trials in advanced solid cancers have documented some clinical responses. Several reports showed that cancer patients achieved complete response, disease stabilization, and partial response, but also no objective response [91,104,125,126,129]. However, widely reviewed preclinical and several clinical studies have shown promising results.

Advantages and disadvantages of epigenetic treatment in anticancer therapy

The most remarkable difference between genetic and epigenetic events in cancer development is that the latter can be reversibly manipulated with pharmaceutical agents aiming to reactivate the silenced tumor suppressor or several DNA repair genes. Despite effective clinical application of aza-nucleoside inhibitors and SAHA in patients with hematopoietic malignancies and intensive development of other new epigenetic inhibitors, predominantly in patients with solid tumors, any therapeutic outcome is difficult to achieve.

After treatment with the widely used drug, DAC, a re-methylating effect within several days [130,131], low drug stability [132], and rapid elimination by patients [133] were observed. In a recent study in colorectal cancer

Table 5 Advantages and disadvantages of epigenetic anti-cancer therapy resulted from pre-clinical studies and initial clinical trials

	Epigenetic therapy	
Inhibitors	Advantages	Disadvantages
DNMTis	High anticancer activities of single drug in hematologic malignancies Specific demethylation effect in proliferating cells Increase of anticancer activity in combination with HDACi, chemotherapy, radiotherapy, immunotherapy, and hormonal therapy Angiostatic effect	Short-term remethylation effect Low drug stability Rapid elimination of drugs Cytotoxicity of drug high doses No gene specificity
HDACis	Complex biological effects on cell growth arrest, apoptosis and terminal differentiation Higher effect in cancer stem cells than in normal stem cells Increase of anticancer activity in combination with DNMTi, chemotherapy, radiotherapy, and hormonal therapy Angiostatic effect	Moderate anticancer activities of single drug Cardiac toxicity

DNMTis, inhibitors of DNA methyltransferases; HDACis, inhibitors of histone deacetylases.

cells treated with DAC, the possible cause of DNA remethylation was identified. Re-expressed genes were surrounded by histones with epigenetic marks of both active and inhibited genes. This semiheterochromatic chromatin structure is likely unable to protect the reactivated tumor suppressors from restoring gene silencing [55]. Rapid remethylation was observed in a study of human MLH1 promoter methylation in xenograft-bearing mice, in which methylation was reversed up to 12 days after a single DAC treatment [30]. A long-term demethylation effect in the *p15* tumor suppressor gene was found after successive pulses of DAC in patients with myelodysplastic syndrome [32]; therefore, optimal treatment schedules need to be included. Many of the initial clinical trials for hematopoietic malignancies used the maximal tolerated dose of AC, but this therapy was often associated with bone marrow toxicity and a low dose response [134]. However, recent trials were characterized by the use of reduced doses and prolonged drug exposure, so the hypomethylation and not the cytotoxic effect of AC or DAC was prevalent. Long-term repeated injection administration of epigenetic drugs is a notable clinical disadvantage for recurrently hospitalized patients. However, oral administration of several agents can partially solve this problem.

Another problem of nucleoside DNMTis is cytosine deamination, which renders the drugs inactive by converting them into 5-azauridine compounds. A recent study of short oligonucleotides containing azapyrimidines, namely S110 dinucleotide, indicated its resistance to cytosine deamination and adequate demethylation effects in tumor cells after the administration of lower doses of DMNTi [135].

Several classes of structurally different HDACi showed, in addition to their role in the epigenetic regulation of gene expression, more complex biological effects including cell growth arrest, apoptosis, and induction of terminal differentiation. However, in single-agent treatments, only moderate and limited anticancer outcomes and several side effects, predominantly cardiac toxicity, were observed [126,129].

In contrast to conventional cytotoxic chemotherapy, epigenetic therapy may target specific cell populations in heterogeneous cancers. In accordance with the mechanism of action of nucleoside DNMTis, only dividing cells allow drug incorporation, whereas nondividing normal cells are left unchanged. This fact explains the higher therapeutic responses to these demethylating agents in hematological malignancies, arising from their higher number of proliferating cells, in the contrast to solid tumors. Moreover, it is possible that epigenetic drugs preferentially activate abnormally silenced genes in tumorigenic cells in contrast to nontumorigenic cells, because of the aberrant chromatin structure not allowing the methylation of their promoter sequences [136]. Furthermore, several studies have shown that cancer stem cells can be more sensitive to epigenetic modulators, predominantly HDACis, than normal stem cells [137,138]. In the contrast to several cell specificity indications, no gene specificity for epigenetic reactivation has yet been reported; therefore, the risk of oncogenes and the expression of other previously nontranscribed sequences, such as latent pathogenic viruses, remains relatively high.

The above-mentioned results of DNMTi and HDACi co-operation in reactivation of silenced genes or inhibition of cell proliferation indicate new possibilities in strategies of anticancer therapy. Moreover, the combinations of epigenetic inhibitors with other therapeutic modalities have a potential to improve the results of anticancer treatment. For example, in a preclinical ovarian cancer study, HDACi increased the activity of several chemotherapeutic agents such as docetaxel, paclitaxel, and carboplatin in the growth inhibition of multidrug-resistant cells [139]. Further studies have shown that cancer cell lines can be sensitized to several cytotoxic drugs acting on the DNA, namely VP-16, ellipticine, doxorubicin, and cisplatin after TSA or SAHA pretreatment. The authors supposed that a more accessible chromatin structure through histone acetylation allows more effective DNA damage by this type of agents [140]. Similarly, HDACis can act by several potential mechanisms such as radiosensitizers for therapeutic ionizing radiation, which has been shown in many clinical studies [125,141]. Moreover, two ERα-negative antihormone-unresponsive breast cancer cell lines were sensitized to tamoxifen through the enhancement of overall *ER* transcription activities, predominantly *ER-β*, after the exposure to TSA [142].

The therapeutic importance of DMNTis and HDACis, however, seems to be more complex. Recent studies showed that these inhibitors also have an angiostatic effect in cancers or indirectly by the reactivation of TSG

which are related with angiogenesis inhibition properties, or directly by decreasing tumor angiogenesis [143]. Furthermore, the interferon-induced apoptosis can be augmented by promoter demethylation of immune-modifying genes as was observed in the DAC-injected nude mice with xenografted melanoma cells. These mice became more sensitive to subsequent interferon treatment, and their tumors were significantly reduced as compared with the controls [144]. Similarly, clinical trials in patients with melanoma and chronic myeloid leukemia treated with interleukin 2 and imatinib mesylate, respectively, documented significantly supported anticancer response after the DAC administration [145,146]. The pluses and minuses of epigenetc therapy presented in the earlier chapters and this chapter are summarized in Table 5.

Cytotoxic chemotherapy could generate drug-resistant variant cells in tumor tissues by increasing the level of DNA methylation. Earlier studies in cancer cell lines and leukemic patients documented that exposure to high doses of variable chemotherapeutic agents led to drug-induced DNA hypermethylation, but only in cells with more then 90% reduction of DNA synthesis. Drug resistance could be induced by the epigenetic inactivation of the genes responsible for drug cytotoxicity [147]. This phenomenon was also indicated by the results in colorectal cancer cell lines exposed to low doses of the DNA damaging agent, 6-thioguanine. These cells manifested increased promoter methylation in the *HPRT* gene involved in cellular sensitivity to this drug and the *CDX1* gene with no functional relationship to this treatment [24].

In the studies mentioned above, single epigenetic drugs or the DNMTi/HDACi combination was administered as the primary treatment to improve the effect of sequential cytotoxic chemotherapy, radiotherapy, hormonal treatment, or immunotherapy. These dual therapies result in tumor stasis or apoptosis induction. However, the promising results in epigenetic activation of tumor suppressor miRNAs could lead to the silencing of oncogenes in cancer tissues [148]. A new strategy in anticancer therapy could present the administration of an epigenetic agent after the reduction of the tumor by conventional chemotherapy. The residual cells resistant to the cytotoxic agent are very probable cancer stem cells, which could differentiate after the subsequent epigenetic intervention [13].

Conclusion

Generally, human cancer represents a heterogeneous group of diseases. The differences in cancer types and individual pathogenic features of patients include wide variabilities that range from molecular alterations in tumor tissues to various clinical manifestations. Therefore, the universal scheme for cancer management remains only wishful thinking, and developing anticancer strategies has been focused on important points of tumorigenesis according to the latest results in cancer research.

Variable epigenetic drugs undoubtedly represent a new approach in anticancer therapy, notwithstanding some side effects and the partial understanding of the molecular mechanisms of its therapeutic outcome. To date, the high response rates of epigenetic therapies, including DMNT and HDAC inhibitors, were observed in hematological malignancies, but the effect of known epigenetic agents is not very encouraging in solid tumors. Actual clinical trials are focused on clinical and molecular identification of a target group of patients who will be more likely sensitive to the epigenetic therapy, defining of an optimal dose and schedule of treatment, and testing of more specific inhibitors for single treatment, or their combinations with conventional anticancer therapies.

Recent progress in the investigation of non-nucleoside DNMTis has shown several small molecules with higher specificity and lower toxicity in numerous preclinical studies. Moreover, most of the HDACis manifested more complete anticancer activities, but the new trend is the development of more effective drugs targeting one or only several types of HDACs. Other epigenetic tools for restoring the epigenetic profiles in tumors could be histone methyltransferases, histone demethylases, MBD proteins, or specific miRNA, which have not yet been investigated extensively. Finally, for a more tailored therapy, it would be helpful to discover drugs specific for cancer stem cells and that would regulate its clonogenic activities. This will presumably require several years of intensive study.

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References

1 Fog CK, Jensen KT, Lung AH. Chromatin-modifying proteins in cancer. *APMIS* 2007; **115**:1060–1089.
2 Hermann A, Gowher H, Jeltsch A. Biochemistry and biology of mammalian DNA methyltransferases. *Cell Mol Life Sci* 2004; **61**:2571–2587.
3 Pradhan S, Esteve PO. Mammalian DNA (cytosine-5) methyltransferases and their expression. *Clin Immunol* 2003; **109**:6–16.
4 Liang G, Chan MF, Tomigahara Y, Tsai YC, Gonzales FA, Li E, *et al.* Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. *Mol Cell Biol* 2002; **22**:480–491.
5 Hermann A, Schmitt S, Jeltsch A. The human Dnmt2 has residual DNA-(cytosine-C5) methyltransferase activity. *J Biol Chem* 2003; **278**:31717–31721.
6 Chen ZX, Mann JR, Hsieh CL, Riggs AD, Chédin F. Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. *J Cell Biochem* 2005; **95**:902–917.
7 Grønbæk K, Hother C, Jones PA. Epigenetic changes in cancer. *APMIS* 2007; **115**:1039–1059.
8 Wajed SA, Laird PW, DeMeester TR. DNA methylation: an alternative pathway to cancer. *Ann Surg* 2001; **234**:10–20.

9 Ehrlich M, Gama-Sosa MA, Huang LH, Midgett RM, Kuo KC, McCune RA, *et al.* Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res* 1982; **10**:2709–2721.

10 Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007; **128**:669–681.

11 Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proc Natl Acad Sci U S A* 2002; **99**:3740–3745.

12 Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007; **8**:286–298.

13 Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 2007; **128**:683–692.

14 Ehrlich M. DNA methylation in cancer: too much, but also too little. *Oncogene* 2002; **21**:5400–5413.

15 Florl AR, Löwer R, Schmitz-Dräger BJ, Schulz WA. DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. *Br J Cancer* 1999; **80**:1312–1321.

16 Jackson K, Yu MC, Arakawa K, Fiala E, Youn B, Fiegl H, *et al.* DNA hypomethylation is prevalent even in low-grade breast cancers. *Cancer Biol Ther* 2004; **3**:1225–1231.

17 Suter CM, Martin DI, Ward RL. Hypomethylation of L1 retrotransposons in colorectal cancer and adjacent normal tissue. *Int J Colorectal Dis* 2004; **19**:95–101.

18 Widschwendter M, Jiang G, Woods C, Muller HM, Fiegl H, Goebel G, *et al.* DNA hypomethylation and ovarian cancer biology. *Cancer Res* 2004; **64**:4472–4480.

19 Roman-Gomez J, Jimenez-Velasco A, Agirre X, Castillejo JA, Navarro G, San Jose-Eneriz E, *et al.* Repetitive DNA hypomethylation in the advanced phase of chronic myeloid leukemia. *Leuk Res* 2008; **32**:487–490.

20 Yegnasubramanian S, Haffner MC, Zhang Y, Gurel B, Cornish TC, Wu Z, *et al.* DNA hypomethylation arises later in prostate cancer progression than CpG island hypermethylation and contributes to metastatic tumor heterogeneity. *Cancer Res* 2008; **68**:8954–8967.

21 Milutinovic S, D'Alessio AC, Detich N, Szyf M. Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. *Carcinogenesis* 2007; **28**:560–571.

22 Goffin J, Eisenhauer E. DNA methyltransferase inhibitors-state of the art. *Ann Oncol* 2002; **13**:1699–1716.

23 Rao SP, Rechsteiner MP, Berger C, Sigrist JA, Nadal D, Bernasconi M. Zebularine reactivates silenced E-cadherin but unlike 5-azacytidine does not induce switching from latent to lytic Epstein–Barr virus infection in Burkitt's lymphoma Akata cells. *Mol Cancer* 2007; **6**:3.

24 Bredberg A, Bodmer W. Cytostatic drug treatment causes seeding of gene promoter methylation. *Eur J Cancer* 2007; **43**:947–954.

25 Keen JC, Davidson NE. The biology of breast carcinoma. *Cancer* 2003; **97**:825–833.

26 Li LC, Carroll PR, Dahiya R. Epigenetic changes in prostate cancer: Implication for diagnosis and treatment. *J Natl Cancer Inst* 2005; **97**:103–115.

27 Walton TJ, Li G, Seth R, McArdle SE, Bishop MC, Rees RC. DNA demethylation and histone deacetylation inhibition co-operate to re-express estrogen receptor beta and induce apoptosis in prostate cancer cell-lines. *Prostate* 2008; **68**:210–222.

28 Ballestar E, Paz MF, Valle L, Wei S, Fraga MF, Espada J, *et al.* Methyl-CpG binding proteins identify novel sites of epigenetic inactivation in human cancer. *EMBO J* 2003; **22**:6335–6345.

29 Villar-Garea A, Fraga MF, Espada J, Esteller M. Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. *Cancer Res* 2003; **63**:4984–4989.

30 Plumb JA, Strathdee G, Sludden J, Kaye SB, Brown R. Reversal of drug resistance in human tumor xenografts by 2′-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. *Cancer Res* 2000; **60**:6039–6044.

31 Scott SA, Lakshimikuttysamma A, Sheridan DP, Sanche SE, Geyer CR, DeCoteau JF. Zebularine inhibits human acute myeloid leukemia cell growth in vitro in association with p15INK4B demethylation and reexpression. *Exp Hematol* 2007; **35**:263–273.

32 Daskalakis M, Nguyen TT, Nguyen C, Guldberg P, Köhler G, Wijermans P, *et al.* Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-aza-2′-deoxycytidine (decitabine) treatment. *Blood* 2002; **100**:2957–2964.

33 Bachman KE, Park BH, Rhee I, Rajagopalan H, Herman JG, Baylin SB, *et al.* Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. *Cancer Cell* 2003; **3**:89–95.

34 Cheng JC, Weisenberger DJ, Gonzales FA, Liang G, Xu GL, Hu YG, *et al.* Continuous zebularine treatment effectively sustains demethylation in human bladder cancer cells. *Mol Cell Biol* 2004; **24**: 1270–1278.

35 Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. *Proc Natl Acad Sci U S A* 2000; **97**:10014–10019.

36 Hrzenjak A, Kremser ML, Strohmeier B, Moinfar F, Zatloukal K, Denk H. SAHA induces caspase-independent, autophagic cell death of endometrial stromal sarcoma cells by influencing the mTOR pathway. *J Pathol* 2008; **216**:495–504.

37 Saito Y, Kanai Y, Nakagawa T, Sakamoto M, Saito H, Ishii H, *et al.* Increased protein expression of DNA methyltransferase (DNMT) 1 is significantly correlated with the malignant potential and poor prognosis of human hepatocellular carcinomas. *Int J Cancer* 2003; **105**:527–532.

38 Girault I, Tozlu S, Lidereau R, Bièche I. Expression analysis of DNA methyltransferases 1, 3A, and 3B in sporadic breast carcinomas. *Clin Cancer Res* 2003; **9**:4415–4422.

39 Ding WJ, Fang JY, Chen XY, Peng YS. The expression and clinical significance of DNA methyltransferase proteins in human gastric cancer. *Dig Dis Sci* 2008; **53**:2083–2089.

40 Rhee I, Jair KW, Yen RW, Lengauer C, Herman JG, Kinzler KW, *et al.* CpG methylation is maintained in human cancer cells lacking DNMT1. *Nature* 2000; **404**:1003–1007.

41 Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, *et al.* DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. *Nature* 2002; **416**:552–556.

42 Robert MF, Morin S, Beaulieu N, Gauthier F, Chute IC, Barsalou A, *et al.* DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. *Nat Genet* 2003; **33**:61–65.

43 Robertson KD, Uzvologyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, *et al.* The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. *Nucleic Acids Res* 1999; **27**:2291–2298.

44 Margueron R, Trojer P, Reinberg D. The key to development: interpreting the histone code? *Curr Opin Genet Dev* 2005; **15**:163–176.

45 Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**:41–45.

46 Kouzarides T. Chromatin modifications and their function. *Cell* 2007; **128**:693–705.

47 Glozak MA, Seto E. Histone deacetylases and cancer. *Oncogene* 2007; **26**:5420–5432.

48 Zheng YG, Wu J, Chen Z, Goodman M. Chemical regulation of epigenetic modifications: opportunities for new cancer therapy. *Med Res Rev* 2008; **28**:645–687.

49 Grønbaek K, Treppendahl M, Asmar F, Guldberg P. Epigenetic changes in cancer as potential targets for prophylaxis and maintenance therapy. *Basic Clin Pharmacol Toxicol* 2008; **103**:389–396.

50 Weichert W. HDAC expression and clinical prognosis in human malignancies. *Cancer Lett* 2009; **280**:168–176.

51 Fritzsche FR, Weichert W, Röske A, Gekeler V, Beckers T, Stephan C, *et al.* Class I histone deacetylases 1, 2 and 3 are highly expressed in renal cell cancer. *BMC Cancer* 2008; **8**:381.

52 Oehme I, Deubzer HE, Wegener D, Pickert D, Linke JP, Hero B, *et al.* Histone deacetylase 8 in neuroblastoma tumorigenesis. *Clin Cancer Res* 2009; **15**:91–99.

53 Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol Cell Biol* 2004; **24**:306–319.

54 Robertson KD, Ait-Si-Ali S, Yokochi T, Wade PA, Jones PL, Wolffe AP. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat Genet* 2000; **25**:338–342.

55 McGarvey KM, Fahrner JA, Greene E, Martens J, Jenuwein T, Baylin SB. Silenced tumor suppressor genes reactivated by DNA demethylation do not return to a fully euchromatic chromatin state. *Cancer Res* 2006; **66**:3541–3549.

56 Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, *et al.* Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. *Nat Genet* 2005; **37**:391–400.

57 Hinshelwood RA, Huschtscha LI, Melki J, Stirzaker C, Abdipranoto A, Vissel B, *et al.* Concordant epigenetic silencing of transforming growth factor-β signaling pathway genes occurs early in breast carcinogenesis. *Cancer Res* 2007; **67**:11517–11527.

58 Espada J, Ballestar E, Fraga MF, Villar-Garea A, Juarranz A, Stockert JC, *et al.* Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J Biol Chem* 2004; **279**:37175–37184.

59 Schulte JH, Lim S, Schramm A, Friedrichs N, Koster J, Versteeg R, *et al.* Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 2009; **69**:2065–2071.

60 Sansom OJ, Maddison K, Clarke AR. Mechanisms of disease: methyl-binding domain proteins as potential therapeutic targets in cancer. *Nat Clin Pract Oncol* 2006; **4**:305–315.

61 Lopez-Serra L, Ballestar E, Fraga MF, Alaminos M, Setien F, Esteller M. A profile of methyl-CpG binding domain protein occupancy of hypermethylated promoter CpG islands of tumor suppressor genes in human cancer. *Cancer Res* 2006; **66**:8342–8346.

62 Cowland JB, Hother C, Grønbaek K. MicroRNAs and cancer. *APMIS* 2007; **115**:1090–1106.

63 Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, *et al.* MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. *Proc Natl Acad Sci U S A* 2007; **104**:15805–15810.

64 Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, *et al.* MicroRNA -29b induces global DNA hypomethylation and tumor suppressor gene re-expression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. *Blood* 2009; **113**:6411–6418.

65 Gowher H, Jeltsch A. Mechanism of inhibition of DNA methyltransferases by cytidine analogs in cancer therapy. *Cancer Biol Ther* 2004; **3**:1062–1068.

66 Jüttermann R, Li E, Jaenisch R. Toxicity of 5-aza-2′-deoxycytidine to mammalian cells is mediated primarily by covalent trapping of DNA methyltransferase rather than DNA demethylation. *Proc Natl Acad Sci USA* 1994; **91**:11797–11801.

67 Griffiths EA, Gore SD. DNA methyltransferase and histone deacetylase inhibitors in the treatment of myelodysplastic syndromes. *Semin Hematol* 2008; **45**:23–30.

68 Kaminskas E, Farrell AT, Wang YC, Sridhara R, Pazdur R. FDA drug approval summary: azacitidine (5-azacytidine, Vidaza) for injectable suspension. *Oncologist* 2005; **10**:176–182.

69 Ghoshal K, Bai S. DNA methyltransferases as targets for cancer therapy. *Drugs Today (Barc)* 2007; **43**:395–422.

70 Laliberté J, Marquez VE, Momparler RL. Potent inhibitors for the deamination of cytosine arabinoside and 5-aza-2-deoxycytidine by human cytidine deaminase. *Cancer Chemother Pharmacol* 1992; **30**:7–11.

71 Momparler RL. Pharmacology of 5-aza-2-deoxycytidine (decitabine). *Semin Hematol* 2005; **42**:S9–S16.

72 Kim CH, Marquez VE, Mao DT, Haines DR, McCormack JJ. Synthesis of pyrimidin-2-one nucleosides as acid-stable inhibitors of cytidine deaminase. *J Med Chem* 1986; **29**:1374–1380.

73 Zhou L, Cheng X, Connolly BA, Dickman MJ, Hurd PJ, Hornby DP. Zebularine: a novel DNA methylation inhibitor that forms a covalent complex with DNA methyltransferases. *J Mol Biol* 2002; **23**:591–599.

74 Lemaire M, Momparler LF, Bernstein ML, Marquez VE, Momparler RL. Enhancement of antineoplastic action of 5-aza-2′-deoxycytidine by zebularine on L1210 leukemia. *Anticancer Drugs* 2005; **16**:301–308.

75 Lemaire M, Momparler LF, Raynal NJ, Bernstein ML, Momparler RL. Inhibition of cytidine deaminase by zebularine enhances the antineoplastic action of 5-aza-2′-deoxycytidine. *Cancer Chemother Pharmacol* 2009; **63**:411–416.

76 Cheng JC, Yoo CB, Weisenberger DJ, Chuang J, Wozniak C, Liang G, *et al.* Preferential response of cancer cells to zebularine. *Cancer Cell* 2004; **6**:151–158.

77 Flotho C, Claus R, Batz C, Schneider M, Sandrock I, Ihde S, *et al.* The DNA methyltransferase inhibitors azacitidine, decitabine and zebularine exert differential effects on cancer gene expression in acute myeloid leukemia cells. *Leukemia* 2009; **23**:1019–1028.

78 Brueckner B, Boy RG, Siedlecki P, Musch T, Kliem HC, Zielenkiewicz P, *et al.* Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res* 2005; **65**:6305–6311.

79 Lee BH, Yegnasubramanian S, Lin X, Nelson WG. Procainamide is a specific inhibitor of DNA methyltransferase 1. *J Biol Chem* 2005; **280**:40749–40756.

80 Lee WJ, Shim JY, Zhu BT. Mechanisms for the inhibition of DNA methyltransferases by tea catechins and bioflavonoids. *Mol Pharmacol* 2005; **68**:1018–1030.

81 Shankar S, Ganapathy S, Srivastava RK. Green tea polyphenols: biology and therapeutic implications in cancer. *Front Biosci* 2007; **12**:4881–4899.

82 Plummer R, Vidal L, Griffin M, Lesley M, de Bono J, Coulthard S, *et al.* Phase I study of MG98, an oligonucleotide antisense inhibitor of human DNA methyltransferase 1, given as a 7-day infusion in patients with advanced solid tumors. *Clin Cancer Res* 2009; **15**:3177–3183.

83 Klisovic RB, Stock W, Cataland S, Klisovic MI, Liu S, Blum W, *et al.* A phase I biological study of MG98, an oligodeoxynucleotide antisense to DNA methyltransferase 1, in patients with high-risk myelodysplasia and acute myeloid leukemia. *Clin Cancer Res* 2008; **14**:2444–2449.

84 Winquist E, Knox J, Ayoub JP, Wood L, Wainman N, Reid GK, *et al.* Phase II trial of DNA methyltransferase 1 inhibition with the antisense oligonucleotide MG98 in patients with metastatic renal carcinoma: a National Cancer Institute of Canada Clinical Trials Group investigational new drug study. *Invest New Drugs* 2006; **24**:159–167.

85 Lin J, Gilbert J, Rudek MA, Zwiebel JA, Gore S, Jiemjit A, *et al.* A phase I dose-finding study of 5-azacytidine in combination with sodium phenylbutyrate in patients with refractory solid tumors. *Clin Cancer Res* 2009; **15**:6241–6249.

86 http://clinicaltrials.gov/

87 Jatoi A, Ellison N, Burch PA, Sloan JA, Dakhil SR, Novotny P, *et al.* A phase II trial of green tea in the treatment of patients with androgen independent metastatic prostate carcinoma. *Cancer* 2003; **97**:1442–1446.

88 Chuang JC, Yoo CB, Kwan JM, Li TW, Liang G, Yang AS, *et al.* Comparison of biological effects of non-nucleoside DNA methylation inhibitors versus 5-aza-2′-deoxycytidine. *Mol Cancer Ther* 2005; **4**:1515–1520.

89 Mai A, Massa S, Rotili D, Cerbara I, Valente S, Pezzi R, *et al.* Histone deacetylation in epigenetics: an attractive target for anticancer therapy. *Med Res Rev* 2005; **25**:261–309.

90 McLaughlin F, La Thangue NB. Histone deacetylase inhibitors open new doors in cancer therapy. *Biochem Pharmacol* 2004; **68**:1139–1144.

91 Mai A, Altucci L. Epi-drugs to fight cancer: From chemistry to cancer treatment, the road ahead. *Int J Biochem Cell Biol* 2009; **41**:199–213.

92 Jakubikova J, Bao Y, Sedlak J. Isothiocyanates induce cell cycle arrest, apoptosis and mitochondrial potential depolarization in HL-60 and multidrug-resistant cell lines. *Anticancer Res* 2005; **25**:3375–3386.

93 Jakubikova J, Bao Y, Bodo J, Sedlak J. Isothiocyanate iberin modulates phase II enzymes, posttranslational modification of histones and inhibits growth of Caco-2 cells by inducing apoptosis. *Neoplasma* 2006; **53**:463–470.

94 Clarke JD, Dashwood RH, Ho E. Multi-targeted prevention of cancer by sulforaphane. *Cancer Lett* 2008; **269**:291–304.

95 Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. *J Biol Chem* 1990; **265**:17174–17179.

96 Xiong Y, Dowdy SC, Podratz KC, Jin F, Attewell JR, Eberhardt NL, *et al.* Histone deacetylase inhibitors decrease DNA methyltransferase-3B messenger RNA stability and down-regulate de novo DNA methyltransferase activity in human endometrial cells. *Cancer Res* 2005; **65**:2684–2689.

97 Su GH, Sohn TA, Ryu B, Kern SE. A novel histone deacetylase inhibitor identified by high-throughput transcriptional screening of a compound library. *Cancer Res* 2000; **60**:3137–3142.

98 Lee EJ, Lee BB, Kim SJ, Park YD, Park J, Kim DH. Histone deacetylase inhibitor scriptaid induces cell cycle arrest and epigenetic change in colon cancer cells. *Int J Oncol* 2008; **33**:767–776.

99 Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, *et al.* Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 1999; **401**:188–193.

100 Detich N, Bovenzi V, Szyf M. Valporate induces replication independent active DNA demethylation. *J Biol Chem* 2003; **278**:27586–27592.

101 Fournel M, Bonfils C, Hou Y, Yan PT, Trachy-Bourget MC, Kalita A, *et al.* MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity in vitro and in vivo. *Mol Cancer Ther* 2008; **7**:759–768.

102 Conley BA, Wright JJ, Kummar S. Targeting epigenetic abnormalities with histone deacetylase inhibitors. *Cancer* 2006; **107**:832–840.

103 Dovic M, Vu J. Vorinostat: a new oral histone deacetylase inhibitor approved for cutaneous T-cell lymphoma. *Expert Opin Investig Drugs* 2007; **16**:1111–1120.

104 Batty N, Malouf GG, Issa JP. Histone deacetylase inhibitors as anti-neoplastic agents. *Cancer Lett* 2009; **280**:192–200.

105 Camacho LH, Olson J, Tong WP, Young CW, Spriggs DR, Malkin MG. Phase I dose escalation clinical trial of phenylbutyrate sodium administered

twice daily to patients with advanced solid tumors. *Invest New Drugs* 2007; **25**:131–138.
106 Daud AI, Dawson J, DeConti RC, Bicaku E, Marchion D, Bastien S, *et al.* Potentiation of a topoisomerase I inhibitor, karenitecin, by the histone deacetylase inhibitor valproic acid in melanoma: translational and phase I/II clinical trial. *Clin Cancer Res* 2009; **15**:2479–2487.
107 De Bono JS, Kristeleit R, Tolcher A, Fong P, Pacey S, Karavasilis V, *et al.* Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**:6663–6673.
108 Glaser KB. HDAC inhibitors: clinical update and mechanism-based potential. *Biochem Pharmacol* 2007; **74**:659–671.
109 http://www.clinicalstudyresults.org/
110 Garcia-Manero G, Assouline S, Cortes J, Estrov Z, Kantarjian H, Yang H, *et al.* Phase 1 study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia. *Blood* 2008; **112**:981–989.
111 Siu LL, Pili R, Duran I, Messersmith WA, Chen EX, Sullivan R, *et al.* Phase I study of MGCD0103 given as a three-times-per-week oral dose in patients with advanced solid tumors. *J Clin Oncol* 2008; **26**:1940–1947.
112 Kummar S, Gutierrez M, Gardner ER, Donovan E, Hwang K, Chung EJ, *et al.* Phase I trial of MS-275, a histone deacetylase inhibitor, administered weekly in refractory solid tumors and lymphoid malignancies. *Clin Cancer Res* 2007; **13**:5411–5417.
113 Gore L, Rothenberg ML, O'Bryant CL, Schultz MK, Sandler AB, Coffin D, *et al.* A phase I and pharmacokinetic study of the oral histone deacetylase inhibitor, MS-275, in patients with refractory solid tumors and lymphomas. *Clin Cancer Res* 2008; **14**:4517–4525.
114 Wang H, Yu N, Song H, Chen D, Zou Y, Deng W, *et al.* N-Hydroxy-1, 2-disubstituted-1H-benzimidazol-5-yl acrylamides as novel histone deacetylase inhibitors: design, synthesis, SAR studies, and in vivo antitumor activity. *Bioorg Med Chem Lett* 2009; **19**:1403–1408.
115 Dos Santos MP, Schwartsmann G, Roesler R, Brunetto AL, Abujamra AL. Sodium butyrate enhances the cytotoxic effect of antineoplastic drugs in human lymphoblastic T-cells. *Leuk Res* 2009; **33**:218–221.
116 Sharma D, Saxena NK, Davidson NE, Vertino PM. Restoration of tamoxifen sensitivity in estrogen receptor-negative breast cancer cells: tamoxifen-bound reactivated ER recruits distinctive co repressor complexes. *Cancer Res* 2006; **66**:6370–6378.
117 Hostetter CL, Licata LA, Keen JC. Timing is everything: Order of administration of 5-aza-2′-deoxycytidine, trichostatin A and tamoxifen changes estrogen receptor mRNA expression and cell sensitivity. *Cancer Lett* 2009; **275**:178–184.
118 Chai G, Li Ch, Zhou W, Wu L, Zhao Y, Wang D, *et al.* HDAC inhibitors act with 5-aza-2′-deoxycytidine to inhibit cell proliferation by suppressing removal of incorporated abases in lung cancer cells. *PLoS ONE* 2008; **3**:e2445.
119 Keen JC, Yan L, Mack KM, Pettit C, Smith D, Sharma D, *et al.* A novel histone deacetylase inhibitor, Scriptaid, enhances expression of functional estrogen receptor á (ER) in ER negative human breast cancer cells in combination with 5-aza 2-deoxycytidine. *Breast Cancer Res Treat* 2003; **81**:177–186.
120 Jakubíková J, Sedlák J. Garlic-derived organosulfides induce cytotoxicity, apoptosis, cell cycle arrest and oxidative stress in human colon carcinoma cell lines. *Neoplasma* 2006; **53**:191–199.
121 Nian H, Delage B, Ho E, Dashwood RH. Modulation of histone deacetylase activity by dietary isothiocyanates and allyl sulfides: studies with sulforaphane and garlic organosulfur compounds. *Environ Mol Mutagen* 2009; **50**:213–221.
122 Cameron EE, Bachman KE, Myöhänen S, Herman JG, Baylin SB. Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet* 1999; **21**:103–107.
123 Baylin SB, Ohm JE. Epigenetic gene silencing in cancer-a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer* 2006; **6**:107–116.
124 Ecke I, Petry F, Rosenberger A, Tauber S, Mőnkemeyer S, Hess I, *et al.* Antitumor effects of a combined 5-aza-2′deoxycytidine and valproic acid treatment on rhabdomyosarcoma and medulloblastoma in ptch mutant mice. *Cancer Res* 2009; **69**:887–895.
125 Nolan L, Johnson PW, Ganesan A, Packham G, Crabb SJ. Will histone deacetylase inhibitors require combination with other agents to fulfil their therapeutic potential? *Br J Cancer* 2008; **99**:689–694.
126 Graham JS, Kaye SB, Brown R. The promises and pitfalls of epigenetic therapies in solid tumours. *Eur J Cancer* 2009; **45**:1129–1136.
127 Mologni L, Cleris L, Magistroni V, Piazza R, Boschelli F, Formelli F, *et al.* Valproic acid enhances bosutinib cytotoxicity in colon cancer cells. *Int J Cancer* 2009; **124**:1990–1996.
128 De Schutter H, Kimpe M, Isebaert S, Nuyts S. A systematic assessment of radiation dose enhancement by 5-aza-2′-deoxycytidine and histone deacetylase inhibitors in head-and-neck squamous cell carcinoma. *Int J Radiat Oncol Biol Phys* 2009; **73**:904–912.
129 Botrugno OA, Santoro F, Minucci S. Histone deacetylase inhibitors as a new weapon in the arsenal of differentiation therapies of cancer. *Cancer Lett* 2009; **280**:134–144.
130 Bender CM, Gonzalgo ML, Gonzales FA, Nguyen CT, Robertson KD, Jones PA. Roles of cell division and gene transcription in the methylation of CpG islands. *Mol Cell Biol* 1999; **19**:6690–6698.
131 Velicescu M, Weisenberger DJ, Gonzales FA, Tsai YC, Nguyen CT, Jones PA. Cell division is required for de novo methylation of CpG islands in bladder cancer cells. *Cancer Res* 2002; **62**:2378–2384.
132 Lin KT, Momparler RL, Rivard GE. High-performance liquid chromatographic analysis of chemical stability of 5-aza-2′-deoxycytidine. *J Pharm Sci* 1981; **70**:1228–1232.
133 Van Groeningen CJ, Leyva A, O'Brien AM, Gall HE, Pinedo HM. Phase I and pharmacokinetic study of 5-aza-2′-deoxycytidine (NSC 127716) in cancer patients. *Cancer Res* 1986; **46**:4831–4836.
134 Glover AB, Leyland-Jones BR, Chun HG, Davies B, Hoth DF. Azacitidine: 10 years later. *Cancer Treat Rep* 1987; **71**:737–746.
135 Yoo CB, Jeong S, Egger G, Liang G, Phiasivongsa P, Tang C, *et al.* Delivery of 5-aza-2′-deoxycytidine to cells using oligodeoxynucleotides. *Cancer Res* 2007; **67**:6400–6408.
136 Liang G, Gonzales FA, Jones PA, Orntoft TF, Thykjaer T. Analysis of gene induction in human fibroblasts and bladder cancer cells exposed to the methylation inhibitor 5-aza-2′-deoxycytidine. *Cancer Res* 2002; **62**:961–966.
137 Han JW, Ahn SH, Park SH, Wang SY, Bae GU, Seo DW, *et al.* Apicidin, a histone deacetylase inhibitor, inhibits proliferation of tumor cells via induction of p21WAF1/Cip1 and gelsolin. *Cancer Res* 2000; **60**:6068–6074.
138 Li H, Wu X. Histone deacetylase inhibitor, Trichostatin A, activates p21WAF1/CIP1 expression through downregulation of c-myc and release of the repression of c-myc from the promoter in human cervical cancer cells. *Biochem Biophys Res Commun* 2004; **324**:860–867.
139 Qian X, LaRochelle WJ, Ara G, Wu F, Petersen KD, Thougaard A, *et al.* Activity of PXD101, a histone deacetylase inhibitor, in preclinical ovarian cancer studies. *Mol Cancer Ther* 2006; **5**:2086–2095.
140 Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F. Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. *Cancer Res* 2003; **63**:7291–7300.
141 Karagiannis TC, El-Osta A. Modulation of cellular radiation responses by histone deacetylase inhibitors. *Oncogene* 2006; **25**:3885–3893.
142 Jang ER, Lim SJ, Lee ES, Jeong G, Kim TY, Bang YJ, *et al.* The histone deacetylase inhibitor trichostatin A sensitizes estrogen receptor alpha-negative breast cancer cells to tamoxifen. *Oncogene* 2004; **23**:1724–1736.
143 Hellebrekers DM, Griffioen AW, Van Engeland M. Dual targeting of epigenetic therapy in cancer. *Biochim Biophys Acta* 2007; **1775**:76–91.
144 Reu FJ, Bae SI, Cherkassky L, Leaman DW, Lindner D, Beaulieu N, *et al.* Overcoming resistance to interferon-induced apoptosis of renal carcinoma and melanoma cells by DNA demethylation. *J Clin Oncol* 2006; **24**: 3771–3779.
145 Gollob JA, Rathmell WK, Richmond TM, Marino CB, Miller EK, Grigson G, *et al.* Phase II trial of sorafenib plus interferon alfa-2b as first- or second-line therapy in patients with metastatic renal cell cancer. *J Clin Oncol* 2007; **25**:3288–3295.
146 Issa JP, Gharibyan V, Cortes J, Jelinek J, Morris G, Verstovsek S, *et al.* Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J Clin Oncol* 2005; **23**:3948–3956.
147 Nyce J. Drug-induced DNA hypermethylation and drug resistance in human tumors. *Cancer Res* 1989; **49**:5829–5836.
148 Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, *et al.* Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 2006; **9**:435–443.