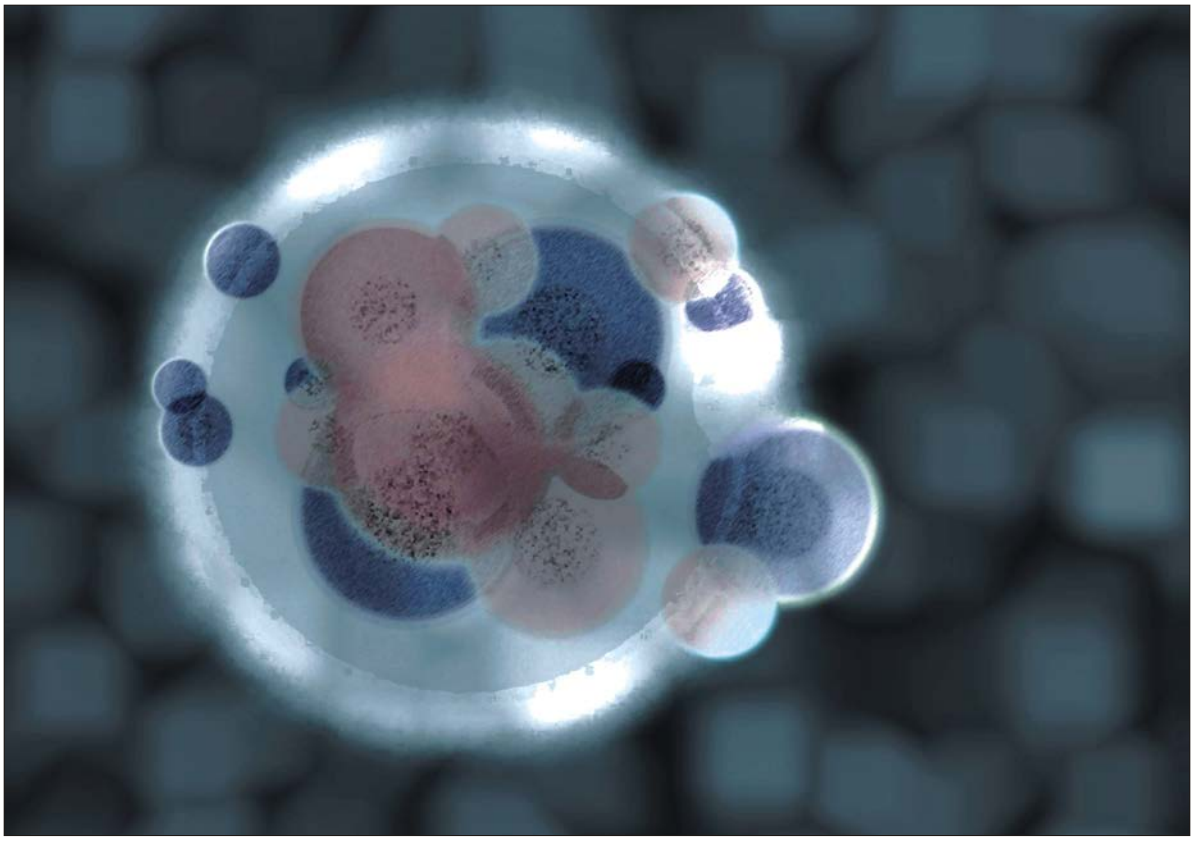

Journal of Biological Sciences

ISSN 1727-3048

science alert

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Journal of Biological Sciences 10 (5): 373-385, 2010
ISSN 1727-3048

Clinical Applications of Epigenetic Markers in Diagnosis and Treatment of Cancer

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Abstract: The best characterized of epigenetic is the modifications that occur on DNA and histones that can specify regulate transcriptional activity. Since discovery of cancer epigenetic in 1983, there is an explosion of interest in the epigenetic of cancer study. The epigenetic of cancer has been associated with all stages of tumor formation and progression. DNA methylation and histone acetylation are important epigenetic mechanisms of gene regulation and play essential roles in tumor initiation and progression. Also, epigenetic alterations are potentially reversible, but epigenetic events can facilitate genetic damage by the increased mutagenicity of 5-methylcytosine by DNA methylation. The advantages of epigenetic changes can be used as powerful marker to detect cancer cells or cancer-derived and have made the way of innovative diagnostic and therapeutic strategies, although only a few genes have given promising results as potential tumor biomarkers.

Key words: Epigenetic, biomarker, tumor, diagnostic, therapeutic

INTRODUCTION

Epigenetic changes, unlike genetic alterations defined as heritable changes in gene expression that are potentially reversible and have essential roles during embryonic development, however appear to contribute to the malignant transformation and progression of cancer (Wolffe and Matzke, 1999; Li, 2002). There are three main types of epigenetic information in the genome which are having important role in the regulation of gene expression as follow. Firstly, DNA methylation that is a covalent modification of DNA in which a methyl group is transferred from S-adenosyl-methionine to the C-5 position of cytosine by a family of cytosine methyl-transferases (DNAMTs). DNA methylation occurs almost exclusively at CpG nucleotides. Secondly, Histone modifications which are including acetylation, methylation and phosphorylation which are stably maintained during cell division. Finally, Genomic imprinting which is parent-of-origin-specific allele silencing or relative silencing of one parental allele compared with the other parental allele (Vaziri Gohar and Mohammadi, 2010).

The goal of this study is the background, promise, status of the applications of epigenetic alterations for the early detection, prevention, diagnostics, therapeutics and risk prediction of cancer.

DNA methylation: DNA methylation in mammalian genomes is a defense mechanism by which repetitive DNA which accounts for at least 50% of genome's content is transcriptionally silenced to prevent it from propagating (Urnov, 2002).

Methyl-cytosine residues are often found in short stretches of CpG-rich regions (CpG islands) that are 0.5-2 kb long and found in the 5′-region of approximately 60% of genes (Gardiner-Garden and Frommer, 1987). When a CpG site is methylated, cytosines on both DNA strands are methylated. At DNA replication, the methylated status is transmitted to daughter DNAs by maintenance DNA methyl-transferase (DNMT), which is present at a replication fork and recognizes hemi-methylated CpG sites. Although a newly synthesized DNA strand does not have methyl groups, maintenance DNA methyl-transferase at the replication fork transfers a methyl group to the newly synthesized strand. Therefore, the methylated or unmethylated status of CpG sites is faithfully copied into daughter DNA in somatic cells (Jones and Baylin, 2002; Herman and Baylin, 2003).

Most CpG islands are unmethylated, with the exception of certain imprinted genes and genes on the inactive X chromosomes of females (Bird, 1986). DNA methylation aberrations can occur as either hypo- or hypermethylation. Both forms can lead to chromosomal instability and transcriptional gene silencing (Baylin *et al*., 1991).

DNMT1 is referred to as the maintenance methylase due to its preference for hemimethylated CpG sites in DNA (Pradhan *et al*., 1999). DNMT3a and DNMT3b are considered to be de novo methylases because they can methylate unmethylated DNA (Okano *et al*., 1999; Pradhan *et al*., 1999). However, all three DNMTs have been shown to act cooperatively and the functional differences between the methylases may to a large extent be due to the genomic regions that they act upon

(Liang *et al*., 2002; El-Osta, 2003). Over-expression of the DNA methyltransferases 1 and 3A was found in the bone marrow of patients with myelodysplastic syndrome (MDS). Upregulation of DNMTs has also been shown in prostate cancer cell lines and tissues (Patra *et al*., 2002; Langer *et al*., 2005).

For detection of cancer cells in body fluids, a high-sensitivity method is necessary. One way is mutation detection in cells. Indeed, because the exact location of a mutation within a gene is usually unknown, many primer sets are necessary for complete analysis. In contrast, aberrant methylation of DNA molecule of cancer cells, even in very few in number, can be sensitively detected by using Methylation-Specific PCR method (MSP), only with one set of PCR primer can be performed on chemically stable DNA, not on RNA (Herman *et al*., 1996; Laird, 2003).

DNA hypermethylation: Methylation of unmethylated DNA, also known as hypermethylation, can repress the gene expression. The gene repression is caused by changes in chromatin structures due to binding of specific proteins to methylated DNA. This binding leads to decreased affinity for binding of some transcriptional factors to methylated CpG sites. Inappropriate silencing of genes can contribute to cancer initiation, progression, pathologic grade, invasion and metastasis (Ushijima *et al*., 2003; Laird *et al*., 2004). Nearly, all types of cancers have transcriptional inactivation of tumor suppressor genes due to DNA hypermethylation (Costello *et al*., 2000; Herman and Baylin, 2003; Ransohoff, 2003; Jones and Baylin, 2007).

DNA hypomethylation: Demethylation of normally methylated DNA, also known as hypomethylation, can disrupt such a defense mechanism, leading to structural and functional alterations of the genome. There are two types of hypomethylation: global and gene-specific hypomethylation, which refers to an overall decrease of 5-methyl-cytosine content in the genome or a gene, respectively (Dunn, 2003). Both global- and gene-specific hypomethylation have hazardous effects and may contribute in human cancer, like DNA hypermethylation. Global DNA hypomethylation has also been found in the premalignant or early stages of some neoplasms. Also, net decreases in the content of methyl-cytosines, often exceed the localized increases in DNA methylation. However, it is unclear whether this epigenetic alteration is a cause or consequence of tumorigenesis and also, whether hypomethylation induced by disrupting DNMT1 does inhibit or promote tumor growth (Feinberg *et al*., 1988; Cravo *et al*., 1996; Baylin *et al*., 2000; Robertson, 2001).

In a murine model of intestinal neoplasia, mice carrying a germ-line mutation in the APC gene (APCMin/_) crossed with mice heterozygous for the DNMT1 mutation had substantially fewer tumors than Min mice with wild-type DNMT1 (Laird *et al*., 1995; Cormier and Dove, 2000). In contrast, genomic hypomethylation has been associated with the induction of T-cell lymphomas in mice carrying a hypomorphic DNMT1 allele, which reduces DNMT1 expression to 10% of wild-type levels and results in substantial genome-wide hypomethylation in all tissues. So, hypomethylation-induced cancer might be related to differences in model systems or tissue specificity (Lengauer, 2003).

It is possible that demethylation could lead to the activation proto-oncogenes (Ehrlich, 2002; Fruhwald and Plass, 2002; Nishigaki *et al*., 2005). Also, genome-wide demethylation may promote genomic instability possibly by activating global demethylation of repetitive sequences such as satellite DNAs can lead to increased chromosomal rearrangements (Dunn, 2003).

Compared with adjacent normal tissues, different types of cancer tissues contain hypomethylated c-jun and c-MYC proto-oncogenes in liver cancer (Tsujiuchi *et al*., 1999; Tao *et al*., 2000), pS2 gene in breast cancer (Fruhwald and Plass, 2002) and PLAU gene in the prostate cancer (Van Veldhuizen *et al*., 1996).

Demethylating agents: Considering that some aberrant DNA methylation is present in early stages of carcinogenesis, there is a possibility that such demethylating agents may protect against some cancers (Laird *et al*., 1995). Demethylating agents are including DNMT1 inhibitors group (Azacitadine, Decitabine, Zebularine and MG98), procainamide, procaine and EGCG (epigallocatechin-3-gallate) (Fang *et al*., 2003; Villar-Garea *et al*., 2003). Inhibitors of DNMTs have been widely used in cell culture systems to reverse abnormal DNA hypermethylation and restore silenced gene expression. However, only limited success has been achieved in clinical trials with these drugs (Thibault *et al*., 1998; Goffin and Eisenhauer, 2002). Also, nucleosides analog inhibitors of DNMTs may promote genomic instability and increase the risk of cancer in other tissues, because have many potential side effects such as myelotoxicity, mutagenesis and tumorigenesis (Jones and Taylor, 1980; Jackson-Grusby *et al*., 1997; Gaudet *et al*., 2003). So, there is an attractive alternative for possible clinical use of non-nucleoside analog DNMT inhibitors.

The use of these drugs raises questions regarding their potential to affect non-cancerous cells epigenetically. However, normal cells divide at a slower rate than malignant cells and incorporate less of these drugs into their DNA resulting in less of an effect on

DNA methylation. As shown in Table 1, methylation pattern of multiple genes have higher role in diagnostic and prognostic possibilities than that of a single gene. However, long-term negative effects of DNA methylation inhibitors in patients have not been found to date (Yang *et al*., 2003).

Azacitadine and decitabine are labil and have acute hematological toxicities. A next generation DNA methylation inhibitor, such as zebularine, might possibly overcome these problems (Marquez *et al*., 2005; Yoo *et al*., 2008). Also, the non-nucleoside analogue inhibitors are not as potent as the nucleoside analogues and therefore this issue needs for improvement (Chuang *et al*., 2005).

DNA methylation as a marker: It has been reported that the pattern of aberrant methylation of individual- or multiple genes can be associated with clinically useful information, such as cancer risk assessment, cancer prognoses, early detection and responses to therapeutics. Therefore, these features make DNA hypermethylation an excellent tumor biomarker candidate (Herman and Baylin, 2003).

In Table 2, aberrant DNA methylation can be applied to detect cancer cells or cancer-derived DNA cancer and diagnostics in several ways. Firstly, if aberrant methylation of some CGIs is specifically present in cancer cells, it can be used to detect cancer cells in biopsy samples or cancer-derived free DNA in plasma. On the

Table 1: DNA methylation-related drugs

Drug name	Structure	Function	Clinical application	Reference(s)
Azacitadine (Vidaza®)	5-azacytidine (5-Aza-CR)	Incorporated into DNA during DNA replication and inhibits DNA methylation by trapping DNMT1 enzyme	It has been FDA approved for treatruent of hematological malignancies (AML, CML and MDS), along with a reversal of p15 hypermethylation in bone marrow	Jones and Taylor (1980), Silverman *et al*. (2002), Momparler (2005), Ghoshal and Bai (2007)
Decitabine (Dacogen®)	5-aza-2´-deoxycitidine (5-Aza-CdR)	Incorporated into DNA during DNA replication and inhibits DNA methylation by irreversible covalent binding and degradation of DNMT1 enzyme	It has been FDA approved for the treatment of (AML, CML and MDS), along with a reversal of p15 hypermethylation in bone marrow. Also, it can restore drug sensitivity to melanoma cell lines that have become unresponsive to chemotherapy by re-expression of a crucial player in the apoptotic pathway, APAF-1	Jones and Taylor (1980) Issa *et al*. (2004), Momparler (2005), Kantarjian *et al*. (2007)
Zebularine	1-(beta-D-ribofuranosyl) 1,2-dihydropyrimidin-2-one	Inhibitor of DNA methylation and exhibits chemical stability and minimal cytotoxicity, both in vitro and *in vivo*	It demethylated various hypermethylated regions, especially CpG-poor regions. It caused a complete depletion of extractable DNMT1 and partial depletion of DNMT3a and DNMT3b3. Also, induces and maintains p16 gene expression	Okochi-Takada *et al*. (2004),
MG98	Antisense oligodeoxynucleotide	It is specific inhibitor of DNA methyltransferase mRNA which targets the 3'-UTR of DNMT1	Partial effective in patients with renal cell carcinoma	Stewart *et al*. (2003), Winquist *et al*. (2006), Gronbaek *et al*. (2007), Klisovic *et al*. (2008)
RG108	Antisense oligodeoxynucleotide (N-Phthalyl-1-tryptophan)	Binds to the catalytic site of DNMTs	Colon cancer cell line	Brueckner *et al*. (2005), Zheng *et al*. (2008)
Procainamide	4-Amino-N-(2-diethylaminoethyl) benzamide hydrochloride	This drug is non-nucleoside inhibitor of methyl transferring from S-adenosyl-methionine to catalytic site of DNMT	It has been FDA approved for treatruent of cardiac arrhythmia It reversed CpG-island hypermethylation of GSTP1 (glutathione S-transferase P1) gene and restored GSTP1 expression in LNCaP prostate cells	Scheinbart *et al*. (1991), Lin *et al*. (2001), Chuang *et al*. (2005)
Synthetic methylated sense oligonucleotide	The cytosine residues are replaced by 5-methylcytosine	It binds to one strand of the DNA and forms a hemimethylated DNA intermediate that has a replication fork–like structure and is a preferred substrate of DNMTs	Introduction into PC-3 prostate cancer cells and in mice with implanted hepatocellular carcinoma targets ESR2 and insulin-like growth factor 2 gene expression, respectively	Lau *et al*. (2000), Yao *et al*. (2003)

Table 2: Clinical association of DNA methylation in diagnostic and prognostic of cancer

Gene(s) methylation	Cancer type/clinical association	Reference
GSTP1, APC, RASSF1 and MDR1 genes	Primary prostate cancer	Yegnasubramanian *et al.* (2004)
hMLH1 mismatch repair gene	Gastric and colon cancer	Ricciardiello *et al.* (2003)
p16, O6-methylguanine DNA methyltransferase (MGMT), retinoic acid receptor beta (RARb), death-associated protein kinase 1 (DAPK), hMLH1, Ecadherin, APC and RASSF1A genes	Lung cancer	Belinsky *et al.* (1998), Ahrendt *et al.* (1999), Kersting *et al.* (2000) Palmisano *et al.* (2000)
GSTP1 gene	Prostate cancer	Goessl *et al.* (2000), Cairn *et al.* (2001)
Cyclin D2, RARb, Twist, GSTP1, p16, p14, RASSF1A and DAPK genes	Breast cancer	Evron *et al.* (2001), Krassenstein *et al.* (2004)
p16, DAPK and MGMT genes	Head and neck cancers	Rosas *et al.* (2001)
DAPK, RARb, E-cadherin, APC, RASSF1A and p14 genes	Bladder cancer	Chan *et al.* (2002), Dulaimi *et al.* (2004)
SFRP2 gene	Colorectal cancer	Muller *et al.* (2004)
APC and p16 tumor suppressor genes	Esophageal adenocarcinomas and squamous cell carcinomas	Kawakami *et al.* (2000), Hibi *et al.* (2001)
Rb1 tumor suppressor gene	Retinoblastoma	Vaziri Gohar *et al.* (2007)
P16 tumor suppressor gene	Early event in lung cancer and also, it can be detected in cancer-free smokers, who were considered to be at high risk	Belinsky *et al.* (1998, 2001) Soria *et al.* (2002)
APC tumor suppressor gene	Gastric and esophageal cancers	Kawakami *et al.* (2000)
VHL tumor suppressor gene (von Hippel-Lindau)	Renal cancer	Battagli *et al.* (2003)
DAPK gene	High risk of recurrence in bladder cancer	Tada *et al.* (2002)
MGMT gene	Predictor of the responsiveness of tumors to alkylating agents in gliomas	Esteller *et al.* (2000a)
BRCA1 tumor suppressor gene	High-grade breast cancers; ovarian cancer	Esteller *et al.* (2000b), Wilson *et al.* (1999)

other hand, incidences of aberrant methylation of specific CGIs are higher than those of mutations. Secondly, if aberrant methylation of some CGIs is associated with a disease phenotype, such as prognosis, responses to chemotherapies or occurrence of adverse effects, it can be used as a marker to predict the phenotype. Finally, if aberrant methylation of some CGIs in non-cancerous tissue is associated with a risk for cancer development, it can be used as a cancer risk marker (Kaneda *et al.*, 2002; Miyamoto *et al.*, 2003; Hagihara *et al.*, 2004).

Histone modification: The N-terminal tails of histones, positioned peripheral to the nucleosome core, are subject to various covalent modifications, such as acetylation, methylation, phosphorylation and ubiquitination by specific chromatin modifying enzymes (Zhang and Reinberg, 2001).

The pattern of these modifications has been referred to as the histone code, and it acts as a second layer of epigenetic regulation of gene expression affecting chromatin structure and remodeling (Jenuwein and Allis, 2001). Histone modification is closely associated with DNA methylation status and is important for gene regulation (Jaenisch and Bird, 2003).

Histone methylation: Histone methylation of histone H3-k4 (Lys4) is associated with active gene transcription. But methylation of histone H3-k9 (Lys9) and H3K27 are normally present at transcriptionally inactive or heterochromatic regions is associated with gene repression (Cao *et al.*, 2002; Nguyen *et al.*, 2002). Lysine residues (lys) can accept up to three methyl groups, which are added by various histone methyltransferases (HMTs) (Fischle *et al.*, 2003; Santos-Rosa and Caldas, 2005).

The methyltransferase MLL, which methylates H3K4, is involved in translocations that lead to the inappropriate expression of various homeotic (Hox) genes, which contributes to leukemic progression (Krivtsov and Armstrong, 2007).

The Polycomb group (PcG) complexes are chromatin modifiers that are crucial to development and have been implicated in the development of cancer (Tonini *et al.*, 2008). These negative regulators of gene expression are very important in sustaining the repressive state of their target genes through the cell cycle (Kingston *et al.*, 1996). Two of the PcG repressive complexes (PRC1 and PRC2) have both been shown to be involved in various cancers. Enhancer of zeste homologue 2 (EZH2), a component of PRC2 with H3K27 methyltransferase activity, is upregulated in mantle cell lymphoma, breast and prostate cancer (Visser *et al.*, 2001; Kleer *et al.*, 2003). RING1, a component of PRC1 that aids in the ubiquitylation of histone H2A lysine 119, is upregulated in prostate cancer (Van Leenders *et al.*, 2007).

Histone acetylation: Histone acetylation is catalyzed by histone acetyltransferases (HATs) and associated with active gene transcription. The basic charges of the histone tails become neutralized upon acetylation. This causes increased accessibility for further modifications or access to the DNA for binding factors and transcriptional machinery (Hebbes *et al.*, 1992; Turner, 1993; Kouzarides, 2007).

Histone deacetylation, mediated by three classes of HDACs which remove the acetyl group from lysine residues and associated with active gene transcription

(Gray and Ekstrom, 2001; Yoshida *et al*., 2001; Marks *et al*., 2004). Inappropriate deacetylation can also contribute to cancer progression. HDACs are upregulated in various types of cancer, such as gastric, prostate, oral squamous cell and lung. Over-expression of HDACs can also lead to the transcriptional inactivation of tumor suppressors, such as p53 (Halkidou *et al*., 2004; Bartling *et al*., 2005; Song *et al*., 2005; Sakuma *et al*., 2006).

HDAC inhibitors are divided into 4 groups based on their structures including hydroxamic acids, cyclic peptides, short chain fatty acids and benzamides (Zheng *et al*., 2008). HDAC inhibitors have pleiotropic effects including inhibition of angiogenesis, induction of apoptosis and cell cycle arrest (Stearns *et al*., 2007). The hydroxamic acid group of HDAC inhibitors has been successful in treating both hematologic malignancies and solid tumors. The X-ray crystallography has shown that the catalytic site of HDACs contains a zinc atom. The hydroxamic acid can fit into the catalytic site of HDACs and bind to the zinc atom of this site thereby inhibiting the HDAC (Marks *et al*., 2000). The shortcoming of these HDAC inhibitors is that a high concentration of drug is required for efficacy resulting in limited use in the clinic (Johnstone, 2002). The list of the HDAC inhibitory agents was shown in Table 3.

Table 3: The anti-cancer drugs which exhibit HDAC inhibitory activity

Drug name	Structure	Clinical application	References
TSA	Trichostatin A	Hyperacetylation of histones	Suenaga *et al*. (2002), Marks *et al*. (2004)
Sodium butyrate	NaB	It played important roles in vitamin D-induced apoptosis through PTEN upregulation. So, it has potential benefits in gastric cancer therapies. Also, it inhibits claudin-1 expression in multiple colon cancer cell lines	Pan *et al*. (2010)
Depsipeptide (FK228)	Cyclic peptide HDAC inhibitor	Hyperacetylation in lung, pancreatic and colon cancer cell lines, while it cause a decrease in methylation of DNA and inhibit growth of human prostate cancer cells. However, it inhibits PC-3 cell growth by suppressing the expression of VEGF mRNA, through accumulation of acetylated histones in chromatin associated with the VEGF gene promoter. It is effective in patients with MDS, AML	Sasakawa *et al*. (2003), Gronbaek *et al*. (2007), Lai *et al*. (2008), Wu *et al*. (2008)
Valproic acid (VPA)	2-Propylpentanoic acid	Originally used to treat epilepsy. It inhibits proliferation and induces differentiation in human neuroblastoma cells. Also, it induces apoptosis in prostate cancer cell by increasing the expression of several pro-apoptotic genes	Cinatl *et al*. (2002), Thelen *et al*. (2004), Blaheta *et al*. (2005)
MS-275	N-(2-aminopherryl)4-[N-(pyridine-3-yl-methoxycarbonyl)aminomethyl] benzamide	Hyperacetylation in prostate cancer cell lines and also, hyperacetylation in peripheral blood mononuclear cells of a variety of solid tumors and lymphoid malignancies	Camphausen *et al*. (2004), Qian *et al*. (2007)
Pyroxamide	Suberoyl-3-aminopyridineamide hydroxamic acid	This is a hybrid polar compound that has potential inhibitory effects on histone deacetylases that induces differentiation and/or apoptosis of various transformed cells such as murine erythroleukemia (MEL), prostate carcinoma, bladder carcinoma and neuroblastoma cells (one possible mechanism is through increased expression of the cell cycle regulator p21/WAF1)	Butler *et al*. (2001)
Pherryl butyrate	Pherryl butyrate	It is in phase I trials for MDS treatment and also, it is safe for treatment of solid tumors	Dyer *et al*. (2002), Gore *et al*. (2002), Camacho *et al*. (2007)
CI-994	N-(2-aminopherryl)-4-acetylaminobenzamide)	It can be used alone or in combination with other chemotherapeutic drugs to treat patients with various types of solid tumors	Prakash *et al*. (2001), Pauer *et al*. (2004)
LBH589	((E)-N-hydroxy-3-[4-[[2-(2-methyl-1H-indol-3-yl) ethylamino]methyl]pherryl] pro p-2-enamide)	Hyperacetylation of histones H3, H4 and Hsp90. It has shown clinical activity in cutaneous T-cell lymphoma (CTCL)	Gore *et al*. (2006), Steele *et al*. (2008)
PXD101	(E)-N-hydroxy-3-[3-(phenylsulfamoyl)pherryl] prop-2-enamide	Effective in refractory solid tumors	Glaser (2007), Steele *et al*. (2008)
SAHA (Vorinostat)	Suberoylanilide hydroxamic acid	It has been FDA approved for treatruent of CTCL. It inhibits the class I and class II of HDACs by binding to active site of the enzyme. Also, it is in phase II trials to treat solid tumors. A recent use of SAHA in women with a recurrence of ovarian cancer showed a progression-free survival	Marks *et al*. (2000), Butler *et al*. (2001), Yoo and Jones (2006), Olsen *et al*. (2007) Xu *et al*. (2007)

Neither of these drugs is as potent as the other classes of HDAC inhibitors and seems to have the greatest effect when used in a combinatorial treatment (Kouraklis and Theocharis, 2006). For example, sodium butyrate and TSA synergize with 1,25-(OH)2-vitamin D3 to inhibit the growth of LNCaP, PC-3 and DU-145 prostate cancer cells by inducing apoptosis (Fruhwald and Plass, 2002).

In prostate cancer, the expression of several genes may be potentially regulated by histone acetylation. Treatment of prostate cancer cells with HDAC inhibitors increased expression of specific genes such as insulin-like growth factor-binding protein 3 (Tsubaki *et al*., 2002) and carboxypeptidase A3 (Huang *et al*., 1999) and thus inferred a role for histone acetylation in gene regulation.

One such gene, coxsackie and adenovirus receptor (CAR), is the primary receptor for group C adenoviruses and is important for adenovirus attachment to the cell membrane. In urogenital cancer cells, including the prostate cancer cell line PC-3, activation of the CAR gene is modulated by histone acetylation and can be induced by depsipeptide, an HDAC inhibitor (Pong *et al*., 2003). Exposing cancer cells to low concentrations of depsipeptide has the functional consequence of preferentially increasing the efficiency of adenoviral transgene expression (Goldsmith *et al*., 2003).

Another gene regulated by histone modification is the vitamin D receptor. 1,25-(OH)2-vitamin D3 acts to antiproliferative effects in a variety of tumor cells, including those of the prostate (Moffatt *et al*., 1999; Zhao *et al*., 2000; Ikeda *et al*., 2003; Yang and Burnstein, 2003).

Prostate cancer cells that are insensitive to 1,25-(OH)2-vitamin D3 have increased levels of nuclear receptor corepressor SMRT (silencing mediator of retinoid and thyroid), which could result in increased deacetylase activity and decreased transcriptional activity of the vitamin D receptor. In addition, combined treatment of prostate cancer cell lines with the HDAC inhibitor trichostatin A (TSA) and 1,25-(OH)2-vitamin D3 synergistically inhibits cell proliferation. This finding may be useful in the clinical setting, in which use of 1,25-(OH)2-vitamin D3 and its analogs in combination with HDAC inhibitors could activate the vitamin D receptor while minimizing unwanted side effects associated with 1,25-(OH)2- vitamin D3, such as hypocalcemia (Banwell *et al*., 2003).

Loss of imprinting (LOI): Also, aberrant methylation of imprinted genes can disturb imprinting. LOI of insulin like growth factor 2 (IGF2) is causally involved in Wilms tumors and colorectal cancers through its overexpression (Feinberg and Tycko, 2004). Loss of IGF2 imprinting in colonic mucosae is associated with an elevated risk of colorectal cancers (Cui *et al*., 1998, 2003; Woodson *et al*., 2004) and LOI in peripheral lymphocyte was also associated with an increased risk (Cui *et al*., 2003). A study using a mouse model for loss of IGF2 imprinting showed that LOI caused less differentiation of normal intestinal epithelium (Sakatani *et al*., 2005).

DISCUSSION

The DNA methylation and histone modifications are as important interconnected epigenetic regulatory mechanisms that they can influence the gene regulation (Fuks *et al*., 2003). DNA methylation is involved in gene silencing through binding of methylated DNA binding proteins such as MeCP2 to gene promoter. This interaction then recruits HDAC to methylated promoters. Therefore, DNA methylation event happens first followed by histone deacetylation and then histone methylation (Antequera and Bird, 1999; Stirzaker *et al*., 2004).

Several studies have shown that the combination of HDAC and DNMT inhibitors can work synergistically to induce the re-expression of such genes like tumor suppressor genes and genes that involved in apoptosis, differentiation, cell growth arrest could enhance the antitumor effects, *in vitro* (Cameron *et al*., 1999; Weiser *et al*., 2001; Ghoshal *et al*., 2002). For example, phenylbutyrate and 5-Aza-CdR have synergistic effects on reducing lung tumor formation in mice more than 5-Aza-CdR alone (Belinsky *et al*., 2003). So, DNA methylation inhibitors and HDAC inhibitors are now used together in the clinic after garnering encouraging results in vitro and it is hypothesized that in a phase II trial, using a longer exposure could further increase the response rate (Gore *et al*., 2006). However, non-specific demethylation has the risk of inducing demethylation of normally methylated sequences, such as retrotransposons and thus retrotranspositions.

Pretreatment with an HDAC inhibitor can greatly increase cytotoxicity in various cell lines when followed by subsequent treatment of a chemotherapeutic drug (kim *et al*., 2003). Likewise, cisplatin resistant cells from head and neck cancer cell lines can be reprogrammed to become responsive after treatment with Phenyl butyrate (Burkitt and Ljungman, 2008). The increased sensitivity to other drugs after use of an epigenetic drug is encouraging since drug resistance does present a challenge in effective cancer treatment.

CONCLUSIONS

As the field of cancer epigenetic advances, a better understanding of the DNA methylation and post-translational histone modifications which play central roles in gene regulation is under developing. The future directions for the development of epigenetic drugs and epigenetic markers will depend on the elucidation of their mechanisms and the downstream effects of treatment.

The use of FDA approved epigenetic drugs has gained momentum and has proven useful in some tumors. The combinatorial use of DNA methylation inhibitors, HDAC inhibitors and non-epigenetic chemotherapeutic drugs in an effort increase response rates and maximize the efficacy of these drugs in the clinic and may have synergistic effects in re-establishing the expression of tumor suppressor genes. However, much work remains in designing drugs that will be more stable, less toxic and more specific in their enzyme inhibition. Future epigenetic drugs can also be designed to target histone methyltransferases, histone demethylases or other chromatin modifiers not yet discovered.

ACKNOWLEDGMENT

I am grateful to all the research scientists, especially to Professors. Andrew Feinberg and Stephen Baylin from School of Medicine at Johns Hopkins University for several years studies on cancer epigenetic.

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