Cell. Mol. Life Sci. (2011) 68:1681–1702
DOI 10.1007/s00018-010-0624-z

Cellular and Molecular Life Sciences

REVIEW

Epigenetic aberrations during oncogenesis

Maria Hatziapostolou · Dimitrios Iliopoulos

Received: 31 August 2010 / Revised: 2 December 2010 / Accepted: 28 December 2010 / Published online: 20 January 2011

Abstract The aberrant epigenetic landscape of a cancer cell is characterized by global genomic hypomethylation, CpG island promoter hypermethylation of tumor suppressor genes, and changes in histone modification patterns, as well as altered expression profiles of chromatin-modifying enzymes. Recent advances in the field of epigenetics have revealed that microRNAs' expression is also under epigenetic regulation and that certain microRNAs control elements of the epigenetic machinery. The reversibility of epigenetic marks catalyzed the development of epigenetic-altering drugs. However, a better understanding of the intertwined relationship between genetics, epigenetics and microRNAs is necessary in order to resolve how gene expression aberrations that contribute to tumorigenesis can be therapeutically corrected.

Keywords Epigenetics · Networks · Methylation · Histones · MicroRNAs · Oncogenic transformation

Introduction

Epigenetic inheritance is defined as cellular information that is heritable during cell division but is not encoded in the sequence of the genome. There are three main, interrelated types of epigenetic inheritance: DNA methylation, genomic imprinting, and histone modification. DNA methylation occurs almost exclusively at CpG nucleotides and contributes in the regulation of gene expression and the silencing of repeat elements in the genome. Histone modifications (including acetylation, methylation, and phosphorylation) are important in transcriptional regulation and are often associated with DNA methylation [1].

Epigenetic inheritance is important in many physiological processes including differentiation, silencing of chromosomal domains such as the X chromosome of female mammals (Xi), stem cell plasticity, aging and genomic imprinting. Genomic imprinting is a parent-of-origin-specific allele silencing or relative silencing of one parental allele compared with the other parental allele. It is maintained by differentially methylated regions within or near imprinted genes. Epigenetic abnormalities also provide information about many pathophysiological conditions, including tumorigenesis [2].

Tumorigenesis (also referred as neoplastic transformation) is regarded as the process whereby cells undergo a change involving uncontrolled proliferation, a loss of checkpoint control tolerating the accumulation of chromosomal aberrations and genomic aneuploidies, and misregulated differentiation. It is commonly thought to be triggered by at least one genetic lesion, such as a point mutation, a deletion or a translocation, disrupting either oncogenes or tumor suppressor genes [3]. In cancer cells, oncogenes are activated through dominant mutations or overexpression of a gene, while tumor suppressor genes become silenced. Accumulation of aberrant epigenetic changes, such as DNA methylation, histone modifications and chromatin remodeling, is also associated with oncogenesis. Thus, neoplastic transformation is a complex multistep process that involves the random activation of oncogenes and/or silencing of tumor suppressor genes,

M. Hatziapostolou · D. Iliopoulos
Department of Cancer Immunology & AIDS,
Dana-Farber Cancer Institute, Boston, MA 02115, USA

M. Hatziapostolou · D. Iliopoulos (✉)
Department of Pathology, Harvard Medical School,
Boston, MA 02115, USA
e-mail: dimitrios_iliopoulos@dfci.harvard.edu

through genetic or epigenetic events, and is referred as the "Knudson two-hit" theory [1].

In this review, we provide an overview of the current knowledge of epigenetic processes and their link to oncogenesis. These events are now transforming the field of cancer biology and provide novel pathways for the development of therapeutic approaches.

Current opinions on the origin of human cancer

The cellular origin of the cancer-initiating cell has been studied and debated for more than 50 years. The focus, until recent years, has largely been on the genetic basis of cancer, particularly on the mutational activation of oncogenes or inactivation of tumor suppressor genes. However, in the modern era, an explosion of data indicates the importance of epigenetic processes, especially those resulting in the silencing of key regulatory genes. The early onset of epigenetic changes and the growing view that stem cells are the target cells for cancer, together with the idea that epigenetic changes probably distinguish stem cells from somatic cells, make it likely that epigenetic disruption of stem cells is a common theme in cancer aetiology. The differences between tumor types might largely be due to the genetic gatekeeper alterations that arise in the background of epigenetically altered stem cells [4]. The main models proposed for tumor initiation and propagation are the following.

The clonal genetic model of oncogenesis

In the past century, the genetic model of cancer has predominated, beginning with Boveri at 1929 who first suggested a role for abnormal chromosomes in cancer. Cancer, as a heterogeneous group of disorders with marked different biologic properties, is thought to arise from a single cell. According to the clonal genetic model, cancer arises through a series of mutations, including dominantly acting oncogenes and recessively acting tumor suppressor genes. Each mutation leads to the selective growth of a monoclonal population of tumor cells, resulting in acquisition of properties such as genetic plasticity, metastasis and drug resistance.

The clonal genetic model of cancer has been particularly successful in predicting the so-called gatekeeper mutations. Gatekeeper mutations are defined as the ones that are necessary for the earliest stages of tumor growth, such as of *APC* in colorectal cancer [5]. Despite its strengths, this classical view has significant limitations. For example, progression-related genetic changes are inconsistent. In colorectal cancer, which might be the best-defined example of genetic changes in tumor progression, no known mutations are necessary and sufficient for specific stages of tumor progression other than the gatekeeper mutation itself. Despite determined efforts by many laboratories, so far no recurrent mutations have been identified that are responsible for invasion or metastasis of that tumor [4].

Epigenetic precedence over genetic alterations

Pathologic epigenetic changes are increasingly considered as alternatives to mutations and chromosomal alterations in disrupting gene function. These include global DNA hypomethylation in tumors, hypermethylation of the CpG islands in the promoter regions of tumor-suppressor genes, chromatin alterations and loss of imprinting (LOI).

Genomic instability has been suggested as a driving force in tumorigenesis. Recent studies, however, indicate that epigenetic changes may precede genetic mutations and genomic instability [6, 7]. DNA hypomethylation contributes to cancer development by inducing generation of chromosomal instability, reactivation of transposable elements, and loss of imprinting [7]. On the other hand, local changes in promoter methylation (hypermethylation) work together with histone modifications to silence well-characterized tumor suppressor genes. Epigenetically silenced genes are located across all chromosome locations in cancer cells. A key concept is that, in order to effectively monitor and control human neoplasia, we might need to explore the cancer cell "epigenome" as well as the mutations in the cancer cell genome [8].

The cancer stem cell hypothesis

The cancer stem cell model is integral to current thinking in the cancer biology field relative to an old, but still vital, concept that each patient's tumor consists of a heterogeneous population of cells, some of which have more tumorigenic and metastatic potential than others. The term cancer stem cell is still an operational term defined as a cancer cell that has the ability to self-renew, and to give rise to another malignant stem cell, as well as a cell that will give rise to phenotypic diverse cancer cells that are known as transient amplifying cells. The transient amplifying cells are believed to be the cells that are responsible for the bulk tumor cell proliferation that is responsive to conventional therapy, whereas the cancer stem-like cells may be left intact and will eventually repopulate the tumor. Although the cancer-initiating cells are still not fully identified or characterized, they seem to display different phenotypes, which can possibly be derived from both embryonic and adult stem cell pools, as well as from progenitor or differentiated cells [7, 9].

Which of the proposed models will dominate? Though we are currently not in position to adopt one model over the

others, it seems more possible that the truth lies somewhere in between. In other words, a combination of events from several models seems likely to provide satisfactory answers to the questions of cancer initiation and growth. A unifying model should pave the way for the development of new targeted therapeutic approaches.

Epigenetic mechanisms of cellular transformation

Several epigenetic mechanisms contribute to the transformation of a normal cell into a cancer cell (Fig. 1). The main mechanisms are described in the following sections.

DNA methylation

Epigenetic regulation is a multifaceted control system, in which DNA methylation is just one of many aspects. DNA methylation though is the only flexible genomic parameter that can change genome function under exogenous influence. Hence, it constitutes the main, so far missing, link between genetics, disease and the environment that is widely thought to play a decisive role in the aetiology of all human pathologies, including cancer. This necessity led to the establishment of human epigenome project (http://www.epigenome.org) which aims to identify and interpret genome-wide methylation patterns of all human genes in all major tissues and advance our ability to understand and diagnose human disease.

The most studied modification in humans is the methylation of the cytosine located within the dinucleotide CpG. CpG dinucleotides are not evenly distributed across the human genome but are instead concentrated in regions of large repetitive sequences (e.g., centromeric repeats, retrotransposon elements, rDNA, etc.) and in short CpG-rich DNA stretches called "CpG islands" [10].

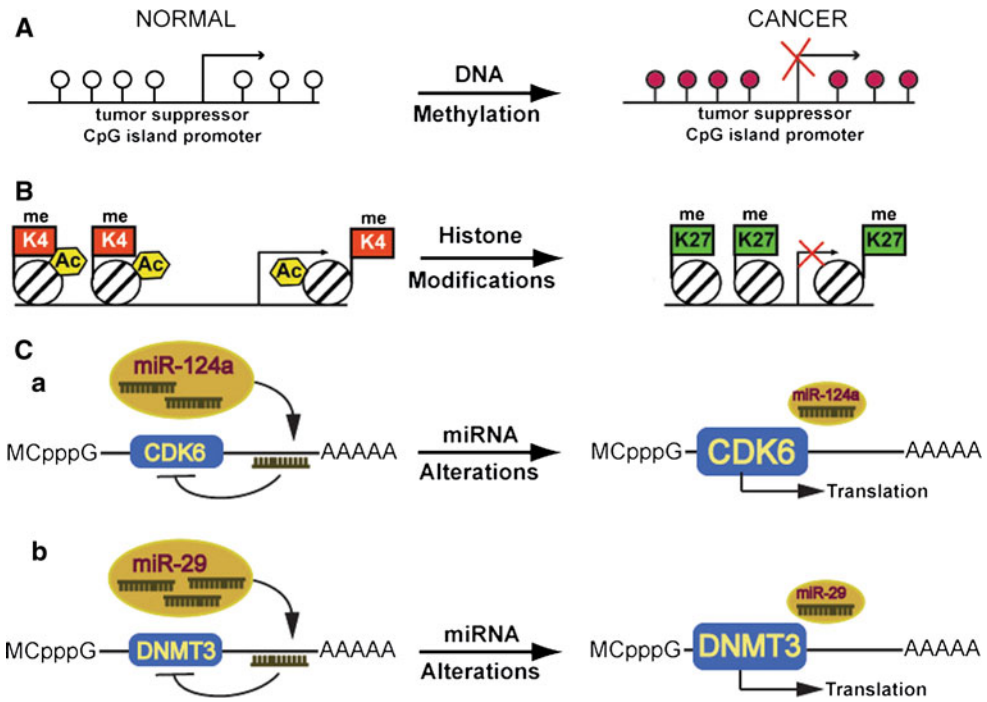

Fig. 1 Epigenetic alterations during oncogenesis. **A** DNA methylation alterations in cancer. In normal cells, CpG island promoters are generally unmethylated (*white circles*) and active, as in the case of tumor suppressor genes. An active gene shows an open chromatin structure with no nucleosome upstream of the transcription start site (*black arrow*). During tumorigenesis, tumor suppressor gene promoters with CpG islands become methylated (*red circles*) resulting in the formation of silent chromatin structure and aberrant silencing (*red X* on the *black arrow*). **B** In normal cells active tumor suppressor genes (*black arrow*) show an enrichment of active histone marks such as acetylation (*yellow polygon*) and K4 methylation (*red rectangular*) of histone H3. In cancer cells, gene silencing of tumor suppressors may occur through the enrichment of repressive histone marks like H3K27 methylation (*green rectangular*) mediated by the PRC complex and the removal of active histone marks (acetylation and H3K4 methylation). **C** Abnormal miRNA expression in cancer. **a** Epigenetic changes like DNA methylation and histone medications are responsible for aberrant miRNA expression. For example, miR-124a is under epigenetic control in acute lymphoblastic leukemia (ALL) due to the fact that is embedded in CpG island regions that are silenced by hypermethylation. The result is that miR-124a is down-regulated in ALL (when compared to normal cells) and its target CDK6 is up-regulated. **b** Epi-miRNAs are regulators of epigenetic effectors. For example, miR-29 family targets DNMT3a and DNMT3b. In normal cells, the levels of miR-29 are increased leading to decreased expression of its targets (DNMT3a and DNMT3b), normal patterns of DNA methylation and expression of tumor suppressor genes. In lung cancer cells, miR-29 is down-regulated resulting in up-regulation of DNMTs and hypermethylation and silencing of tumor suppressor genes

DNA methylation is catalyzed by enzymes known as DNA methyltransferases. The de novo DNA methyltransferases, DNMT3A and DNMT3B, show specificity for both the unmethylated and hemimethylated DNA and function independently of replication. The maintaining DNA methyltransferase, DNMT1, shows a preference for hemimethylated DNA and functions during DNA replication. The concerted function of DNA methyltransferases results in the generation and maintenance of the heritable DNA methylation patterns observed in the mammalian genome [11].

DNA methylation uses a variety of mechanisms to heritably silence genes and non-coding genomic regions. Thus, DNA methylation can lead to gene silencing by either preventing or promoting the recruitment of regulatory proteins to DNA. For example, it can inhibit transcriptional activation by blocking transcription factors from accessing target binding sites [12] or by providing binding sites for methyl-binding domain proteins, which can mediate gene repression through interactions with histone deacetylases (HDACs) [13].

There are at least three major routes by which CpG methylation can contribute to oncogenesis. These include hypomethylation of the cancer genome, hypermethylation of the promoters of tumor suppressor genes and direct mutagenesis. Furthermore, methylation of CpG sites increases the binding of some chemical carcinogens to DNA and increases the rate of UV-induced mutations.

DNA hypomethylation

Normally, the repetitive genomic sequences that are scattered all over the human genome are heavily methylated. One of the most exciting possibilities for the normal function of DNA methylation outside the CpG islands is its role in repressing parasitic DNA sequences. Human genome is plagued with transposons and endogenous retroviruses. Maybe DNA methylation is the main line of defense against the large burden of parasitic sequence elements (>35% of our genome). Furthermore, DNA methylation prevents chromosomal instability by silencing non-coding DNA and transposable DNA elements [14].

Loss of DNA methylation at CpG dinucleotides was the first epigenetic abnormality to be identified in cancer cells [15]. The loss of methylation involved every tumor type studied, both benign and malignant; furthermore, pre-malignant adenomas universally had also altered DNA methylation [16]. Global DNA hypomethylation in cancer cells occurs at various genomic sequences including repetitive elements, retrotransposons, CpG poor promoters, introns and gene deserts [17].

Three mechanisms have been proposed to explain the contribution of DNA hypomethylation to carcinogenesis:

- First, DNA hypomethylation can lead to genomic instability. Undermethylation of DNA at repeat sequences might favor mitotic recombination, leading to deletion and translocations, and it can also promote chromosomal rearrangements [6, 18]. For example, many human cancers (ovarian and breast carcinomas, Wilms tumors) that frequently contain unbalanced chromosomal translocations (with breakpoints in the pericentromeric DNA of chromosomes 1 and 16) are characterized by severe hypomethylation in the pericentromeric satellite sequences. These unbalanced chromosomal translocations produce loss of heterozygosity (LOH) for markers on chromosome 16, which in turn strongly correlates with tumor anaplasia [19]. Demethylation of satellite sequences might predispose to their breakage and recombination. Additionally, extensive demethylation in centromeric sequences of cancer cells may play a role in aneuploidy. As evidence of this, depletion of DNA methylation by the disruption of DNMTs causes aneuploidy [20] while patients with germline mutations in DNMT3B are known to have numerous chromosomal alterations [21]. Hypomethylation of retrotransposons can result in their activation and translocation to other genomic regions, thereby further disrupting the genome. For example, hypomethylation of L1 retrotransposons (long interspersed nuclear elements) is a characteristic of colorectal cancer which might promote chromosomal rearrangement [22].
- Second, DNA hypomethylation can lead to gene activation. Specific genes in the normal somatic tissue are silenced because CpG islands in the promoter region are methylated. In some cancer cells, by contrast, these promoter regions undergo demethylation, and the usually repressed genes become expressed [1]. Examples of genes that are affected by hypomethylation include R-Ras, cyclin D2 and maspin in gastric cancer, MAGE in melanoma, HPV16 (human papillomavirus 16) in cervical cancer, S100A4 in colon cancer and various genes in pancreatic cancer [1, 23]. In addition, the hypomethylation mechanism has recently been proposed for the activation of the let-7a-3 miRNA gene, which has been implicated in endometrial and colon cancer [24].
- Third, the loss of methyl groups from DNA can also disrupt genomic imprinting. For example, loss of imprinting of IGF2, an important autocrine growth factor, results in increased risk of colorectal neoplasia [25] and contributes to the development of Wilms tumor [26].

Hypermethylated gene promoters

Differences between normal and cancer cells CpG islands are CpG-rich regions preferentially located at the 5′ end of genes that occupy ~60% of human gene promoters. They are excellent markers of the beginning of a gene since they frequently span from the promoter to the untranslated region and further to exon 1 of a number of genes. While most of the CpG sites in the genome are methylated (large repetitive CpG sequences), the majority of CpG islands are usually unmethylated in all normal tissues [14]. If the corresponding transcription factors are available, histone modifications are in a permissive state and the CpG-island remains in an unmethylated state, that particular gene will be transcribed.

This basic relationship between CpG-island methylation and gene inactivation, as well as the identification of CpG islands themselves, came from early studies of the X chromosome inactivation. Genes on the inactive X chromosome in women (Xi) are normally methylated on CpG islands, thus inactive [27]. A similar phenomenon of gene dosage reduction (in order to avoid redundancy) is also observed in genomic or parental imprinting. During this process, DNA hypermethylation in one allele of a gene early on in the male or female germline leads to monoallelic expression. Methylation of CpG islands has also been postulated as a mechanism for silencing tissue-specific genes in cell types in which they should not be expressed [28]. Of course, like most biological processes, gene silencing is much more complicated and seems to involve the cooperation of multiple processes, including covalent modifications of chromatin, physical alterations in nucleosomal positioning, non-coding RNAs, and DNA methylation among others.

In contrast, cancer cell genomes are characterized by hypermethylation of the CpG islands in the promoter regions of tumor suppressor genes in the context of a massive global hypomethylation. In contrast to hypomethylation, which increases genomic instability and activates proto-oncogenes, site-specific hypermethylation contributes to tumorigenesis by silencing tumor suppressor genes (Table 1). The first link between hypermethylation and tumor suppressor genes was made on the first known tumor suppressor gene—the retinoblastoma gene, RB [29]. Key tumor suppressor proteins, such as the cell cycle inhibitor p16INK4a, the p-53 regulator p14ARF, the cell adherence protein E-cadherin, or the estrogen and retinoid receptors, were shown to undergo methylation-associated silencing in cancer cell lines and primary tumors [28, 30].

Table 1 Hypermethylated genes in different types of cancer

DNA hypermethylation		
Type	Target gene	References
Brain, thyroid cancer	PTEN	[44, 45]
Glioma	p16	[46]
Lung, ovarian cancer	BRCA1, BRCA2, XRCC5	[47, 48]
Lung, breast, colorectal cancer	APC	[49, 50]
Lymphoma	p15, p16	[51]
Multiple myeloma	p15, p16	[52]
Endometrial cancer	hMLH1	[53]
Prostate cancer	Estrogen receptor	[54]

Methylation profiling data of the CpG islands in tumor suppressor genes led to the observation that each type of cancer can be assigned a specific, defining DNA "hypermethylome". Mapping the hypermethylation of CpG islands suggested the occurrence of 100–400 hypermethylated CpG islands in the promoter regions of a given tumor [6, 31]. This information in combination with the genetic and cytogenetic markers can be very useful in classifying tumor subtypes according to their aggressiveness or sensitivity to chemotherapy.

In addition to direct inactivation of tumor suppressor genes, DNA hypermethylation can also indirectly inactivate additional classes of genes by silencing transcription factors and DNA repair genes. Promoter hypermethylation-induced silencing of transcription factors, such as RUNX3 in esophageal cancer [32] and GATA-4 and -5 in colorectal and gastric cancer [33], leads to inactivation of their downstream targets. Silencing of DNA repair genes like BRCA1 blocks the repair of genetic mistakes and enables cells to accumulate further genetic lesions leading to neoplastic transformation of the cell [34].

The role of DNA methyltransferases (DNMTs) The role of DNMTs in the establishment and maintenance of abnormal DNA methylation has received a great deal of attention in recent years. DNMT1 overexpression appears to be a common feature of tumor tissues [35]. Interestingly, 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, develop aggressive T cell lymphomas [36]. The role of the de novo DNMTs in tumor development has also been explored. Both DNMT3a and DNMT3b are frequently expressed in cancer tissue, but only DNMT3b has been implicated in tumor development. Overexpression of DNMT3a and DNMT3b in $Apc^{MIN/+}$ mice indicated that only DNMT3b and not DNMT3a enhances the number of colon tumors and induces methylation and silencing of certain tumor suppressor genes [37]. It has also been proposed that DNMT1 and DNMT3b may cooperatively maintain DNA methylation and gene silencing in colon

cancer cells. Genetic disruption of both DNMT1 and DNMT3b reduced genomic DNA methylation by 95% and resulted in the abrogation of p16 silencing and growth suppression [38]. The mammalian DNMTs appear to have complex functions that include not only DNA methylation catalytic activity but also direct transcriptional repression activities [39, 40]. Di Croce et al. [41] showed that a complex between the promyelocytic leukemia (PML)/retinoic acid receptor (RAR) fusion protein and DNMT1 and DNMT3a contributes to the hypermethylation of the RARB2 promoter and suggested that oncogenic transcription factors may aberrantly recruit DNMTs to target promoters, inducing de novo methylation. Thus, the role of DNMTs has potentially many facets in gene silencing, from initiation to maintenance, which may or may not include steps of DNA methylation.

Mechanism of CpG hypermethylation The mechanism of how specific gene promoters are targeted for CpG hypermethylation during tumorigenesis remains unclear. One possibility is that hypermethylation of CpG islands occur as a result of their location inside genomic regions that have undergone large-scale epigenetic reprogramming. Another one is that promoters of some genes are specifically targeted by DNMTs through the interaction of the latter with oncogenic transcription factors (see above [41]). Finally, another mechanism proposes a role of histone marks in the tumor-specific targeting of de novo methylation and will be discussed in detail in the next section.

What has not been clear is if hypermethylation is the primary mechanism of gene silencing, and this has been the subject for some debate [42]. For instance, it has been shown that activation of MLH1 by the DNA demethylating agent 5-azaCdR is rapidly reversed spontaneously [43]. This observation leads to the conclusion that methylation changes could arise secondarily to other epigenetic changes, such as histone modifications, by contributing to the maintenance of the silenced state. Furthermore, in the context of DNA methylation aberrations at the early stages of carcinogenesis, global hypomethylation seems to arise earlier leading to chromosomal instability and loss of imprinting, while promoter hypermethylation can arise secondarily resulting in the silencing of tumor suppressor genes [1, 8].

Histone modifications: crosstalk with DNA methylation

Histone modifications in normal cells

Chromatin can be classified into two forms: the accessible, de-condensed and early-replicating euchromatin and the inaccessible, gene-dense and late-replicating heterochromatin. Chromatin is composed of nucleosome particles which consist of a protein octamer around which 147 bp of DNA is wrapped. Protein octamers contain two molecules of each canonical histone protein (H2A, H2B, H3 and H4). Histones are small and highly basic proteins and are composed of a globular C-terminal domain and a flexible unstructured N-terminal tail [91]. Histone tails, which protrude from the surface of the nucleosome, can undergo a variety of post-translational covalent modifications. Histone globular domains and chromatin-associated proteins are also subject to covalent post-translational modifications. These modifications involve different chemical groups (e.g., methyl, acetyl and phosphate) and different degrees of methylation (e.g., mono-, di- and tri-methylation). Additional covalent histone modifications have been identified and characterized: ubiquitination, sumoylation, ADP-ribosylation, biotinylation, and proline isomerization [92]. Modifications occur in different histone proteins and in different histone residues such as lysine, arginine and serine.

Histone tail modifications are established ("written") or removed ("erased") by the catalytic action of a large number of chromatin-associated enzymatic systems (Table 2). All these enzymes often reside in large multi-subunit complexes that can catalyze the incorporation or removal of covalent modifications from both histone and non-histone targets. Moreover, many of these enzymes catalyze their reactions with specificity both to target residue and cellular context (e.g., dependent on external or intrinsic signals). The major enzymatic systems are: histone acetyltransferases (HATs) add acetyl groups on lysine residues while histone deacetylases (HDACs) remove them [93]. Histone kinase families add phosphate groups on specific serine or threonine residues while phosphatases (PPTases) remove them. Histone methyltransferases (HMTs) add methyl groups on lysine residues (HKMTs, histone lysine methyltransferases) [94] or on arginine residues (PRMTs, protein arginine methyltransferases). Both arginine and lysine methylation is now appreciated as a reversible process which is catalyzed by the action of histone demethylases (HDMs) [95, 96].

Histone tail modifications are known to play a critical role in chromatin packaging and cellular processes such as replication, transcription and repair [97]. In addition to the amino termini, modifications in the globular histone fold domains have recently been shown to affect chromatin structure and assembly thereby influencing gene expression and DNA damage repair [98]. Histone modifications work either by changing the accessibility of the chromatin or by recruiting and/or occluding non-histone effector proteins. Acetylation of lysine residues alter charge and so change the bulk electrostatic properties of nucleosomes, while methylation provides specific binding platforms for

Table 2 Histone modifications and the respective enzymatic systems in cancer

Type		Enzyme	References
Histone acetylation			
Ovarian, colorectal cancer		PCAF	[55]
Colorectal, gastric cancer, leukemia		P300	[56–58]
Lung, AML		CBP	[59, 60]
AML		MOZ/MYST3	[61, 62]
Gastric, prostate, colon, breast		HDAC1	[63–65]
Gastric cancer		HDAC2	[66]
Breast cancer		HDAC6	[67]
Lung cancer		HDAC10	[68]
Thyroid cancer		SIRT7	[69]
APL		PML–RARa	[70]
AML		AML1–ETO	[71]
Poly-ADP-ribosylation			
Breast, lung, laryngeal, endometrial, colorectal, hepatocellular cancer		PARP-1	[72–75]
Histone methylation			
H3K4me	Renal cell carcinoma	MLL2	[76]
	Solid tumors	ING1-5	[77]
	ML	PHF23	[78]
	Breast cancer	PYGO2	[79]
	Neuroblastoma, breast, prostate, bladder cancer	LSD1	[80]
	ML	JARID1A	[78]
	Breast, prostate cancer	JARID1B/PLU-1	[81, 82]
	Renal cell carcinoma, gastric cancer	JARID1C	[76]
H3K36me	Breast cancer, leukemia	JHDM1B/NDY1	[83]
H3K27me	Lymphomas, breast, prostate, lung, skin, colon cancer	EZH2	[84, 85]
	Lung, liver cancer	JMJD3	[86, 87]
	MM, esophageal squamous cell carcinoma, renal cell carcinoma	UTX	[88]
H3K9me	Prostate cancer	JMJD2A	[89]
	Prostate cancer	JMJD2B	[89]
	Esophageal squamous cell carcinoma, desmoplastic medulloblastoma, metastatic lung sarcomatoid carcinoma, breast, prostate cancer	JMJD2C	[90]

AML acute myeloid leukemia, *APL* acute promyelocytic leukemia, *ML* myeloid leukemia, *MM* multiple myeloma

chromatin-associated proteins such as the α, β and γ-isoforms of heterochromatin-associated protein 1 (HP1) [99].

Unlike DNA methylation, histone modifications can lead to either activation or repression depending on the type of modification and the residues that are modified. Studies of many model systems have shown that lysine acetylation correlates with transcriptional activation [100], whereas lysine methylation leads to transcriptional activation or repression depending on the locus of the residue that is modified and the degree of methylation. For example, trimethylation of lysine 4 on histone H3 (H3K4me3) is correlated with active gene promoters [101], whereas trimethylation of H3K9 (H3K9me3) and H3K27 (H3K27me3) is correlated with repressed gene promoters [97]. The latter two modifications together constitute the two main silencing mechanisms in mammalian cells, H3K9me3 working in concert with DNA methylation and H3K27me3 largely working exclusive of DNA methylation. With regard to histone H4, modifications at lysines 16 and 20 have an important role in determining chromatin structure and function. Deacetylation of K16 and trimethylation of K20 are hallmarks of heterochromatic domains in mammalian cells [102]. Overall, histone hypoacetylation and hypermethylation is characteristic of DNA sequences methylated and repressed in normal cells, such as the X chromosome (Xi) in females, imprinted genes and tissue-specific genes.

Histone modifications, as exemplified by H3K4me3 and H3K27me3 in this review, survive mitosis, and it has been proposed that they store the epigenetic memory inside the cell in the form of "histone code". However, it should be noted that not all histone modifications maintain heritable information. For example, most acetylations facilitate gene expression in response to regulation. Unlike the genetic code (four-base alphabet in the DNA) that uses an invariant and nearly universal language, "histone code" is not likely to be universal if one considers the dynamic nature of histone modifications varying in space and time. Histone code differs from one cell type to another and is proposed to play a key role in determining cellular identity [103]. Chromatin-remodeling complexes, which involve proteins from the Trithorax and Polycomb families, are necessary for the establishment of phenotypic plasticity in embryonic stem (ES) cells. The trithorax group catalyzes the activating H3K4 trimethylation and the polycomb group catalyzes the repressive H3K27 trimethylation. These coexisting marks at the promoters of developmentally important genes enable ES cells to tightly regulate gene expression during different developmental processes. Differentiated cells lose this plasticity and acquire a more rigid chromatin structure [104].

In addition to performing their individual roles, histone modifications and DNA methylation interact with each other at multiple levels to determine processes such as gene expression, chromatin organization and cellular identity. A

key link between histone modifications and DNA methylation was established by the pioneering experiments of Nan et al. [13] who showed that cytosine methylation could attract methylated DNA binding proteins and histone deacetylases to methylated CpG islands during chromatin compaction and gene silencing. Furthermore, several HMTs, including G9a and PRMT5, can directly recruit DNMTs to specific genomic targets leading to their stable silencing [105, 106]. DNMTs can in turn recruit HDACs and methyl-binding proteins to achieve gene silencing and chromatin condensation [13, 107]. HMTs and demethylases also regulate the stability of DNMT proteins, thus influencing the DNA methylation levels [108, 109]. Furthermore, DNA methylation can also direct H3K9 methylation through effector proteins, such as MECP2, thereby establishing a repressive chromatin state [110].

Histone modifications in cancer cells

Recently, a profile of overall histone modifications and their genomic locations in the transformed cell was established. In human and mouse tumors, histone H4 undergoes a loss of monoacetylated and trimethylated lysines 16 and 20, respectively. Most importantly, these alterations occur within the context of the repetitive DNA sequences that also become hypomethylated in transformed cells. These changes appear early and accumulate during the development of tumor [111]. They have been found in breast and liver cancer [112, 113]. Global histone modification patterns also predict the risk for prostate cancer recurrence. Dimethylation of K4 and acetylation of K18 of histone H3 have been proposed as markers [114]. Other evidence for global changes being involved in carcinogenesis comes from studies in the Polycomb group gene family, which is highly conserved throughout evolution [115]. For example, the polycomb gene BMI1, a component of PRC1, is overexpressed in several human cancers so that it might be expected that aberrations in this system would give rise to global alterations in gene silencing in cancer [115].

During tumor progression, levels of epigenetic promoter silencing seem to increase with respect to the individual chromatin determinants that are involved and the density of CpG island methylation [116]. This could result in permanent gene-silencing in neoplastic cells and their progeny. Silencing of tumor suppressor genes in cancer cells has been associated with a particular combination of histone marks: deacetylation of histones H3 and H4, loss of H3K4 trimethylation and gain of H3K9 methylation and H3K27 trimethylation [117]. An interesting model of the relationship between DNA methylation and histone modifications in the promoter CpG island region of a gene has been proposed to explain gene silencing in cancer cells. In the expressed gene (normal cell), the CpG island that surrounds the transcription site resides in a zone that is protected from DNA methylation. CpG sites in regions flanking this protective zone are, in contrast, methylated and associated with key silencing marks such as H3K9 methylation. In the protected zone, though, key histone tail amino-acids like H3K9 are in the acetylated state and transcription factors have access to the transcription start-site region. When the same gene is aberrantly silenced in a cancer cell, the CpG island that surrounds the transcription start-site is DNA hypermethylated. This methylation is maintained by complexes that are composed by DNMTs, HDACs and HMTs. The result is that DNA hypermethylation in the transcription start-site is accompanied by deacetylation of key histone residues (by HDACs) and the presence of repressive histone methylation marks on residues like H3K9 (by HMTs). The final outcome of these orchestrated epigenetic changes is the exclusion of transcriptional activating complexes from the transcription start-site [118].

The presence of the hypo-acetylated and hypermethylated histones H3 and H4 silences certain genes with tumor suppressor-like properties, such as $p21^{WAF1}$, despite the absence of hypermethylation of the CpG island [119]. This observation, along with the fact that HMTs recruit DNMTs to gene promoters, gave rise to the hypothesis that histone methylation occurs during the initial phases of gene silencing in cancer and DNA methylation might then spread over the promoter with time [116]. Furthermore, this proposed sequential appearance of gene silencing events in cancer has been supported by another attractive model appeared in the work of Rauscher and colleagues [120]. In human cells, transient induction of silencing was triggered by binding of a specific transcription–repressor complex to a reporter gene. This binding induces methylation of H3K9 in the promoter region. When the transcriptional repressor is removed, most clones revert to active transcription. However, some clones retain the promoter–repressor marks, fail to reactivate transcription and, overtime, stably repress transcription through promoter DNA methylation. Additionally, this model along with pharmacological studies [118, 121] reinforces the notion that DNA methylation, even if occurs after histone methylation, is the dominant factor in maintaining gene silencing.

Expression patterns of histone-modifying enzymes distinguish cancer tissues from their normal counterparts, and they differ according to tumor type [122]. In leukemias, chromosomal translocations that involve histone-modifier genes like HATs and HMTs can induce global alterations in both histone acetylation and methylation. In particular, chromosomal translocations of HAT and HAT-related genes (e.g., MOZ, MORF, CBP and p300) lead to the aberrant formation of deleterious fusion proteins [57]. In

solid tumors, there is amplification of genes for HMTs or HDMs. For example, EZH2, which is the H3K27 HMT, is overexpressed in breast and prostate cancer [115], and G9a, the H3K9 HMT, is increased in liver cancer [123]. In addition to HMTs, lysine-specific demethylases are also implicated in cancer progression. LSD1 can effectively remove both activating and repressing remarks (H3K4 and H3K9 methylation, respectively) depending on its specific binding partners [124, 125], thus acting as either a co-repressor or a co-activator.

Chromatin remodeling and histone variants

DNA methylation and histone modifications occur in the context of a higher order chromatin structure. The general process of inducing changes in chromatin structure is called chromatin remodeling. The most common use of chromatin remodeling is to change the organization of nucleosomes at the promoter of a gene that is to be transcribed. This is required to allow the transcription apparatus to gain access to the promoter. The remodeling most often takes the form of displacing one or more histone octamers. Nucleosome-free regions (NFRs) present at both the 5′ and 3′ ends of genes are thought to provide the sites for assembly/disassembly of the transcription machinery [126]. Chromatin remodeling is undertaken by large ATP-dependent complexes, such as SWI/SNF and NuRD, which catalyze both sliding and ejection of nucleosomes. The interaction of nucleosome remodeling machinery with DNA methylation and histone modifications plays a pivotal role in establishing global gene expression patterns and chromatin architecture [127].

Emerging data have revealed that DNA methylation-induced silencing of tumor suppressor genes in cancer involves distinct changes in nucleosome positioning resulting in nucleosome occupancy at the transcription start-site. Reactivation of such silenced genes, using DNMT inhibitors, is accompanied by a loss of nucleosomes from the promoter region [128]. Recent work has also indicated that nucleosome remodeling can lead to aberrant gene silencing via the transmission of repressive chromatin marks to tumor suppressor gene promoters. In particular, it has been shown in leukemia that NuRD facilitates recruitment of PRC2 and DNMT3A to PML-RARa target gene promoters leading to their permanent silencing [129].

The causative role of nucleosomal remodeling in the development of cancer has been demonstrated by mutations of the SWI/SNF complex. SNF5 mutants cooperate with p53 to induce oncogenic transformation and leads to the inactivation of p16 and p21 pathways [130]. On the other hand, it has recently been proposed that BRG1 contributes to cancer development by constraining p53 activity through the destabilization of the p53 protein [131].

Compositional differences of chromatin that occur through the presence of histone variants contribute to the indexing of chromosomal regions for specialized functions. Each histone variant represents a substitute for a particular core histone. Histone H2A has three variants: (1) the H2A.Z variant correlates with transcriptional activity and can index the 5′ end of nucleosome free promoters, while H2A.Z incorporation may also contribute to gene activation by protecting genes against DNA methylation; (2) the variant H2A.X, together with other histone marks, is associated with sensing DNA damage and appears to index a DNA lesion for recruitment of DNA repair complexes; and (3) MacroH2A is a histone variant that specifically associates with the inactive X chromosome (Xi) in mammals. Histone H3 has two universal variants: (1) CENP-A, the centromere-specific H3 variant, is essential for centromeric function and hence chromosome segregation; and (2) the H3.3 variant, like H2A.Z, is enriched at promoters of active genes. In contrast to the commonly held textbook notion that histones are synthesized and deposited only during S phase, synthesis and substitution of many of these histone variants occurs throughout the cell cycle [132]. Like canonical histones, histone variants also undergo various post-translational modifications which determine their nuclear localization and function. For instance, acetylation of H2A.Z associates with active genes in euchromatin, whereas ubiquitination of H2A.Z associates with facultative heterochromatin [133].

The role of histone variants has also been implicated in cancer progression. CENP-A variant becomes overproduced in colorectal cancer and it has been suggested that is the responsible factor leading to aneuploidy [134]. H2A.Z is overexpressed in several types of cancer and it has been implicated in the possible destabilization of chromosomal boundaries, which in turn leads to the spreading of repressive chromatin domains and the de novo hypermethylation of tumor suppressor gene promoters [133].

Epigenetic regulation of MicroRNAs

MicroRNAs (miRNAs) are endogenous small non-coding RNAs which regulate gene expression in a sequence-specific manner. MiRNAs target the 3′-untranslated region (3′-UTR) or the 5′-UTR of the target mRNA resulting in mRNA degradation and/or inhibition of translation [135, 136]. MiRNAs are involved in crucial biological processes, including development, differentiation, cell cycle regulation and metabolism [137]. The full spectrum of miRNAs expressed in a specific cell type (the miRNAome) varies between normal and pathologic tissues, and specific

signatures of deregulated miRNAs harbor diagnostic and prognostic implications. It is now widely accepted that miRNAs undergo the same regulatory mechanisms as any other protein-coding gene (PCG) including epigenetic regulation. Intriguingly, a subgroup of miRNAs (epi-miRNAs) target, directly or indirectly, effectors of the epigenetic machinery such as DNMTs, HDACs and polycomb genes. In this way, miRNAs can also indirectly regulate gene expression by directly regulating epigenetic processes [138].

An emerging role of miRNAs in the regulation of stem cell self-renewal and differentiation has been revealed [139], indicating that they are crucial for proper stem cell function and maintenance. Coordinated transcription factor networks involving OCT4, SOX2 and Nanog have currently emerged as the master regulatory elements of stem cell pluripotency and differentiation, and miRNAs to OCT4, SOX2 and Nanog coding regions are found to modulate embryonic stem cell differentiation [140]. Interestingly, miR-302 that can target OCT4/SOX2/NANOG has been shown to have a role in converting differentiated cells to induced pluripotent stem cells [141].

A causal role for miRNAs in cancer was first suggested in 2002 by Croce and colleagues [142] with the discovery that miR-15 and miR-16 were located on chromosome 13q14, a region frequently deleted in chronic lymphocytic leukemia (CLL). They also noted that the 13q14 deletion was frequently the only genetic abnormality in patients and thus the deletion of miR-15/16 may be a direct cause for CLL. Upon examination of genomic locations of microRNAs in cancers, the same group reported that miRNAs are frequently located in cancer-associated genomic regions, which include minimal regions of amplification, loss of heterozygosity, common breakpoint regions in or near oncogenes and tumor suppressor genes and fragile sites (preferential sites of chromatin exchange, deletion, translocation, amplification, or integration of plasmid DNA and tumor associated viruses) [143].

Deregulation of miRNA expression is involved in the initiation and progression of tumorigenesis and has been investigated in almost all kinds of human cancer [138]. Some miRNAs act mainly as tumor suppressors while others have a well-established role as oncogenes, depending upon their target genes (Table 3). For example, the miR-15/16-1 cluster, that targets BCL2, acts as a tumor suppressor in CLL, whereas let7, that targets the oncogene RAS, acts as a tumor suppressor in lung cancer. Oncogenic miRNAs, which target growth inhibitory pathways, are often upregulated in cancer. MiR-155 induces leukemia in transgenic murine models, and miR-21, which targets PTEN1 and PDCD4, is induced in several neoplasms [144]. Furthermore, several microRNAs have been reported as mediators of inflammation-induced carcinogenesis, like miR-21, miR-181b-1 and let-7 [145, 146].

Many mechanisms underlie the abnormal miRNA expression in cancer including transcriptional deregulation, mutations, DNA copy number abnormalities and defects in the miRNA biogenesis machinery. Epigenetic changes like DNA methylation and histone modifications are also responsible for aberrant miRNA expression. Earlier studies indicate that treatment of different cancer cell lines with the DNA demethylating agent (5-aza-2′-deoxycitidine) and/or HDAC inhibitors is able to alter the expression levels of miRNAs [147, 148]. Several miRNAs (miR-1, miR-124a and miR-127) are under epigenetic control in human cancer due to the fact that they are embedded in CpG island regions and are epigenetically silenced by promoter hypermethylation and histone modifications [148–150]. Recently, in acute lymphoblastic leukemia (ALL), 13 miRNAs were identified embedded in CpG islands, with high heterochromatic markers (such as high levels of K9H3me2 and/or low levels of K4H3me3) [151, 152]. Furthermore, it has been proposed that transcription factors can recruit epigenetic effectors at miRNA promoter regions and contribute to the regulation of their expression [153].

On the other hand, certain miRNAs (epi-miRNAs) are regulators of epigenetic effectors (Table 3). Epi-miRNAs regulate the expression of DNMT3a, DNMT3b and DNMT1 (miR-29a, -29b, -29c) in lung cancer and AML, RBL2 the inhibitor of DNMT3 genes (miR-290), HDACs (miR-1, miR-140, and miR-449a) in prostate cancer and skeletal muscle tissue and polycomb genes like EZH2 (miR-101) in prostate and bladder cancer [138]. The discovery of epi-miRNAs and that miRNAs undergo epigenetic regulation introduces new layers of complexity in understanding how gene expression aberrations contribute to human carcinogenesis.

Epigenetic switch regulates neoplastic transformation

An epigenetic switch occurs when a stable cell type changes to another cell type without any change in DNA sequence. Epigenetic switches require an initiating event, but the phenotypes of the new cell type are inherited in the absence of the initiating signal [145]. There are two ways for an epigenetic switch to be established: a double-negative loop in which the product of a gene turns off the expression of another gene and vice versa; and a positive feedback loop, in which a gene activates its own transcription [179]. Epigenetic switches occur in many developmental pathways in eukaryotes as well as in prokaryotes, indicating that the presence of chromatin is not a requirement.

Table 3 Epigenetic microRNAs and their targets in different types of cancer

miRNA	Cancer or tissue type	Target	References
Epigenetically regulated miRNAs			
miR-1	Colorectal cancer	HDAC4	[149]
	Hepatocellular carcinoma		
miR-9	Breast cancer	N/A	[154, 155]
	Metastases		
miR-29	Rabdomyosarcoma	N/A	[156]
miR-34b/c	Colorectal cancer	E2F3 CDK6, c-MYC	[155, 157]
	Metastases		
miR-107	Pancreatic cancer	CDK6	[158]
miR-124a	Colorectal cancer	CDK6, vimentin, SMYD3	[151, 152, 159, 160]
	ALL		
	Gastric cancer		
miR-124	Hepatocellular carcinoma		
miR-126	Prostate cancer	N/A	[161]
	Bladder cancer		
miR-127	Bladder cancer	BCL6	[161]
miR-137	Colorectal cancer	LSD1	[162]
miR-148a	Breast cancer	TGIF2	[154, 155]
	Metastases		
miR-181c	Gastric cancer	NOTCH4, KRAS	[163]
miR-193b	Prostate cancer	N/A	[164]
miR-196b	Gastric cancer	N/A	[165]
miR-200a, -200b	Pancreatic cancer	ZFHX1B, ZEB2	[166, 167]
	Breast cancer		
miR-203	Hepatocellular carcinoma	CDK6, ABCE1	[160]
miR-223	AML	NFI-A, MEF2C	[153]
miR-342	Colorectal cancer	N/A	[168]
let-7	Colorectal cancer	RAS, IL-6, IGF2	[24, 145, 169]
	Hepatocellular carcinoma		
	Lung cancer		
	Prostate cancer		
	Breast cancer		
miRNAs regulating elements of the epigenetic machinery			
miR-1	Skeletal muscle tissue	HDAC4	[170]
miR-29a, -29b, -29c	Lung cancer AML	DNMT 3a, 3b, 1	[171] [172]
miR-101	Prostate cancer	EZH2	[173] [174]
	Bladder cancer		
miR-137	Colorectal cancer	LSD1	[162]
miR-140	Mouse cartilage tissue	HDAC4	[175]
miR-148a, -148b	Cervical cancer	DNMT3b	[176]
miR-290 cluster	Mouse ES cells	RBL2	[177]
miR-449a	Prostate cancer	HDAC1	[178]

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia

Epigenetic switching has been used recently to explain how normal cells can be converted to transformed cells. In particular, it has been proposed that "epigenetic switching" of the plastic polycomb mark (H3K27 methylation) with the more stable DNA methylation (H3K9 methylation) results in the permanent silencing of key regulatory genes that may contribute to cell proliferation and tumorigenesis [180].

We have recently demonstrated that epigenetic switch provides the link between inflammation and cellular transformation. Specifically, we used an inducible model of oncogenic transformation in order to identify transcriptional regulatory circuits important in oncogenesis. In this inducible transformation model, the switch is triggered by an initial inflammatory signal (transient activation of the Src kinase oncoprotein) that activates NF-κB transcription factor. NF-κB generates high levels of IL-6 by direct activation of IL-6 transcription and indirect activation via the miRNA processing factor Lin28B, which leads to inhibition of let-7 miRNA. The resulting high levels of IL-6 activate NF-κB, thereby completing a positive feedback loop that maintains the transformed phenotype and tumor formation in nude mice in the absence of the triggering event (Src activation). Furthermore, this positive feedback loop is required for the self-renewing capacity of tumor-initiating cells (cancer stem cells) [145]. This is not the first time a transient signal has been found to induce stem cell formation. For example, it is well established that reprogramming of adult fibroblasts back to a stem cell-like state can occur after transient expression of the reprogramming factors OCT4, SOX2, c-MYC and KLF4 [181], pointing to the involvement of a positive feedback loop. Our results suggest that the IL6/NF-κB/LIN28B/let-7 loop could also be at work in the reprogramming of fibroblasts.

In addition to its role in the positive feedback loop, IL-6 activates STAT3, a transcription factor that is critical for transformation. This observation is consistent with the conventional view that STAT3 is a downstream effector of IL-6 [182], but not part of the central regulatory circuit that mediates the epigenetic switch. In contrast to this view, our recent data indicate that STAT3 is not simply a downstream effector of IL-6 but is part of the epigenetic switch that links inflammation to cancer. In particular, we demonstrated that STAT3 directly activates transcription of miR-21 and miR-181b-1 miRNAs during the transformation process. STAT3, miR-21 and miR-181b-1 are required for cellular transformation, tumorigenicity and maintenance of the transformed state in vitro as well as tumor growth in vivo. The resulting inhibition of PTEN and CYLD expression (downstream of miR-21 and miR-181b-1, respectively) lead to NF-κB activation which is required to maintain the transformed state [146]. However, activation of the inflammatory regulatory circuit that we proposed is not sufficient to induce transformation of normal cells. Instead, this circuit is likely to be relevant in "predisposed" cells that are genetically altered to be at an intermediate stage in the transition between a primary cell and a cancer cell. In such cells, anything that activates the positive factors (NF-κB, Lin28B, IL-6, STAT3, miR-21, miR-181b-1) or inhibits the negative factors (let-7, PTEN, CYLD) will trigger the inflammatory feedback loop that induces and maintains the transformed state.

DNA methylation and miRNA profiling as a new clinical tool

There is increasing evidence that both DNA-methylation patterns and miRNA profiles could serve as biomarkers with potential use in diagnosis—the process of identifying the nature of a disease—and prognosis—a prediction of the probable course and outcome of a disease. The methylation silence of some particular genes and miRNAs signatures are also potential predictors of the response of cancers to various treatments. Alterations of DNA methylation and transcription of miRNAs are very stable phenomena in tissues and body fluids and suitable for sensitive detection. DNA methylation techniques such as methylation-specific PCR (MSP), high performance liquid chromatography (HPLC) and high performance capillary electrophoresis (HPCE) permit the sensitive and quantitative detection of hypermethylated tumor suppressor genes in all types of biological fluids and biopsy specimens. Circulating miRNAs have also high stability in tissues and body fluids and sensitive detection techniques are already available. Oligonucleotide miRNA microarray analysis is the most commonly used high-throughput technique, while other developments include bead-based flow cytometric technique and quantitative real-time PCR for precursor and active miRNAs [183].

DNA methylation

One good example of a hypermethylated gene that can be used as a diagnostic marker is the glutathione S-transferase gene (GSTP1). GSTP1 is hypermethylated in 80–90% of patients with prostate cancer but is not in benign hyperplastic prostate tissue [184]. Another example is the detection of hypermethylated p16 and/or MGMT up to 3 years before the diagnosis of squamous cell lung carcinoma (SCC) [185]. Other potential methylation candidates that are emerging for detection of cancers at early stages are Septin 9 and MLH1 in colorectal cancer (CRC), p16 in gastric cancer and p15 in acute myeloid leukemia (AML) [183].

The disease's prognosis is also another important parameter of clinical importance. Hypermethylation of p16 has been linked to poor outcomes in patients with CRC and lung cancer. DAPK methylation has been reported in different cancer types and could be used as a prognostic biomarker in lung, bladder and cervical cancer [6]. On the other hand, hypomethylation of LINE-1 elements is a poor

prognostic factor in CRC and chronic myeloid leukemia (CML). Other genes like SFRP1 and EMP3 could be candidate markers of poor prognosis in breast and brain cancer, respectively. Analyses of hypermethylated markers and CpG-island microarrays complementary to gene-expression microarray analyses will be an important clinical tool for the diagnosis and prognosis of certain types of cancer [183].

Once the disease has been diagnosed, it is important to administer the appropriate therapy. In this context, the detection of hypermethylated genes that participate in the repair of DNA damage could be of great importance in chemotherapeutic approaches that utilize alkylating agents. The toxicity of these agents could be restrained in the presence of DNA-repair enzymes (e.g., MGMT, MLH1, etc.). The best example of response to treatment is MGMT, since it has been shown that hypermethylation of its promoter is a predictor of a favorable response of gliomas to carmustine or temozolomide [186]. The potential of methylation status of other genes for predicting the response to chemotherapy has also been seen between cisplatin and methylation of MLH1, between methotrexate and RFC methylation, and between tamoxifen and PGR/HSD17B4/CDH13/MYOD1/BRCA1 methylation [183].

MicroRNAs

There is increasing evidence that the detection of circulating miRNAs in the bloodstream could have diagnostic significance. Recent studies have shown that the plasma levels of miR-92 and serum levels of miR-141 could detect individuals with CRC and prostate cancer, respectively. In addition, significant increase of circulating miR-210 is observed among patients with pancreatic and breast cancers. Nevertheless, further work should be done to investigate whether miRNAs are suitable for detection of cancers at early stages and prediction of malignant potential of precancerous lesions [183].

In contrast, there are a number of association studies on miRNAs and prognosis of cancers. The best examples are the oncosuppressor let-7 and the oncogenic miR-21. The first report on associations with cancer prognosis demonstrated that a miRNA expression signature of the let-7 family of miRNAs could predict survival in lung cancer patients [187]. Later studies have shown that increased miR-155 and decreased let-7α are correlated with poor prognosis in patients with lung cancer [188]. MiR-21 is another candidate prognosis factor that could be used in many types of cancer, such as breast cancer, lung cancer, diffuse large B-cell lymphoma, head and neck squamous cell carcinoma and CRC [189]. Genome-wide miRNA expression studies have identified additional miRNA expression profiles that can predict prognosis in other cancer types including liver cancer, esophageal cancer and ovarian cancer [190, 191].

Recent reports are also indicating the potential use of miRNAs as predictors of the response to treatment. For example, inhibition of miR-21 expression in malignant cholangiocytes increases the sensitivity to gemcitabine. In the same line, inhibition of miR-34 expression in patients with chronic lymphocytic leukemia increases the sensitivity to fludarabine-refractory. On the other hand, downregulation of let-7i in patients with epithelial ovarian cancer is associated with resistance to chemotherapy [183]. The field of miRNAs and chemosensitivity is new, but more evidence will be very useful for the development of personalized cancer treatments.

Manipulation of the epigenome as a therapeutic approach

The reversibility of epigenetic changes, their prevalence in cancer genome and their causative role in tumor biology has catalyzed the development, testing and implementation of epigenome-altering anticancer drugs (Table 4). The purpose of the epigenetic therapy is to reverse the causal epigenetic aberrations that occur in cancer and restore the "normal epigenome". It is almost 30 years since the first demonstration that azanucleoside drugs, 5-azacytidine and 5-aza-2′-deoxycitidine (5-aza-CR and 5-aza-CdR, respectively), have remarkable effects on the differentiated state of cells. These cytotoxic compounds inhibit DNA methylation and lead to the re-expression of DNA-methylated silenced genes. The mechanism of their action is that they get incorporated into the DNA of dividing cells during replication and trap DNMTs onto the DNA, leading to their depletion inside the cell [192]. 5-aza-CR (Vidaza) and 5-aza-CdR (Decitabine) have been approved as treatments for the myeloblastic syndrome and leukemia [193, 194]. Part of their activity in patients might be due to their ability to reactivate growth suppressors, such as INK4B (p15) cyclin-dependent kinase inhibitor [195]. However, these demethylating agents have no clinical activity against solid tumors [196].

The fact that nucleosides require incorporation into DNA in order to be fully effective raises concerns regarding their potential toxic effects on normal cells. On the other hand, since these drugs only act on dividing cells, one can argue that they will target only cancer cells [127, 197]. Nevertheless, an alternative approach to discover other inhibitors of DNA methylation that might act without incorporation into the DNA is now being pursued. The description of small molecule inhibitors such as RG101, RG108 and MG98 [198] is a promising development in that direction. These molecules block the catalytic/

Table 4 Epigenetic Therapy

Drug	Use	References
DNMT inhibitors		
Vidaza (5-azacytidine)	MDS leukemia	[193, 205]
Decitabine (5-aza-2′-deoxycitidine)	MDS leukemia	[193, 205]
Zebularine	Urinary bladder cancer	[206]
Procainamide	Prostate cancer	[207]
Procaine	Breast cancer	[208]
EGCG	Cancer of cervix	[209, 210]
	Photocarcinogenesis	
DNMT1 ASO	Solid tumors	[211]
HDACs inhibitors		
Trichostatin A	Breast cancer	[212, 213]
	Ovarian cancer	
Panobinostat (LBH589)	Leukemias	[214]
Vorinostat (SAHA)	Leukemias	[202, 214]
	Solid tumors	
Belinostat (PXD101)	Leukemias	[215]
Depsipeptide (FK228)	Leukemias	[216–218]
	Melanoma	
	Colon cancer	
Valproic acid	Bipolar disorder	[213, 219]
	Leukemias	
	Breast and ovarian cancer	
HMTs inhibitors		
DZNep	Breast cancer	[220]

cofactor-binding sites of DNMTs or target their regulatory mRNA sequences; however, their efficacy is limited.

The demonstration that histone deacetylase inhibitors have antitumor potential [199] has led to the development of a series of inhibitors (Table 4). HDACs inhibitors are a heterogeneous group of chemical compounds that exert their inhibitory effect through their interaction with the catalytic pocket of HDACs. On the basis of their antiproliferative capabilities, they were classified as anticancer drugs in erythroleukemic cells [200]. However, the molecular mechanism of their action was discovered years later when HDAC inhibition was observed following trichostatin A treatment of several cell lines [201]. HDACs inhibitors induce apoptosis through extrinsic (for example, death receptors) and intrinsic (for example, mitochondria) pathways, cell cycle arrest, induction of differentiation, antiangiogenic, anti-invasive and immunomodulatory functions [199, 202]. Interestingly, most of these effects are seen in transformed cells, whereas normal cells appear to be more resistant [202]. Nevertheless, in clinical trials, HDACs inhibitors are associated with a low incidence of adverse events [196]. An important number of HDACs inhibitors have progressed to clinical trials for a wide spectrum of hematological malignancies [203]. The first HDACs inhibitor, suberoylanilide hydroxamic acid (SAHA or vorinostat), has been approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma [204].

Many studies have demonstrated the synergistic activities of DNA-demethylating agents and HDACs inhibitors [121, 221]. For example, treatment of cancer cells with HDAC inhibitors fails to transcriptionally activate tumor suppressor genes with dense promoter methylation until DNA-demethylating agents have first been applied [121]. The proven synergy of DNA-demethylating agents and HDAC inhibitors as well as the dynamic nature of epigenetic processes led to the design of combinatorial cancer treatment strategies. These treatment strategies have been proved to be more effective than individual treatment approaches. For instance, the combination of 5-aza-CdR with different HDAC inhibitors has been proved to be more effective in leukemic cells [221]. Furthermore, combinatorial treatment strategies using 5-aza-CR with different HDAC inhibitors are now under clinical trials for the treatment of hematologic malignancies, such as AML, Hodgkin's and Non-Hodgkin's lymphomas [203].

Histone methyltransferases represent another valid target for the discovery of new drugs that can reactivate silenced genes. One HMT inhibitor, DZNep, was shown to induce apoptosis in cancer cells by selectively targeting polycomb repressive complex 2 proteins, which are generally overexpressed in cancer [220]. Furthermore, overexpression of a dominant-negative point mutant, H3-K27R, in ovarian cancer cells was shown to result in loss of H3K27 methylation, global reduction of DNA methylation, increased expression of tumor suppressor genes, and sensitization to chemotherapy by cisplatin [222]. These findings reinforce the development of specific histone methylation inhibitors.

As active players in important human oncogenic signaling pathways, miRNAs may affect the development of new strategies for cancer therapy. The potential to therapeutically regulate miRNA levels in cancer and possibly to regulate the immune system is now being explored. Inhibiting oncogenic miRNAs or reintroduction of tumor suppressor miRNAs may serve as useful strategies to treat cancer. The development of modified miRNA molecules with longer in vivo half-life and efficacy, such as the locked nucleic acid (LNA)-modified oligonucleotides [223], the anti-miRNA oligonucleotides (AMOs) [224] and the "antagomirs" [225], is the first step towards bringing the fundamental research advances into medical practice. Inhibition of specific miRNAs has been achieved in vivo in mice [225]. Other technologies are being developed to

reintroduce miRNAs back into cells to mimic their function.

Introduction of synthetic miRNAs, which mimic tumor suppressor miRNAs, in malignant cells can provide new avenues for developing cancer therapeutics. A recent study clearly demonstrated this potential in mouse models of hepatocellular carcinoma. MiR-26a is a tumor suppressor miRNA that is reduced in hepatocellular carcinoma. In an MYC-inducible model of liver cancer, animals were treated systemically with miR-26a using adeno-associated virus for delivery. Treated animals showed significant tumor regression without toxicity, indicating that reintroduction of miR-26a may be an effective strategy to treat cancer [226]. The possibility of rescuing the growth-inhibitory effects of miRNAs by means of DNA-demethylation treatment suggests new epigenetic treatment strategies that are also worthy of further exploration [6].

A major impediment to the use of epigenome-altering drugs is that they are nonspecific and can lead to the activation and silencing of genes nondiscriminately. The search for a more specific epigenetic therapy that targets the crucial epigenetic modification or gene target—for example, by using engineered targeting miRNAs—remains a high priority.

Conclusions

An explosion of data in the field of epigenetics during the last decades has resulted in a better understanding of the epigenetic processes and has established the notion that cancer is at least in part an epigenetic disease. Genetic and epigenetic alterations work in concert for the initiation and maintenance of many human tumor types. The common characteristic of self-renewal in stem cells and cancer cells gave rise to the concept of the cancer stem cell-like phenotype. Therapeutic strategies that combine the conventional chemotherapy and the use of epigenome-altering anticancer drugs are now in progress. However, the design of therapies that eradicate cancer stem-like cells, a refractory to standard chemotherapy, without affecting normal stem cells is of great importance. A better understanding of the mechanisms by which miRNAs and chromatin complexes balance self-renewal and differentiation will be necessary for a deeper insight into tumorigenesis and for the development of new therapeutic strategies.

References

1. Feinberg AP, Tycko B (2004) The history of cancer epigenetics. Nat Rev Cancer 4(2):143–153
2. Jones PA, Baylin SB (2007) The epigenomics of cancer. Cell 128(4):683–692
3. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
4. Feinberg AP, Ohlsson R, Henikoff S (2006) The epigenetic progenitor origin of human cancer. Nat Rev Genet 7(1):21–33
5. de la Chapelle A (2004) Genetic predisposition to colorectal cancer. Nat Rev Cancer 4(10):769–780
6. Esteller M (2008) Epigenetics in cancer. N Engl J Med 358(11):1148–1159
7. Tysnes BB (2010) Tumor-initiating and -propagating cells: cells that we would like to identify and control. Neoplasia 12(7):506–515
8. Baylin SB, Ohm JE (2006) Epigenetic gene silencing in cancer: a mechanism for early oncogenic pathway addiction? Nat Rev Cancer 6(2):107–116
9. Houghton J, Morozov A, Smirnova I, Wang TC (2007) Stem cells and cancer. Semin Cancer Biol 17(3):191–203
10. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16(1):6–21
11. Kim GD, Ni J, Kelesoglu N, Roberts RJ, Pradhan S (2002) Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. EMBO J 21(15):4183–4195
12. Prendergast GC, Ziff EB (1991) Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. Science 251(4990):186–189
13. Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, Bird A (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393(6683):386–389
14. Suzuki MM, Bird A (2008) DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet 9(6):465–476
15. Feinberg AP, Vogelstein B (1983) Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature 301(5895):89–92
16. Feinberg AP, Gehrke CW, Kuo KC, Ehrlich M (1988) Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res 48(5):1159–1161
17. Rodriguez J, Frigola J, Vendrell E, Risques RA, Fraga MF, Morales C, Moreno V, Esteller M, Capella G, Ribas M, Peinado MA (2006) Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. Cancer Res 66(17):8462–9468
18. Eden A, Gaudet F, Waghmare A, Jaenisch R (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300(5618):455
19. Yeh A, Wei M, Golub SB, Yamashiro DJ, Murty VV, Tycko B (2002) Chromosome arm 16q in Wilms tumors: unbalanced chromosomal translocations, loss of heterozygosity, and assessment of the CTCF gene. Genes Chromosomes Cancer 35(2):156–163
20. Karpf AR, Matsui S (2005) Genetic disruption of cytosine DNA methyltransferase enzymes induces chromosomal instability in human cancer cells. Cancer Res 65(19):8635–8639
21. Xu GL, Bestor TH, Bourc'his D, Hsieh CL, Tommerup N, Bugge M, Hulten M, Qu X, Russo JJ, Viegas-Pequignot E (1999) Chromosome instability and immunodeficiency syndrome caused by mutations in a DNA methyltransferase gene. Nature 402(6758):187–191
22. Howard G, Eiges R, Gaudet F, Jaenisch R, Eden A (2008) Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. Oncogene 27(3):404–408

23. Wilson AS, Power BE, Molloy PL (2007) DNA hypomethylation and human diseases. Biochim Biophys Acta 1775(1): 138–162
24. Brueckner B, Stresemann C, Kuner R, Mund C, Musch T, Meister M, Sultmann H, Lyko F (2007) The human let-7a-3 locus contains an epigenetically regulated microRNA gene with oncogenic function. Cancer Res 67(4):1419–1423
25. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, Wu Y, He X, Powe NR, Feinberg AP (2003) Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 299(5613):1753–1755
26. Feinberg AP (1999) Mendel stayed home. Genomic imprinting, environmental disease susceptibility. National Institute of Environmental Health Sciences, Duke University Medical Center, Durham, NC, USA, 8–10 October 1998. Trends Genet 15(2):46
27. Mohandas T, Sparkes RS, Shapiro LJ (1981) Reactivation of an inactive human X chromosome: evidence for X inactivation by DNA methylation. Science 211(4480):393–396
28. Esteller M (2006) The necessity of a human epigenome project. Carcinogenesis 27(6):1121–1125
29. Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP (1991) Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. Am J Hum Genet 48(5): 880–888
30. Herman JG, Baylin SB (2003) Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349(21):2042–2054
31. Esteller M (2007) Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 8(4):286–298
32. Long C, Yin B, Lu Q, Zhou X, Hu J, Yang Y, Yu F, Yuan Y (2007) Promoter hypermethylation of the RUNX3 gene in esophageal squamous cell carcinoma. Cancer Invest 25(8): 685–690
33. Akiyama Y, Watkins N, Suzuki H, Jair KW, van Engeland M, Esteller M, Sakai H, Ren CY, Yuasa Y, Herman JG, Baylin SB (2003) GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. Mol Cell Biol 23(23):8429–8439
34. Dobrovic A, Simpfendorfer D (1997) Methylation of the BRCA1 gene in sporadic breast cancer. Cancer Res 57(16): 3347–3350
35. Issa JP, Vertino PM, Wu J, Sazawal S, Celano P, Nelkin BD, Hamilton SR, Baylin SB (1993) Increased cytosine DNA-methyltransferase activity during colon cancer progression. J Natl Cancer Inst 85(15):1235–1240
36. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R (2003) Induction of tumors in mice by genomic hypomethylation. Science 300(5618): 489–492
37. Linhart HG, Lin H, Yamada Y, Moran E, Steine EJ, Gokhale S, Lo G, Cantu E, Ehrich M, He T, Meissner A, Jaenisch R (2007) Dnmt3b promotes tumorigenesis in vivo by gene-specific de novo methylation and transcriptional silencing. Genes Dev 21(23):3110–3122
38. Rhee I, Bachman KE, Park BH, Jair KW, Yen RW, Schuebel KE, Cui H, Feinberg AP, Lengauer C, Kinzler KW, Baylin SB, Vogelstein B (2002) DNMT1 and DNMT3b cooperate to silence genes in human cancer cells. Nature 416(6880):552–556
39. Rountree MR, Bachman KE, Baylin SB (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 25(3):269–277
40. Fuks F, Burgers WA, Godin N, Kasai M, Kouzarides T (2001) Dnmt3a binds deacetylases and is recruited by a sequence-specific repressor to silence transcription. EMBO J 20(10):2536–2544
41. Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M, Fuks F, Lo Coco F, Kouzarides T, Nervi C, Minucci S, Pelicci PG (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science 295(5557):1079–1082
42. Bestor TH (2003) Unanswered questions about the role of promoter methylation in carcinogenesis. Ann N Y Acad Sci 983:22–27
43. Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, Li GM, Drummond J, Modrich PL, Sedwick WD, Markowitz SD (1998) Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc Natl Acad Sci USA 95(15):8698–8702
44. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275(5308):1943–1947
45. Alvarez-Nunez F, Bussaglia E, Mauricio D, Ybarra J, Vilar M, Lerma E, de Leiva A, Matias-Guiu X (2006) PTEN promoter methylation in sporadic thyroid carcinomas. Thyroid 16(1): 17–23
46. Costello JF, Berger MS, Huang HS, Cavenee WK (1996) Silencing of p16/CDKN2 expression in human gliomas by methylation and chromatin condensation. Cancer Res 56(10): 2405–2410
47. Lee MN, Tseng RC, Hsu HS, Chen JY, Tzao C, Ho WL, Wang YC (2007) Epigenetic inactivation of the chromosomal stability control genes BRCA1, BRCA2, and XRCC5 in non-small cell lung cancer. Clin Cancer Res 13(3):832–838
48. Chiang JW, Karlan BY, Cass L, Baldwin RL (2006) BRCA1 promoter methylation predicts adverse ovarian cancer prognosis. Gynecol Oncol 101(3):403–410
49. Virmani AK, Rathi A, Sathyanarayana UG, Padar A, Huang CX, Cunnigham HT, Farinas AJ, Milchgrub S, Euhus DM, Gilcrease M, Herman J, Minna JD, Gazdar AF (2001) Aberrant methylation of the adenomatous polyposis coli (APC) gene promoter 1A in breast and lung carcinomas. Clin Cancer Res 7(7):1998–2004
50. Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Gonzalez S, Tarafa G, Sidransky D, Meltzer SJ, Baylin SB, Herman JG (2000) Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. Cancer Res 60(16):4366–4371
51. Garcia MJ, Castrillo JM, Granizo JJ, Cazorla A, Rivas C (2002) Clinicopathological correlation, p16-p15 methylation status and outcome predictors in anaplastic large cell lymphoma. Br J Haematol 119(3):877–878
52. Ng MH, Chung YF, Lo KW, Wickham NW, Lee JC, Huang DP (1997) Frequent hypermethylation of p16 and p15 genes in multiple myeloma. Blood 89(7):2500–2506
53. Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, Baylin SB, Herman JG (1999) hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. Am J Pathol 155(5):1767–1772
54. Li LC, Chui R, Nakajima K, Oh BR, Au HC, Dahiya R (2000) Frequent methylation of estrogen receptor in prostate cancer: correlation with tumor progression. Cancer Res 60(3):702–706
55. Ozdag H, Batley SJ, Forsti A, Iyer NG, Daigo Y, Boutell J, Arends MJ, Ponder BA, Kouzarides T, Caldas C (2002) Mutation analysis of CBP and PCAF reveals rare inactivating mutations in cancer cell lines but not in primary tumours. Br J Cancer 87(10):1162–1165

56. Muraoka M, Konishi M, Kikuchi-Yanoshita R, Tanaka K, Shitara N, Chong JM, Iwama T, Miyaki M (1996) p300 gene alterations in colorectal and gastric carcinomas. Oncogene 12(7):1565–1569
57. Yang XJ (2004) The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. Nucleic Acids Res 32(3):959–976
58. Iyer NG, Ozdag H, Caldas C (2004) p300/CBP and cancer. Oncogene 23(24):4225–4231
59. Kishimoto M, Kohno T, Okudela K, Otsuka A, Sasaki H, Tanabe C, Sakiyama T, Hirama C, Kitabayashi I, Minna JD, Takenoshita S, Yokota J (2005) Mutations and deletions of the CBP gene in human lung cancer. Clin Cancer Res 11(2 Pt 1):512–519
60. Fog CK, Jensen KT, Lund AH (2007) Chromatin-modifying proteins in cancer. APMIS 115(10):1060–1089
61. Rozman M, Camos M, Colomer D, Villamor N, Esteve J, Costa D, Carrio A, Aymerich M, Aguilar JL, Domingo A, Sole F, Gomis F, Florensa L, Montserrat E, Campo E (2004) Type I MOZ/CBP (MYST3/CREBBP) is the most common chimeric transcript in acute myeloid leukemia with t(8;16)(p11;p13) translocation. Genes Chromosomes Cancer 40(2):140–145
62. Deguchi K, Ayton PM, Carapeti M, Kutok JL, Snyder CS, Williams IR, Cross NC, Glass CK, Cleary ML, Gilliland DG (2003) MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. Cancer Cell 3(3):259–271
63. Choi JH, Kwon HJ, Yoon BI, Kim JH, Han SU, Joo HJ, Kim DY (2001) Expression profile of histone deacetylase 1 in gastric cancer tissues. Jpn J Cancer Res 92(12):1300–1304
64. Halkidou K, Gaughan L, Cook S, Leung HY, Neal DE, Robson CN (2004) Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. Prostate 59(2):177–189
65. Ishihama K, Yamakawa M, Semba S, Takeda H, Kawata S, Kimura S, Kimura W (2007) Expression of HDAC1 and CBP/p300 in human colorectal carcinomas. J Clin Pathol 60(11):1205–1210
66. Song J, Noh JH, Lee JH, Eun JW, Ahn YM, Kim SY, Lee SH, Park WS, Yoo NJ, Lee JY, Nam SW (2005) Increased expression of histone deacetylase 2 is found in human gastric cancer. APMIS 113(4):264–268
67. Zhang Z, Yamashita H, Toyama T, Sugiura H, Omoto Y, Ando Y, Mita K, Hamaguchi M, Hayashi S, Iwase H (2004) HDAC6 expression is correlated with better survival in breast cancer. Clin Cancer Res 10(20):6962–6968
68. Osada H, Tatematsu Y, Saito H, Yatabe Y, Mitsudomi T, Takahashi T (2004) Reduced expression of class II histone deacetylase genes is associated with poor prognosis in lung cancer patients. Int J Cancer 112(1):26–32
69. De Nigris F, Cerutti J, Morelli C, Califano D, Chiariotti L, Viglietto G, Santelli G, Fusco A (2002) Isolation of a SIR-like gene, SIR-T8, that is overexpressed in thyroid carcinoma cell lines and tissues. Br J Cancer 87(12):1479
70. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, Fanelli M, Ruthardt M, Ferrara FF, Zamir I, Seiser C, Lazar MA, Minucci S, Pelicci PG (1998) Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature 391(6669):815–818
71. Jones LK, Saha V (2002) Chromatin modification, leukaemia and implications for therapy. Br J Haematol 118(3):714–727
72. Masutani M, Nakagama H, Sugimura T (2003) Poly(ADP-ribose) and carcinogenesis. Genes Chromosomes Cancer 38(4):339–348
73. Cao WH, Wang X, Frappart L, Rigal D, Wang ZQ, Shen Y, Tong WM (2007) Analysis of genetic variants of the poly(ADP-ribose) polymerase-1 gene in breast cancer in French patients. Mutat Res 632(1–2):20–28
74. Shimizu S, Nomura F, Tomonaga T, Sunaga M, Noda M, Ebara M, Saisho H (2004) Expression of poly(ADP-ribose) polymerase in human hepatocellular carcinoma and analysis of biopsy specimens obtained under sonographic guidance. Oncol Rep 12(4):821–825
75. Ratnam K, Low JA (2007) Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology. Clin Cancer Res 13(5):1383–1388
76. Dalgliesh GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, Davies H, Edkins S, Hardy C, Latimer C, Teague J, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Forbes S, Jia M, Jones D, Knott H, Kok CY, Lau KW, Leroy C, Lin ML, McBride DJ, Maddison M, Maguire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, O'Meara S, Pleasance E, Rajasingham A, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turrell K, Dykema KJ, Khoo SK, Petillo D, Wondergem B, Anema J, Kahnoski RJ, Teh BT, Stratton MR, Futreal PA (2010) Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature 463(7279):360–363
77. Coles AH, Jones SN (2009) The ING gene family in the regulation of cell growth and tumorigenesis. J Cell Physiol 218(1):45–57
78. Wang GG, Song J, Wang Z, Dormann HL, Casadio F, Li H, Luo JL, Patel DJ, Allis CD (2009) Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. Nature 459(7248):847–851
79. Andrews PG, Lake BB, Popadiuk C, Kao KR (2007) Requirement of Pygopus 2 in breast cancer. Int J Oncol 30(2):357–363
80. Wang Y, Zhang H, Chen Y, Sun Y, Yang F, Yu W, Liang J, Sun L, Yang X, Shi L, Li R, Li Y, Zhang Y, Li Q, Yi X, Shang Y (2009) LSD1 is a subunit of the NuRD complex and targets the metastasis programs in breast cancer. Cell 138(4):660–672
81. Yamane K, Tateishi K, Klose RJ, Fang J, Fabrizio LA, Erdjument-Bromage H, Taylor-Papadimitriou J, Tempst P, Zhang Y (2007) PLU-1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. Mol Cell 25(6):801–812
82. Scibetta AG, Santangelo S, Coleman J, Hall D, Chaplin T, Copier J, Catchpole S, Burchell J, Taylor-Papadimitriou J (2007) Functional analysis of the transcription repressor PLU-1/JARID1B. Mol Cell Biol 27(20):7220–7235
83. Tzatsos A, Pfau R, Kampranis SC, Tsichlis PN (2009) Ndy1/KDM2B immortalizes mouse embryonic fibroblasts by repressing the Ink4a/Arf locus. Proc Natl Acad Sci USA 106(8):2641–2646
84. Simon JA, Lange CA (2008) Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 647(1–2):21–29
85. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, Shashkin P, Charlot JF, Tcherpakov M, Corbett R, Tam A, Varhol R, Smailus D, Moksa M, Zhao Y, Delaney A, Qian H, Birol I, Schein J, Moore R, Holt R, Horsman DE, Connors JM, Jones S, Aparicio S, Hirst M, Gascoyne RD, Marra MA (2010) Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet 42(2):181–185
86. Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenfuhr M, Maertens G, Banck M, Zhou MM, Walsh MJ, Peters G, Gil J (2009) Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. Genes Dev 23(10):1177–1182

87. Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J, Helin K (2009) The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. Genes Dev 23(10):1171–1176
88. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, Edkins S, Hardy C, O'Meara S, Teague J, Butler A, Hinton J, Latimer C, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Cole J, Forbes S, Jia M, Jones D, Kok CY, Leroy C, Lin ML, McBride DJ, Maddison M, Maquire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, Pleasance E, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Collins VP, Ichimura K, Law S, Wong J, Yuen ST, Leung SY, Tonon G, DePinho RA, Tai YT, Anderson KC, Kahnoski RJ, Massie A, Khoo SK, Teh BT, Stratton MR, Futreal PA (2009) Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet 41(5):521–523
89. Lim S, Metzger E, Schule R, Kirfel J, Buettner R (2010) Epigenetic regulation of cancer growth by histone demethylases. Int J Cancer (in press)
90. Wissmann M, Yin N, Muller JM, Greschik H, Fodor BD, Jenuwein T, Vogler C, Schneider R, Gunther T, Buettner R, Metzger E, Schule R (2007) Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. Nat Cell Biol 9(3):347–353
91. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ (1997) Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 389(6648):251–260
92. Vaquero A, Loyola A, Reinberg D (2003) The constantly changing face of chromatin. Sci Aging Knowledge Environ 2003(14):RE4
93. Grozinger CM, Schreiber SL (2002) Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem Biol 9(1):3–16
94. Lachner M, O'Sullivan RJ, Jenuwein T (2003) An epigenetic road map for histone lysine methylation. J Cell Sci 116(Pt 11):2117–2124
95. Chi P, Allis CD, Wang GG (2010) Covalent histone modifications—miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer 10(7):457–469
96. Polytarchou C, Pfau R, Hatziapostolou M, Tsichlis PN (2008) The JmjC domain histone demethylase Ndy1 regulates redox homeostasis and protects cells from oxidative stress. Mol Cell Biol 28(24):7451–7464
97. Kouzarides T (2007) Chromatin modifications and their function. Cell 128(4):693–705
98. Vidanes GM, Bonilla CY, Toczyski DP (2005) Complicated tails: histone modifications and the DNA damage response. Cell 121(7):973–976
99. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T (2001) Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 410(6824):116–120
100. Hebbes TR, Thorne AW, Crane-Robinson C (1988) A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J 7(5):1395–1402
101. Liang G, Lin JC, Wei V, Yoo C, Cheng JC, Nguyen CT, Weisenberger DJ, Egger G, Takai D, Gonzales FA, Jones PA (2004) Distinct localization of histone H3 acetylation and H3–K4 methylation to the transcription start sites in the human genome. Proc Natl Acad Sci USA 101(19):7357–7362
102. Fraga MF, Ballestar E, Villar-Garea A, Boix-Chornet M, Espada J, Schotta G, Bonaldi T, Haydon C, Ropero S, Petrie K, Iyer NG, Perez-Rosado A, Calvo E, Lopez JA, Cano A, Calasanz MJ, Colomer D, Piris MA, Ahn N, Imhof A, Caldas C, Jenuwein T, Esteller M (2005) Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 37(4):391–400
103. Mikkelsen TS, Wakefield MJ, Aken B, Amemiya CT, Chang JL, Duke S, Garber M, Gentles AJ, Goodstadt L, Heger A, Jurka J, Kamal M, Mauceli E, Searle SM, Sharpe T, Baker ML, Batzer MA, Benos PV, Belov K, Clamp M, Cook A, Cuff J, Das R, Davidow L, Deakin JE, Fazzari MJ, Glass JL, Grabherr M, Greally JM, Gu W, Hore TA, Huttley GA, Kleber M, Jirtle RL, Koina E, Lee JT, Mahony S, Marra MA, Miller RD, Nicholls RD, Oda M, Papenfuss AT, Parra ZE, Pollock DD, Ray DA, Schein JE, Speed TP, Thompson K, VandeBerg JL, Wade CM, Walker JA, Waters PD, Webber C, Weidman JR, Xie X, Zody MC, Graves JA, Ponting CP, Breen M, Samollow PB, Lander ES, Lindblad-Toh K (2007) Genome of the marsupial *Monodelphis domestica* reveals innovation in non-coding sequences. Nature 447(7141):167–177
104. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 125(2):315–326
105. Tachibana M, Matsumura Y, Fukuda M, Kimura H, Shinkai Y (2008) G9a/GLP complexes independently mediate H3K9 and DNA methylation to silence transcription. EMBO J 27(20):2681–2690
106. Zhao Q, Rank G, Tan YT, Li H, Moritz RL, Simpson RJ, Cerruti L, Curtis DJ, Patel DJ, Allis CD, Cunningham JM, Jane SM (2009) PRMT5-mediated methylation of histone H4R3 recruits DNMT3A, coupling histone and DNA methylation in gene silencing. Nat Struct Mol Biol 16(3):304–311
107. Jones PL, Veenstra GJ, Wade PA, Vermaak D, Kass SU, Landsberger N, Strouboulis J, Wolffe AP (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nat Genet 19(2):187–191
108. Esteve PO, Chin HG, Benner J, Feehery GR, Samaranayake M, Horwitz GA, Jacobsen SE, Pradhan S (2009) Regulation of DNMT1 stability through SET7-mediated lysine methylation in mammalian cells. Proc Natl Acad Sci USA 106(13):5076–5081
109. Wang J, Hevi S, Kurash JK, Lei H, Gay F, Bajko J, Su H, Sun W, Chang H, Xu G, Gaudet F, Li E, Chen T (2009) The lysine demethylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat Genet 41(1):125–129
110. Fuks F, Hurd PJ, Wolf D, Nan X, Bird AP, Kouzarides T (2003) The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. J Biol Chem 278(6):4035–4040
111. Fraga MF, Esteller M (2005) Towards the human cancer epigenome: a first draft of histone modifications. Cell Cycle 4(10):1377–1381
112. Tryndyak VP, Kovalchuk O, Pogribny IP (2006) Loss of DNA methylation and histone H4 lysine 20 trimethylation in human breast cancer cells is associated with aberrant expression of DNA methyltransferase 1, Suv4–20h2 histone methyltransferase and methyl-binding proteins. Cancer Biol Ther 5(1):65–70
113. Pogribny IP, Ross SA, Wise C, Pogribna M, Jones EA, Tryndyak VP, James SJ, Dragan YP, Poirier LA (2006) Irreversible global DNA hypomethylation as a key step in hepatocarcinogenesis induced by dietary methyl deficiency. Mutat Res 593(1–2):80–87
114. Seligson DB, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdistani SK (2005) Global histone modification patterns predict risk of prostate cancer recurrence. Nature 435(7046):1262–1266
115. Valk-Lingbeek ME, Bruggeman SW, van Lohuizen M (2004) Stem cells and cancer; the polycomb connection. Cell 118(4):409–418

116. Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3(6):415–428
117. Jones RS (2007) Epigenetics: reversing the 'irreversible'. Nature 450(7168):357–359
118. Fahrner JA, Eguchi S, Herman JG, Baylin SB (2002) Dependence of histone modifications and gene expression on DNA hypermethylation in cancer. Cancer Res 62(24):7213–7218
119. Richon VM, Sandhoff TW, Rifkind RA, Marks PA (2000) Histone deacetylase inhibitor selectively induces p21WAF1 expression and gene-associated histone acetylation. Proc Natl Acad Sci USA 97(18):10014–10019
120. Ayyanathan K, Lechner MS, Bell P, Maul GG, Schultz DC, Yamada Y, Tanaka K, Torigoe K, Rauscher FJ 3rd (2003) Regulated recruitment of HP1 to a euchromatic gene induces mitotically heritable, epigenetic gene silencing: a mammalian cell culture model of gene variegation. Genes Dev 17(15): 1855–1869
121. Cameron EE, Bachman KE, Myohanen S, Herman JG, Baylin SB (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. Nat Genet 21(1):103–107
122. Ozdag H, Teschendorff AE, Ahmed AA, Hyland SJ, Blenkiron C, Bobrow L, Veerakumarasivam A, Burtt G, Subkhankulova T, Arends MJ, Collins VP, Bowtell D, Kouzarides T, Brenton JD, Caldas C (2006) Differential expression of selected histone modifier genes in human solid cancers. BMC Genomics 7:90
123. Kondo Y, Shen L, Suzuki S, Kurokawa T, Masuko K, Tanaka Y, Kato H, Mizuno Y, Yokoe M, Sugauchi F, Hirashima N, Orito E, Osada H, Ueda R, Guo Y, Chen X, Issa JP, Sekido Y (2007) Alterations of DNA methylation and histone modifications contribute to gene silencing in hepatocellular carcinomas. Hepatol Res 37(11):974–983
124. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA (2004) Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 119(7):941–953
125. Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH, Gunther T, Buettner R, Schule R (2005) LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature 437(7057):436–439
126. Jiang C, Pugh BF (2009) Nucleosome positioning and gene regulation: advances through genomics. Nat Rev Genet 10(3):161–172
127. Sharma S, Kelly TK, Jones PA (2010) Epigenetics in cancer. Carcinogenesis 31(1):27–36
128. Lin JC, Jeong S, Liang G, Takai D, Fatemi M, Tsai YC, Egger G, Gal-Yam EN, Jones PA (2007) Role of nucleosomal occupancy in the epigenetic silencing of the MLH1 CpG island. Cancer Cell 12(5):432–444
129. Morey L, Brenner C, Fazi F, Villa R, Gutierrez A, Buschbeck M, Nervi C, Minucci S, Fuks F, Di Croce L (2008) MBD3, a component of the NuRD complex, facilitates chromatin alteration and deposition of epigenetic marks. Mol Cell Biol 28(19):5912–5923
130. Chai J, Charboneau AL, Betz BL, Weissman BE (2005) Loss of the hSNF5 gene concomitantly inactivates p21CIP/WAF1 and p16INK4a activity associated with replicative senescence in A204 rhabdoid tumor cells. Cancer Res 65(22):10192–10198
131. Naidu SR, Love IM, Imbalzano AN, Grossman SR, Androphy EJ (2009) The SWI/SNF chromatin remodeling subunit BRG1 is a critical regulator of p53 necessary for proliferation of malignant cells. Oncogene 28(27):2492–2501
132. Jin C, Felsenfeld G (2007) Nucleosome stability mediated by histone variants H3.3 and H2A.Z. Genes Dev 21(12):1519–1529
133. Svotelis A, Gevry N, Gaudreau L (2009) Regulation of gene expression and cellular proliferation by histone H2A.Z. Biochem Cell Biol 87(1):179–188
134. Tomonaga T, Matsushita K, Yamaguchi S, Oohashi T, Shimada H, Ochiai T, Yoda K, Nomura F (2003) Overexpression and mistargeting of centromere protein-A in human primary colorectal cancer. Cancer Res 63(13):3511–3516
135. Lee I, Ajay SS, Yook JI, Kim HS, Hong SH, Kim NH, Dhanasekaran SM, Chinnaiyan AM, Athey BD (2009) New class of microRNA targets containing simultaneous 5′-UTR and 3′-UTR interaction sites. Genome Res 19(7):1175–1183
136. Zhou X, Duan X, Qian J, Li F (2009) Abundant conserved microRNA target sites in the 5′-untranslated region and coding sequence. Genetica 137(2):159–164
137. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297
138. Valeri N, Vannini I, Fanini F, Calore F, Adair B, Fabbri M (2009) Epigenetics, miRNAs, and human cancer: a new chapter in human gene regulation. Mamm Genome 20(9–10):573–580
139. Hatfield S, Ruohola-Baker H (2008) microRNA and stem cell function. Cell Tissue Res 331(1):57–66
140. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I (2008) MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. Nature 455(7216):1124–1128
141. Lin SL, Chang DC, Chang-Lin S, Lin CH, Wu DT, Chen DT, Ying SY (2008) Mir-302 reprograms human skin cancer cells into a pluripotent ES-cell-like state. RNA 14(10):2115–2124
142. Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM (2002) Frequent deletions and down-regulation of micro- RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 99(24):15524–15529
143. Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan S, Bullrich F, Negrini M, Croce CM (2004) Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci USA 101(9):2999–3004
144. Ventura A, Jacks T (2009) MicroRNAs and cancer: short RNAs go a long way. Cell 136(4):586–591
145. Iliopoulos D, Hirsch HA, Struhl K (2009) An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell 139(4):693–706
146. Iliopoulos D, Jaeger SA, Hirsch HA, Bulyk ML, Struhl K (2010) STAT3 Activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell 39(4):493–506
147. Scott GK, Mattie MD, Berger CE, Benz SC, Benz CC (2006) Rapid alteration of microRNA levels by histone deacetylase inhibition. Cancer Res 66(3):1277–1281
148. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, Jones PA (2006) Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. Cancer Cell 9(6):435–443
149. Datta J, Kutay H, Nasser MW, Nuovo GJ, Wang B, Majumder S, Liu CG, Volinia S, Croce CM, Schmittgen TD, Ghoshal K, Jacob ST (2008) Methylation mediated silencing of MicroRNA-1 gene and its role in hepatocellular carcinogenesis. Cancer Res 68(13):5049–5058
150. Lujambio A, Ropero S, Ballestar E, Fraga MF, Cerrato C, Setien F, Casado S, Suarez-Gauthier A, Sanchez-Cespedes M, Git A, Spiteri I, Das PP, Caldas C, Miska E, Esteller M (2007) Genetic unmasking of an epigenetically silenced microRNA in human cancer cells. Cancer Res 67(4):1424–1429
151. Agirre X, Vilas-Zornoza A, Jimenez-Velasco A, Martin-Subero JI, Cordeu L, Garate L, San Jose-Eneriz E, Abizanda G, Rodriguez-Otero P, Fortes P, Rifon J, Bandres E, Calasanz MJ,

Martin V, Heiniger A, Torres A, Siebert R, Roman-Gomez J, Prosper F (2009) Epigenetic silencing of the tumor suppressor microRNA Hsa-miR-124a regulates CDK6 expression and confers a poor prognosis in acute lymphoblastic leukemia. Cancer Res 69(10):4443–4453

152. Roman-Gomez J, Agirre X, Jimenez-Velasco A, Arqueros V, Vilas-Zornoza A, Rodriguez-Otero P, Martin-Subero I, Garate L, Cordeu L, San Jose-Eneriz E, Martin V, Castillejo JA, Bandres E, Calasanz MJ, Siebert R, Heiniger A, Torres A, Prosper F (2009) Epigenetic regulation of microRNAs in acute lymphoblastic leukemia. J Clin Oncol 27(8):1316–1322
153. Fazi F, Racanicchi S, Zardo G, Starnes LM, Mancini M, Travaglini L, Diverio D, Ammatuna E, Cimino G, Lo-Coco F, Grignani F, Nervi C (2007) Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. Cancer Cell 12(5):457–466
154. Lehmann U, Hasemeier B, Christgen M, Muller M, Romermann D, Langer F, Kreipe H (2008) Epigenetic inactivation of microRNA gene hsa-mir-9-1 in human breast cancer. J Pathol 214(1):17–24
155. Lujambio A, Calin GA, Villanueva A, Ropero S, Sanchez-Cespedes M, Blanco D, Montuenga LM, Rossi S, Nicoloso MS, Faller WJ, Gallagher WM, Eccles SA, Croce CM, Esteller M (2008) A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci USA 105(36):13556–13561
156. Wang H, Garzon R, Sun H, Ladner KJ, Singh R, Dahlman J, Cheng A, Hall BM, Qualman SJ, Chandler DS, Croce CM, Guttridge DC (2008) NF-kappaB-YY1-miR-29 regulatory circuitry in skeletal myogenesis and rhabdomyosarcoma. Cancer Cell 14(5):369–381
157. Toyota M, Suzuki H, Sasaki Y, Maruyama R, Imai K, Shinomura Y, Tokino T (2008) Epigenetic silencing of microRNA-34b/c and B-cell translocation gene 4 is associated with CpG island methylation in colorectal cancer. Cancer Res 68(11):4123–4132
158. Lee KH, Lotterman C, Karikari C, Omura N, Feldmann G, Habbe N, Goggins MG, Mendell JT, Maitra A (2009) Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. Pancreatology 9(3):293–301
159. Lujambio A, Esteller M (2007) CpG island hypermethylation of tumor suppressor microRNAs in human cancer. Cell Cycle 6(12):1455–1459
160. Furuta M, Kozaki KI, Tanaka S, Arii S, Imoto I, Inazawa J (2010) miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis 31(5):766–776
161. Saito Y, Friedman JM, Chihara Y, Egger G, Chuang JC, Liang G (2009) Epigenetic therapy upregulates the tumor suppressor microRNA-126 and its host gene EGFL7 in human cancer cells. Biochem Biophys Res Commun 379(3):726–731
162. Balaguer F, Link A, Lozano JJ, Cuatrecasas M, Nagasaka T, Boland CR, Goel A (2010) Epigenetic silencing of miR-137 is an early event in colorectal carcinogenesis. Cancer Res 70(16):6609–6618
163. Hashimoto Y, Akiyama Y, Otsubo T, Shimada S, Yuasa Y (2010) Involvement of epigenetically silenced microRNA-181c in gastric carcinogenesis. Carcinogenesis 31(5):777–784
164. Rauhala HE, Jalava SE, Isotalo J, Bracken H, Lehmusvaara S, Tammela TL, Oja H, Visakorpi T (2010) miR-193b is an epigenetically regulated putative tumor suppressor in prostate cancer. Int J Cancer 127(6):1363–1372
165. Tsai KW, Hu LY, Wu CW, Li SC, Lai CH, Kao HW, Fang WL, Lin WC (2010) Epigenetic regulation of miR-196b expression in gastric cancer. Genes Chromosomes Cancer (in press)
166. Li A, Omura N, Hong SM, Vincent A, Walter K, Griffith M, Borges M, Goggins M (2010) Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels. Cancer Res 70(13):5226–5237
167. Iliopoulos D, Polytarchou C, Hatziapostolou M, Kottakis F, Maroulakou IG, Struhl K, Tsichlis PN (2009) MicroRNAs differentially regulated by Akt isoforms control EMT and stem cell renewal in cancer cells. Sci Signal 2(92):ra62
168. Grady WM, Parkin RK, Mitchell PS, Lee JH, Kim YH, Tsuchiya KD, Washington MK, Paraskeva C, Willson JK, Kaz AM, Kroh EM, Allen A, Fritz BR, Markowitz SD, Tewari M (2008) Epigenetic silencing of the intronic microRNA hsa-miR-342 and its host gene EVL in colorectal cancer. Oncogene 27(27):3880–3888
169. Lu L, Katsaros D, de la Longrais IA, Sochircа O, Yu H (2007) Hypermethylation of let-7a-3 in epithelial ovarian cancer is associated with low insulin-like growth factor-II expression and favorable prognosis. Cancer Res 67(21):10117–10122
170. Chen JF, Mandel EM, Thomson JM, Wu Q, Callis TE, Hammond SM, Conlon FL, Wang DZ (2006) The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 38(2):228–233
171. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA 104(40):15805–15810
172. Garzon R, Heaphy CE, Havelange V, Fabbri M, Volinia S, Tsao T, Zanesi N, Kornblau SM, Marcucci G, Calin GA, Andreeff M, Croce CM (2009) MicroRNA 29b functions in acute myeloid leukemia. Blood 114(26):5331–5341
173. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM (2008) Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322(5908):1695–1699
174. Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, Zhou X, Jones PA (2009) The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res 69(6):2623–2629
175. Tuddenham L, Wheeler G, Ntounia-Fousara S, Waters J, Hajihosseini MK, Clark I, Dalmay T (2006) The cartilage specific microRNA-140 targets histone deacetylase 4 in mouse cells. FEBS Lett 580(17):4214–4217
176. Duursma AM, Kedde M, Schrier M, le Sage C, Agami R (2008) miR-148 targets human DNMT3b protein coding region. RNA 14(5):872–877
177. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W (2008) MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. Nat Struct Mol Biol 15(3):259–267
178. Noonan EJ, Place RF, Pookot D, Basak S, Whitson JM, Hirata H, Giardina C, Dahiya R (2009) miR-449a targets HDAC-1 and induces growth arrest in prostate cancer. Oncogene 28(14):1714–1724
179. Ptashne M (2009) Binding reactions: epigenetic switches, signal transduction and cancer. Curr Biol 19(6):R234–R241
180. Gal-Yam EN, Egger G, Iniguez L, Holster H, Einarsson S, Zhang X, Lin JC, Liang G, Jones PA, Tanay A (2008) Frequent switching of Polycomb repressive marks and DNA

hypermethylation in the PC3 prostate cancer cell line. Proc Natl Acad Sci USA 105(35):12979–12984
181. Soldner F, Hockemeyer D, Beard C, Gao Q, Bell GW, Cook EG, Hargus G, Blak A, Cooper O, Mitalipova M, Isacson O, Jaenisch R (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5):964–977
182. Yu H, Pardoll D, Jove R (2009) STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 9(11):798–809
183. Deng D, Liu Z, Du Y (2010) Epigenetic alterations as cancer diagnostic, prognostic, and predictive biomarkers. Adv Genet 71:125–176
184. Jeronimo C, Usadel H, Henrique R, Oliveira J, Lopes C, Nelson WG, Sidransky D (2001) Quantitation of GSTP1 methylation in non-neoplastic prostatic tissue and organ-confined prostate adenocarcinoma. J Natl Cancer Inst 93(22):1747–1752
185. Palmisano WA, Divine KK, Saccomanno G, Gilliland FD, Baylin SB, Herman JG, Belinsky SA (2000) Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res 60(21):5954–5958
186. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, Baylin SB, Herman JG (2000) Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N Engl J Med 343(19):1350–1354
187. Takamizawa J, Konishi H, Yanagisawa K, Tomida S, Osada H, Endoh H, Harano T, Yatabe Y, Nagino M, Nimura Y, Mitsudomi T, Takahashi T (2004) Reduced expression of the let-7 microRNAs in human lung cancers in association with shortened postoperative survival. Cancer Res 64(11):3753–3756
188. Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC (2006) Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 9(3):189–198
189. Schetter AJ, Heegaard NH, Harris CC (2010) Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis 31(1):37–49
190. Budhu A, Jia HL, Forgues M, Liu CG, Goldstein D, Lam A, Zanetti KA, Ye QH, Qin LX, Croce CM, Tang ZY, Wang XW (2008) Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology 47(3):897–907
191. Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S (2008) MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res 14(9):2690–2695
192. Constantinides PG, Jones PA, Gevers W (1977) Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment. Nature 267(5609):364–366
193. Muller CI, Ruter B, Koeffler HP, Lubbert M (2006) DNA hypermethylation of myeloid cells, a novel therapeutic target in MDS and AML. Curr Pharm Biotechnol 7(5):315–321
194. Oki Y, Issa JP (2007) Treatment options in advanced myelodysplastic syndrome, with emphasis on epigenetic therapy. Int J Hematol 86(4):306–314
195. Daskalakis M, Nguyen TT, Nguyen C, Guldberg P, Kohler G, Wijermans P, Jones PA, Lubbert M (2002) Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2′-deoxycytidine (decitabine) treatment. Blood 100(8):2957–2964
196. Mack GS (2006) Epigenetic cancer therapy makes headway. J Natl Cancer Inst 98(20):1443–1444
197. Yang AS, Estecio MR, Garcia-Manero G, Kantarjian HM, Issa JP (2003) Comment on. Chromosomal instability and tumors promoted by DNA hypomethylationand "Induction of tumors in nice by genomic hypomethylation". Science 302(5648):1153, author reply 1153
198. Cortez CC, Jones PA (2008) Chromatin, cancer and drug therapies. Mutat Res 647(1–2):44–51
199. Minucci S, Pelicci PG (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6(1):38–51
200. Leder A, Orkin S, Leder P (1975) Differentiation of erythroleukemic cells in the presence of inhibitors of DNA synthesis. Science 190(4217):893–894
201. Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265(28):17174–17179
202. Dokmanovic M, Clarke C, Marks PA (2007) Histone deacetylase inhibitors: overview and perspectives. Mol Cancer Res 5(10):981–989
203. Villagra A, Sotomayor EM, Seto E (2010) Histone deacetylases and the immunological network: implications in cancer and inflammation. Oncogene 29(2):157–173
204. Marks PA (2007) Discovery and development of SAHA as an anticancer agent. Oncogene 26(9):1351–1356
205. Oki Y, Aoki E, Issa JP (2007) Decitabine—bedside to bench. Crit Rev Oncol Hematol 61(2):140–152
206. Foubister V (2003) Drug reactivates genes to inhibit cancer. Drug Discov Today 8(10):430–431
207. Lin X, Asgari K, Putzi MJ, Gage WR, Yu X, Cornblatt BS, Kumar A, Piantadosi S, DeWeese TL, De Marzo AM, Nelson WG (2001) Reversal of GSTP1 CpG island hypermethylation and reactivation of pi-class glutathione S-transferase (GSTP1) expression in human prostate cancer cells by treatment with procainamide. Cancer Res 61(24):8611–8616
208. Villar-Garea A, Fraga MF, Espada J, Esteller M (2003) Procaine is a DNA-demethylating agent with growth-inhibitory effects in human cancer cells. Cancer Res 63(16):4984–4989
209. Noguchi M, Yokoyama M, Watanabe S, Uchiyama M, Nakao Y, Hara K, Iwasaka T (2006) Inhibitory effect of the tea polyphenol, (–)-epigallocatechin gallate, on growth of cervical adenocarcinoma cell lines. Cancer Lett 234(2):135–142
210. Mittal A, Piyathilake C, Hara Y, Katiyar SK (2003) Exceptionally high protection of photocarcinogenesis by topical application of (–)-epigallocatechin-3-gallate in hydrophilic cream in SKH-1 hairless mouse model: relationship to inhibition of UVB-induced global DNA hypomethylation. Neoplasia 5(6):555–565
211. Davis AJ, Gelmon KA, Siu LL, Moore MJ, Britten CD, Mistry N, Klamut H, D'Aloisio S, MacLean M, Wainman N, Ayers D, Firby P, Besterman JM, Reid GK, Eisenhauer EA (2003) Phase I and pharmacologic study of the human DNA methyltransferase antisense oligodeoxynucleotide MG98 given as a 21-day continuous infusion every 4 weeks. Invest New Drugs 21(1):85–97
212. Joung KE, Kim DK, Sheen YY (2004) Antiproliferative effect of trichostatin A and HC-toxin in T47D human breast cancer cells. Arch Pharm Res 27(6):640–645
213. Reid G, Metivier R, Lin CY, Denger S, Ibberson D, Ivacevic T, Brand H, Benes V, Liu ET, Gannon F (2005) Multiple mechanisms induce transcriptional silencing of a subset of genes, including oestrogen receptor alpha, in response to deacetylase inhibition by valproic acid and trichostatin A. Oncogene 24(31):4894–4907
214. Frew AJ, Johnstone RW, Bolden JE (2009) Enhancing the apoptotic and therapeutic effects of HDAC inhibitors. Cancer Lett 280(2):125–133
215. Gimsing P (2009) Belinostat: a new broad acting antineoplastic histone deacetylase inhibitor. Expert Opin Investig Drugs 18(4):501–508

216. Zhou W, Zhu WG (2009) The changing face of HDAC inhibitor depsipeptide. Curr Cancer Drug Targets 9(1):91–100
217. Klisovic DD, Klisovic MI, Effron D, Liu S, Marcucci G, Katz SE (2005) Depsipeptide inhibits migration of primary and metastatic uveal melanoma cell lines in vitro: a potential strategy for uveal melanoma. Melanoma Res 15(3):147–153
218. Xiao JJ, Huang Y, Dai Z, Sadee W, Chen J, Liu S, Marcucci G, Byrd J, Covey JM, Wright J, Grever M, Chan KK (2005) Chemoresistance to depsipeptide FK228 [(E)-(1S, 4S, 10S, 21R)-7-[(Z)-ethylidene]-4, 21-diisopropyl-2-oxa-12, 13-dithi a-5, 8, 20, 23-tetraazabicyclo[8, 7, 6]-tricos-16-ene-3, 6, 9, 22-pentanone] is mediated by reversible MDR1 induction in human cancer cell lines. J Pharmacol Exp Ther 314(1):467–475
219. Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA (2008) Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev 34(3):206–222
220. Tan J, Yang X, Zhuang L, Jiang X, Chen W, Lee PL, Karuturi RK, Tan PB, Liu ET, Yu Q (2007) Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev 21(9):1050–1063
221. Klisovic MI, Maghraby EA, Parthun MR, Guimond M, Sklenar AR, Whitman SP, Chan KK, Murphy T, Anon J, Archer KJ, Rush LJ, Plass C, Grever MR, Byrd JC, Marcucci G (2003) Depsipeptide (FR 901228) promotes histone acetylation, gene transcription, apoptosis and its activity is enhanced by DNA methyltransferase inhibitors in AML1/ETO-positive leukemic cells. Leukemia 17(2):350–358
222. Abbosh PH, Montgomery JS, Starkey JA, Novotny M, Zuhowski EG, Egorin MJ, Moseman AP, Golas A, Brannon KM, Balch C, Huang TH, Nephew KP (2006) Dominant-negative histone H3 lysine 27 mutant derepresses silenced tumor suppressor genes and reverses the drug-resistant phenotype in cancer cells. Cancer Res 66(11):5582–5591
223. Orom UA, Kauppinen S, Lund AH (2006) LNA-modified oligonucleotides mediate specific inhibition of microRNA function. Gene 372:137–141
224. Weiler J, Hunziker J, Hall J (2006) Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? Gene Ther 13(6):496–502
225. Krutzfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M (2005) Silencing of microRNAs in vivo with 'antagomirs'. Nature 438(7068):685–689
226. Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekanandan P, Torbenson M, Clark KR, Mendell JR, Mendell JT (2009) Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Cell 137(6):1005–1017