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Epigenetic Therapies for Cancer

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1. Introduction

At the cellular level, cancers originate from the monoclonal expansion of a mutant cell leading to accumulation of aberrant cells that continue to lose differentiated features and acquire different biological properties in their progression toward disseminated or metastatic disease. The onset and progression of cancer involves genomic derangements that can be manifested in two ways: 1) Genetic and gross structural defects (e.g. single nucleotide polymorphism (SNP), classic deletion, insertion mutation, chromosomal deletion/inversion/translocation, allelic loss/gain, gene amplification/ deletion), and 2) Aberrant epigenetic covalent modifications (e.g. DNA methylation, histone acetylation, methylation, phosphorylation, citrullination, sumoylation, and ADP ribosylation).

Genomic instability can be triggered by chemical carcinogens, radiation, stress, oncogenic DNA viruses and the aging process. In almost all cancers, genomic instability in the form of genetic alterations or epigenetic modifications affects four classes of genes: oncogenes, tumor suppressor genes, apoptotic genes and/or DNA repair genes. Oncogenes encode proteins that function as positive proliferative signals for tumors. Tumor suppressor genes negatively regulate cell proliferation and are inactivated in many tumors. Apoptotic genes encode proteins that instruct the cell to commit suicide, while DNA repair genes encode proteins that maintain the fidelity of DNA sequences during transcription and replication. The uncontrolled expression of oncogenes or the silencing of tumor suppressor genes can lead to immortalization of cells. For example, in neuroblastoma, the overexpression of N-myc oncogene correlates with aggressive tumor behavior (Seeger et al., 1985). The ras oncogene is activated in more than half of the tumors studied in humans (Barbacid et al., 1987), and both relapse and decreased survival in breast cancer patients have been associated with overexpression of Her-2 oncogene (Slamon et al., 1987). The tumor suppressor and cell cycle regulator gene, p53, is mutated or deleted in more than 50% of human tumors (Hollstein M et al., 1991). p53 gene is described as the guardian of the genome because it can activate DNA repair genes when DNA is damaged, or induce apoptosis when DNA damage is sensed to be irreparable.

Despite the presence of defective genes in tumors, tumors actually arise through many different combinations of genetic alterations. The phenotypic diversity observed between normal and cancer cells cannot be explained simply by structural and genetic alterations. Epigenetic mechanisms have been shown to activate or inactivate genes. Conrad Waddington first coined the term epigenetic to mean changes above and beyond (epi) the primary DNA sequence (Waddington, 1939). The term epigenetic refers to heritable genetic

variations that give rise to distinct patterns of terminal differentiation phenotypes (Waddington, 1952; Ruden et al., 2005; Goldberg et al., 2007). These heritable variations are independent of the DNA sequence, can be reversible, and often are self-perpetuating (Bonasia et al 2010). Epigenetic modifications can include DNA methylation, histone acetylation, histone methylation, histone phosphorylation, citrullination, sumoylation, and ADP ribosylation. A classic example of epigenetic control is seen as cells undergo differentiation during development (Figure 1). While every cell in the human body has identical DNA sequence (except for T and B cells), epigenetic patterns lead the same cells to differentiate into a wide array of cell types and the formation of different tissues or organs (Figure 1). Also, animals cloned from the same donor DNA are not identical and develop diseases with different penetrance from the donor parent (Esteller, 2008; Rideout 3rd et al., 2001). The methylation patterns (Fraga et al., 2005b; Kaminsky et al., 2009), and histone modification profiles (Kaminsky et al., 2009), are different in monozygote twins, indicating that while epigenetic patterns are stable and heritable, they are also dynamic in the sense that genes can be silenced or activated due to changes in cellular environment (Figure 1). In normal cells, epigenetic patterns are in dynamic equilibrium (Szyf, 2007). Many diseases, of which cancer is no exception, arise when different types of epigenetic patterns are introduced at the wrong time, and/or the wrong place. For example, the hypermethylation of tumor suppressor genes, *t16^{INK4a}*, *p14^{ARF}* and *MGMT*, has been reported as an early event

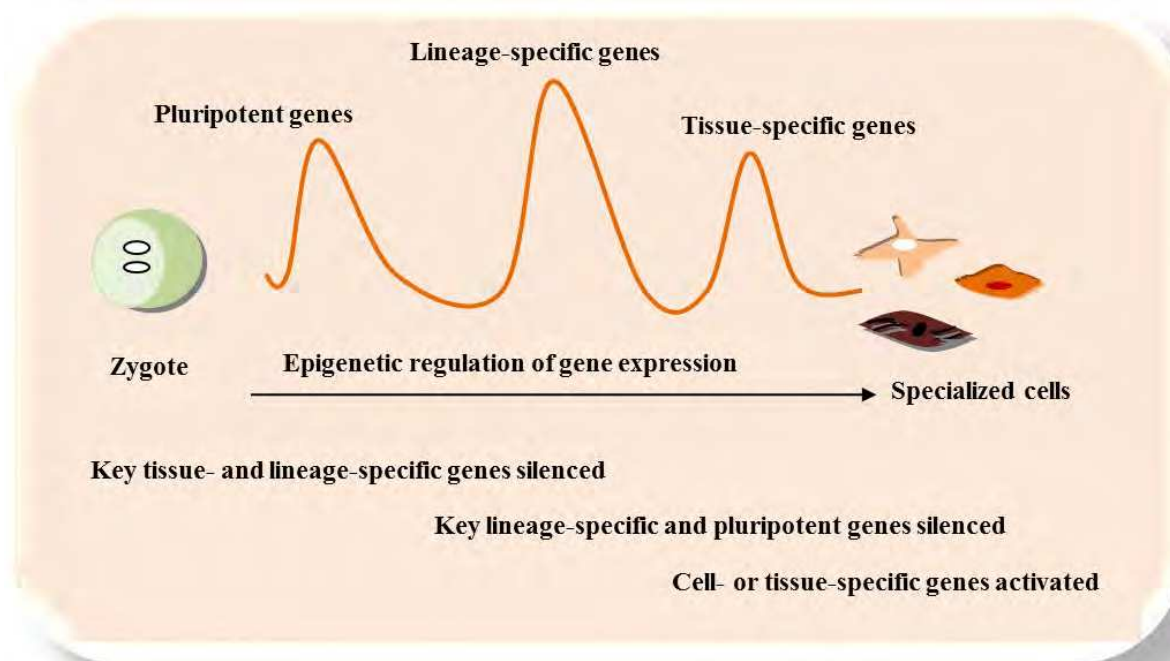


Fig. 1. Epigenetic changes leading to activation or silencing of genes during development. Depicted in the figure is the time dependent activation or inactivation of key genes during development (i.e. from zygote to specialized cells). While every cell has exactly the same DNA composition, the interplay between epigenetic modifications such as DNA methylation or histone modifications (e.g. histone methylation, acetylation, phosphorylation, ubiquitination and sumoylation) can lead to the expression or silencing of pluripotent genes, lineage specific genes or tissue specific genes during development leading to the formation of specialized cells or organs. Peaks depict activation and troughs denote silencing.

in tumorigenesis (Esteller et al., 2007). Because such epigenetic changes are reversible, the epigenome of cancer cells represents an ideal target for cancer treatments. Tumor suppressor genes turned off can be switched back on, and oncogenes turned on can be switched off to restore the epigenetic balance of cancer cells. How epigenetic modifications occur, and how epigenetic profiles define the genomic landscape of cancerous cells and their response to treatment will be the focus of this chapter.

2. Fundamentals of epigenetics

Epigenetic modifications can broadly be classified into three categories: DNA methylation, histone modification and nucleosome positioning. Epigenetic phenotypes result from the interplay between these three categories. Within the microenvironment of the cell, epigenetic patterns are established by transiently activated, and/or stably expressed factors that respond to environmental stimuli, developmental cues, or internal events. Of particular importance within the context of cancer, is the impact of exogenous substances on epigenetic modifications of gene regulation. Since the treatment of many cancers involves exposure to toxic substances, it is important to understand how some of these substances regulate epigenetic modifications. Hence the trajectory of cancer treatment is leaning toward a treatment regimen that includes epigenetic drugs capable of modifying the neoplastic phenotypes to induce shifts in phenotypic expression rather than cytotoxic alterations.

2.1 DNA methylation

DNA methylation was the first identified epigenetic regulation of gene expression in mammals (Holliday and Pugh, 1975; Riggs, 1975). It occurs by enzymatic transfer of a methyl group to carbon 5 of the pyrimidine base, cytosine, in the 5'-3' cytosine guanine (CpG) dinucleotide sequence. Mainly, S-adenosylmethionine (SAM) acts as methyl donor in this reaction. DNA methylation can be classified into four categories based on the region of the genome where methylation occurs. The first identified DNA methylation occurs exclusively in CpG dinucleotides. CpG dinucleotides normally cluster in regions of DNA called CpG islands. They are regions of DNA approximately 200-500 bases long with a G + C content greater than 50% and CpG to GC ratio of at least 0.6 (Bird, 1986; Gardiner-Garden and Frommer, 1987). CpG dinucleotides constitute 1% of the genome and are normally found in the promoter region of genes (Figures 2a and 2b). For example, the promoters of 50-70% of known human genes contain CpG dinucleotides (Bird et al., 1987; Larson et al., 1982; Wang and Lung, 2004). The second kind of DNA methylation is called gene body methylation (Hellman and Chess, 2007). This type of methylation occurs in the open reading frame of genes (Figure 2b) and functions to prevent spurious transcriptional initiation (Hellman and Chess, 2007), or alternative splicing of mainly ubiquitously expressed genes (Zilberman et al., 2007). Recently it has been suggested that gene body methylation also occurs at non CpG dinucleotides (i.e. CHG or CHH sites where H = A, C or T) (Lister et al., 2009; Laurent et al., 2010). A third kind of DNA methylation occurs at CpG island shores (Irizarry et al., 2009; Doi et al., 2009). The term CpG island shores refers to regions of the genome with lower density of CpG dinucleotides that lay approximately 2000 bases away from the CpG islands (Figure 2d). The CpG island shores determines the methylation pattern of tissues (Irizarry et al., 2009; Doi et al., 2009), and the reprogramming of stem cells (Doi et al., 2009; Ji et al., 2010). A fourth kind of DNA methylation occurs at repetitive sequences (e.g. transposable elements and microsatellite regions) (Figure 2e and 2f). The

transposable sequences constitute about 47% of the entire genome (Babushok and Kazazian, 2007). Examples of transposable elements are the DNA transposons (e.g. Mer1/2) and retrotransposons (e.g. non-terminal repeat retrotransposon, such as Lines and Sines and long terminal repeat retrotransposon, such as HERVs and IAPs). When activated, these repetitive sequences can move from one region of the genome to another through a so called “cut and paste” or “copy and paste” mechanisms, respectively. Microsatellites are tandem repeats of DNA embedded in various regions of the genome, and their methylation can result in either gene silencing or chromosome instability if it occurs in centromeric regions of the chromosome.

All four types of DNA methylation are catalyzed by a class of enzymes called DNA methyltransferases (DNMT). In mammals, there are five DNMT isoforms: DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L (Siedlecki and Zielenkiewicz, 2006). The catalytic domain is conserved between the DNMTs (except DNMT3L), while the regulatory domain responsible for a protein-protein interaction is variable. DNMT1 is a maintenance methyltransferase that ensures the pattern of DNA methylation is transferred from daughter to parent. DNMT1 and proliferating cell nuclear antigen (PCNA) colocalize to DNA replication foci in early S phase (Chuang et al., 1997). Loss of DNMT1 causes cell cycle arrest and apoptosis (Chen et al., 2007). DNMT2 has little DNA methylation activity and DNMT2 knockout mice display no aberrant DNA methylation patterns (Okano et al., 1998). Studies by Goll et al. (2006) have shown that the main function of DNMT2 is to methylate tRNA outside the nucleus. DNMT3a and DNMT3b are *de novo* methyltransferases responsible for establishing methylation patterns during early development. These *de novo* DNMTs are highly expressed in embryonic stem cells and their expression is downregulated after differentiation (Esteller et al., 2007). DNMT3L lacks the catalytic domain and is involved in establishing maternal genomic imprinting by acting as a stimulatory factor for DNMT3a and DNMT3b (Bourc’his et al., 2001). DNMT3L has been shown to interact and colocalize with both DNMT3a and DNMT3b in the nucleus (Chen et al 2005; Holt-Schietinger et al., 2010).

In general, DNA methylations (except gene body methylation) inhibit gene expression. DNA methylation at CpG islands, CpG island shores and repetitive sequence alters the conformation of the DNA in such a way that it prevents recruitment of transcription factors and positive regulators. In addition, methylated DNA promotes the recruitment of Methyl-CpG binding (MBD) proteins (Lopez-Serra and Esteller, 2008). Five classes of MBDs have been identified: MeCP2, MBD1, MBD2, MBD3, and MBD4. MBDs recruit histone deacetylases (HDACs) and histone methyltransferase (HMTs). HMTs methylate histone, with methylated histones being recognized and bound by heterochromatin complexes, such as heterochromatin protein 1 (HP1). These conditions combine to create a chromatin structure (heterochromatin) that favors transcriptional repression. Conversely, unmethylated DNA favors the formation of active chromatin (euchromatin). The formation of active chromatin results in recruitment of histone acetyltransferases and methyltransferases which create domains characterized by high levels of acetylation and trimethylation at H3K4, H3K36 and H3K79 leading to unwinding of chromatin and binding of transcriptional factors that lead to gene expression.

Compared to normal cells, cancer cells are characterized by global hypomethylation (overall 20-60 less CpG methylation) (Goel et al., 1985). Hypomethylation in cancer cells results in the induction of oncogenic genes, loss of imprinting, activation of transposable elements and microsatellite instability (Dunn, 2003; Esteller, 2008) (Figure 2). Hypomethylation at specific promoters can lead to aberrant expression of oncogenes and loss of imprinting (Figure 2a).

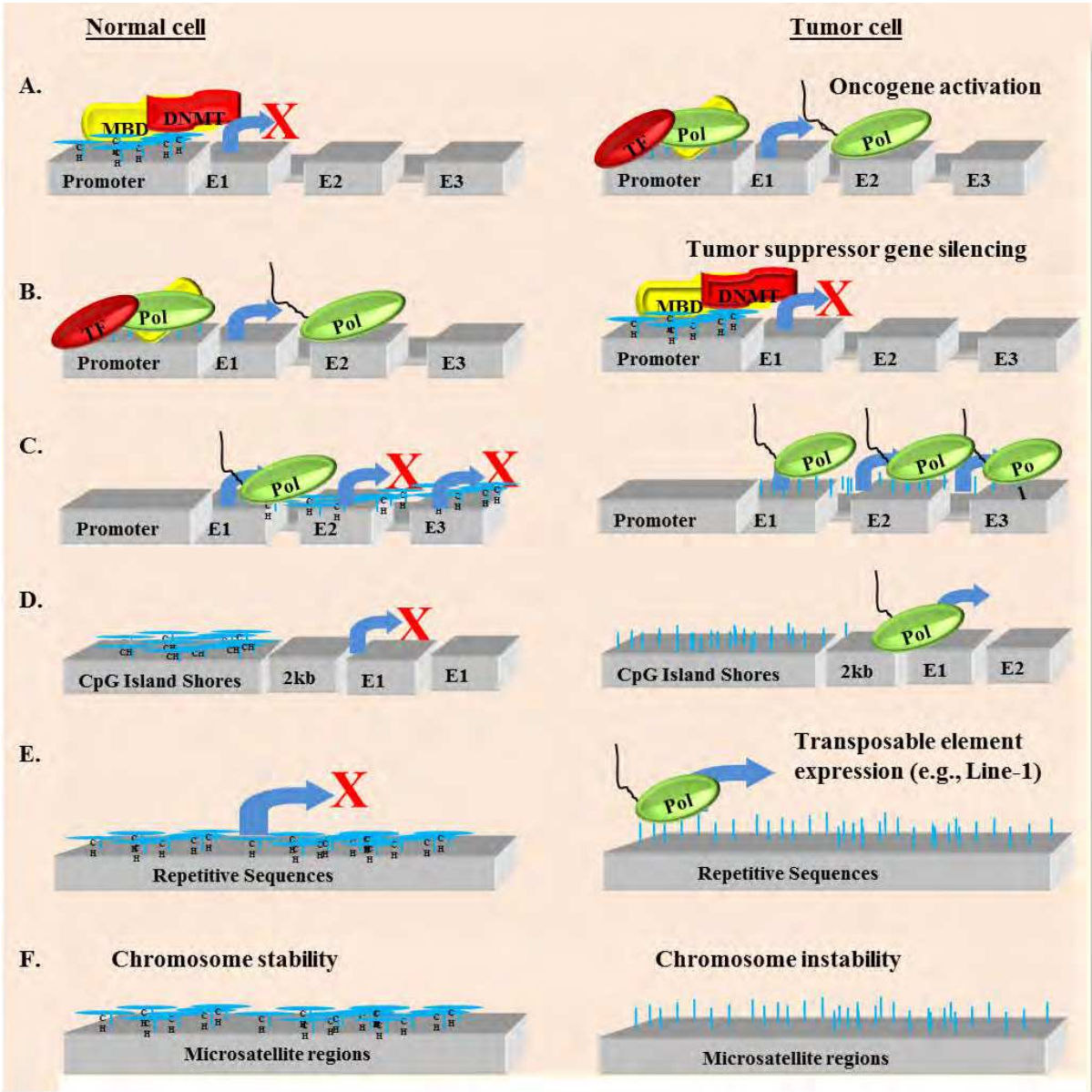


Fig. 2. Methylation patterns in normal versus cancer cells. A. Hypomethylation of oncogene leads to their activation in cancer cells. B. Hypermethylation at the promoter region of a tumor suppressor gene leads to their silencing in cancer cells. C. Gene body hypomethylation leads to spurious transcription initiation in ubiquitously expressed genes. D. Hypomethylation at CpG island shores leads to ubiquitous expression of key regulatory genes. E & F. Hypomethylation at repetitive or microsatellite sequences leads to expression of transposable elements and chromosomal instability, respectively.

Oncogenes such as S100P, SNCG, melanoma-associated gene (MAGE) and dipeptidyl peptidase 6 (DPP6) are hypomethylated in pancreatic cancer, breast cancer and melanomas, respectively (Wilson et al., 2007; Irizarry et al., 2009). This hypomethylation converts the expression of these genes into aberrantly expressed genes leading to increase growth and metastatic advantage. Loss of imprinting due to hypomethylation has been reported for insulin-like growth factor 2 (IGF2) gene in breast, liver, lung and colon cancers (Ito et al.,

2008). Repetitive sequences are hypomethylated and reactivated in many cancers (Goel et al., 1985; Gaudet et al., 2003; Futscher et al., 2004), as documented for lung, breast, bladder, and liver cancers (Wilson et al., 2007). Lastly, hypomethylation of microsatellite regions at the pericentromeric region leads to genomic instability (Kuismanen et al., 1999).

While the genomes of cancer cells are globally hypomethylated, some aberrant DNA methylation occurs at specific regions of the genome (Figure 2b). These aberrant methylations affect the expression of genes involved in cell cycle control (e.g. p53, Rb, p16^{INK4a}, p15^{INK4b}), apoptosis (TMSI, DAPKI, WIF-1 and SERP1) and DNA repair (e.g. BRAC1, WRN, MGMT, hMLH1). For example, hypermethylation of the CpG dinucleotide in the promoter region of the tumor suppressor gene p16 occurs in 20% of human cancers (Merlo et al., 1995). In addition, the guardian of the genome, p53, is epigenetically silenced in a large proportion of human cancers (Hollstein et al 1991; Jirtle, 1999, Jirtle and Skinner 2007; Jones and Baylin, 2004). Hypermethylation of the promoter region of *MASPIN* gene was reported as an early event in breast cancer (Futcher et al., 2004). Hypermethylation at CpG regions is also prone to spontaneous point mutations. Methylated cytosines can undergo spontaneous deamination and a subsequent conversion to uracil leading to C-T transition. This results in a rate of mutation at methylated CpG regions that is 42 times higher than predicted for random mutation (Cooper and Youssoufian, 1998). Point mutation (C-T) is frequently seen in p53 and Rb genes leading to loss of function of these proteins (Cooper and Youssoufian, 1988; Magewu and Jone, 1994; Tornaletti and Pfeifer, 1995; Manici et al., 1997). In addition, CpG sites are favored binding sites for carcinogens which also lead to increase rates of CpG mutations (Magewu and Jone, 1994; Yoon et al., 2001).

The question of why some regions of the genome are hypermethylated and others hypomethylated in cancer is poorly understood. However, most of the hypomethylated regions in tumors lie outside the so called CpG islands. In normal cells, these non CpG regions are methylated. Also the patterns of hypermethylation are tumor-specific leading to the idea that selection pressures in favor of growth for clonal cells might lead to different patterns of methylation. Another explanation for the site-specific hypermethylation could be due to recruitment of DNMTs by accessory proteins in cancer cells. The identity of these accessory proteins may be unique to the cancer cell phenotype, and also exhibit specificity for different cancer subtypes. Finally, epigenetic profiles in cancer may involve dysregulation of DNMT expression. This suggestion is consistent with the finding that DNMT1 and DNMT3b are overexpressed in many tumors (Miremadi et al., 2007). In addition, many tumors are characterized by downregulation of miRNAs due to methylation at their promoters (Saito et al., 2006; Melo et al., 2009). Conversely, miRNAs have also been shown to target DNMTs. In fact, miR-29 has been shown to target and downregulate both DNMT3a and DNMT3b, and indirectly DNMT3L (Garzon et al., 2009). Compared to normal cells, it is possible that regulation of DNMTs by miRNAs is dysregulated in cancer cells resulting in unique patterns of DNMT expression.

2.2 Nucleosome positioning

Total eukaryotic DNA is 2m long and must fit into the nucleus which has an approximate size of 2 μ m³. This is accomplished by an elaborate interaction between DNA, four core histone proteins (i.e. H2A, H2B, H3 and H4) and one linker histone. The core histone proteins are arranged into two sets of H3/H4 and H2A/H2B heterodimers. This arrangement forms into an octamer shaped structure, called the nucleosome, around which ~146bp of DNA is wrapped (Kornberg 1974). The interaction between the nucleosome and

DNA is facilitated by the positively charged amino acids of the histone proteins and the negatively charged phosphate backbone of the DNA. Nucleosomes are separated from each other by 20-100 bp linker regions. The linker region is bound by another histone protein called histone 1 (H1). H1 and its variants function to promote the coiling of nucleosomes into fiber like structures in cells (Bedner et al., 1998). Linker histones are distinguished from other histones because of special modifications at key amino acids, or in their tail regions or domain structures (Li et al., 2007). Nucleosomes, DNA and linker histones are packaged to form chromatin. The conformation of chromatin is determined by the positioning of the nucleosome and its level of modification. For example, loss of nucleosomes at the transcription start site is directly correlated to gene expression (Figure 3), and occlusion of the nucleosome at transcription start sites is correlated with transcriptional repression (Schomes et al., 2008; Cairns et al., 2009). At this level, nucleosomes act as barriers to transcription because they block access to binding sites for transcription factors and regulators (Figure 3), or block elongation by sterically hindering the movement of RNA polymerase II. The precise position of the nucleosome is influenced by linker histones (Zilberman et al., 2007), and chromatin remodeling complexes (Clapier et al., 2009). Incorporation of different histone variants can influence transcription and the methylation landscape in eukaryotic cells (Chodavarapu et al., 2010). For example, incorporation of histone linker H2A.Z protects genes against DNA methylation (Zilberman et al., 2008), thereby playing a role in epigenetic activation of transcription.

The chromatin remodeling complexes are classified into four families: mating type switch/sucrose non-fermenting (SWI/SNF) family, chromodomain helicase DNA-binding (CHD) family, Imitation SWItch (ISWI) family and Inositol/choline responsive element dependent gene activation mutant-80 (INO80) family. These four families are distinguished by unique features within their catalytic subunits that allow them to read specific histone post-translational modifications that stabilize their interaction with chromatin. They also differ in the composition of the other subunits (Ho and Crabtree, 2010). Chromatin remodeling complexes function by moving, ejecting, destabilizing or restructuring the nucleosome in an ATP dependent manner. The remodeling machinery is also influenced by both DNA methylation (Harikrisnan et al., 2005), and histone modifications (Wysoca et al., 2006).

Mammalian SWI/SNF enzymes are multisubunit complexes of 1-2MDa and consist of 9-12 subunits, one of which is an ATPase (De la Serna et al., 2006). The ATPase subunit is identified as either Brahma (BRM) or brama/swi2-related gene-1(BRGI) which have been recognized as human homologs. The BRGI subunit is 75% identical between SWI/SNF family members. Functionally, SWI/SNFs are master regulators of gene expression. For example, SWI/SNFs are involved in regulating the expression of FOS, CRYAB, MIM-1, p21 and CSF-1. These complexes are also involved in alternative splicing (Reisman et al., 2009).

The CHD family of chromatin remodeling complexes is distinguished by having two chromodomains that have affinity for methylated histones (Marfella et al., 2007). There are nine CHD proteins that can be divided into three subfamilies based on the presence of other conserved domains and interacting factors: I. (CHD1 and 2), II. (CHD3 and 4), III. (CHD 5-9). Some CHD family members are involved in the sliding and ejection of the nucleosome thereby promoting transcriptional activation. Others like Mi-2/NuRD have HDAC activity and can also act as methyl binding protein (Clapier et al., 2009). These family members at this role act as transcriptional repressors.

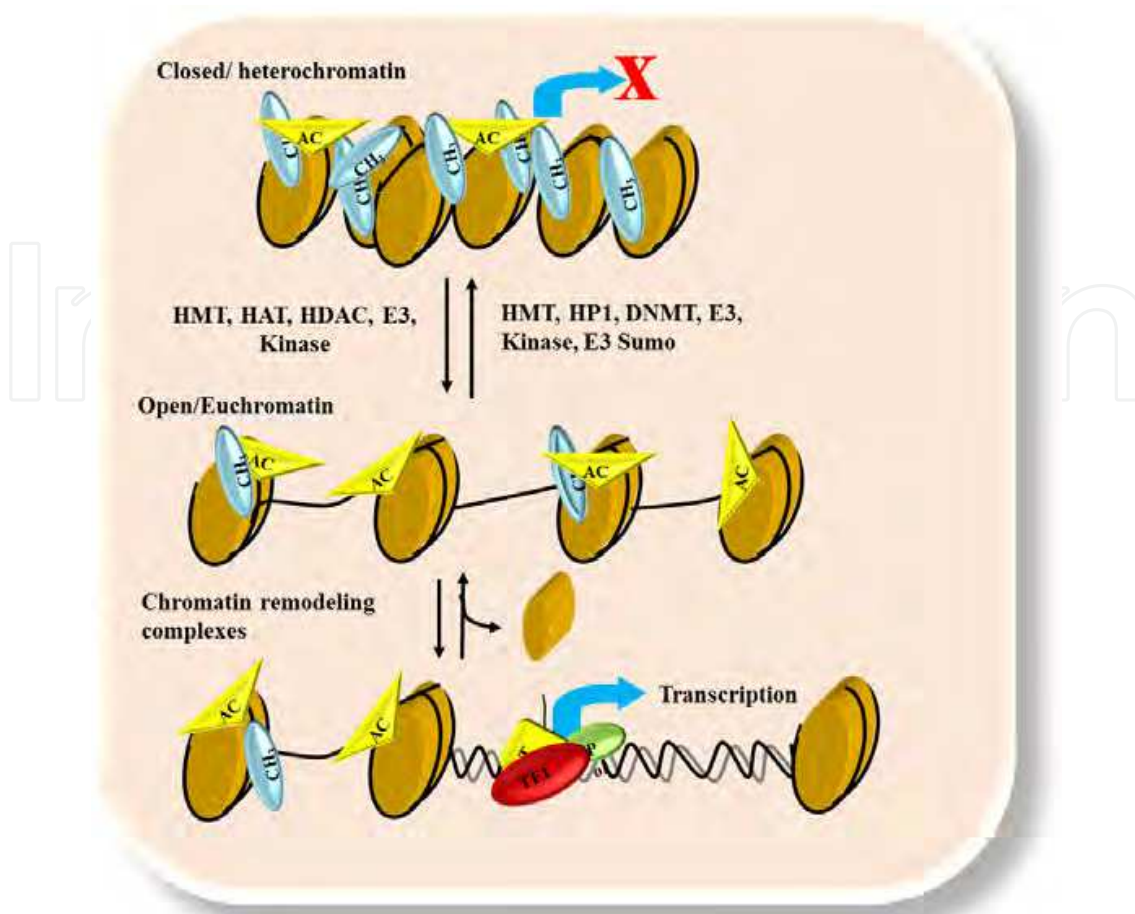


Fig. 3. Chromatin exists in a transcriptionally repressed (heterochromatin) or transcriptionally active (Euchromatin) states. Epigenetic modifications such as DNA methylation, histone methylation, phosphorylation, sumoylation and ubiquitination favor the formation of heterochromatin and lead to transcriptional repression. Conversely, histone acetylation, methylation, phosphorylation and ubiquitination favor the formation of euchromatin and transcriptional activation. Note that epigenetic modifications, such as histone methylation, phosphorylation and ubiquitination, favor both the formation of euchromatin and heterochromatin. This depends on the composition of trans-acting factors, cross talk between histone marks, microdomain created by the histone marks and the chromatin remodeling complexes. Additional details can be found in the text. HMT: Histone methyltransferase, HAT: Histone acetyltransferase; DNMT: DNA methyltransferase; E3: E3 ubiquitin ligase; E3 Sumo: E3 sumo ligase; HP1: heterochromatin protein 1; TF: transcription factor; Pol: Polymerase; Ac: Acetylation mark; CH₃: Methylation mark.

Imitation SWItch (ISWI) complexes were initially identified and purified from *Drosophila* embryo extracts as NUCleosome Remodeling Factor (NURF), ATP dependent Chromatin assembly and remodeling Factor (ACF), and CHRomatin Accessibility Complex (CHRAC) complexes. Currently ISWI complexes have been found in a variety of organisms from yeast to humans (Tsukiyama and Wu, 1995; Tsukiyama et al., 1995; Ito et al., 1997; Varga-Weisz et al., 1997). The ISWI family members such as ACT and CHRAC are involved in promoting chromatin assembly, thus acting as transcriptional repressors. However, the NURF complex has been shown to activate RNA polymerase II, thus acting as a transcriptional activator.

INO80 was identified as a gene encoding an ATPase that is incorporated into a large multisubunit complex (Ebbert et al., 1999). Biochemical characterization of the yeast INO80 complex has revealed the presence of chromatin remodeling activity and 3'-5' helicase activity (Shen et al., 2000). Yeast cells that lack either INO80 or one of its core subunits (Arp5 and Arp8) exhibit hypersensitivity to DNA damaging agents, suggesting that the INO80 complex is involved in DNA repair (Morrison et al., 2004; van Attikum et al., 2004). In accord with these findings, the INO80 complex is recruited to sites of double strand DNA breaks through interaction with phosphorylated H2A. From these findings and others, members of the INO80 family are believed to be involved in DNA repair, chromosomal segregation, DNA replication, telomere regulation and transcriptional activation (Ho et al., 2010). However, SWRI functions to restructure the nucleosome by removing the H2A-H2B dimers and replacing them with H2A.Z-H2B dimers (Clapier et al., 2009).

All four families of chromatin remodeling complexes are believed to be involved in tumorigenesis. For example, the BRGI subunit of the SWI/SNF families has been characterized as a tumor suppressor, and is silenced in about 20% of non-small lung cancer (Medina et al., 2008). BRGI subunit also functions to destabilize p53 hence acting as a tumor suppressor (Naidu et al., 2009). SWI/SNF complexes have been shown to interact with oncogenes, such as Rb, Myc, p53, breast cancer 1 (BRCA1) and myeloid/lymphoid or mixed-lineage leukemia (MLL) (Roberts et al., 2004). Point mutations in the SNF subunit of the SWI/SNF complex have been implicated in renal tumors, choroid plexus carcinomas, medulloblastoma and neuroectodermal tumors (Robert et al., 2004). In colon cancer, promoter hypermethylation of the *MLH1* gene results in occlusion of the transcription start site by nucleosomes (Lin et al., 2007). The CHD5 complexes are targets of CpG hypermethylation (Mulero-Navarro and Esteller, 2008) resulting in downregulation of these complexes. In addition, crosstalk between chromatin remodeling complexes and histone modifications is vital to transcription regulation and tumorigenesis. For example, members of the CHD family are components of the NRD deacetylating and the SAGA acetyltransferase complexes (Tong et al., 1998). In accord with these findings, The H3K4 methyltransferase MLL has been shown to interact with SNF5/BRG1-associated factors (BAF) 47 (Rozenblatt-Rosen et al., 1998), and H3K4 methylation has been shown to recruit and mediate association of ISWI with chromatin to initiate transcription (Santos-Rosa et al., 2003). Acetylation of H3K56 leads to recruitment of SWI/SNF complexes (Xu et al., 2005). Linker histones have also been shown to play a role in tumorigenesis, with increased expression of linker histone macroH2A in senescent lung cells (Sporn et al., 2009), indicating that lung tumors with high expression of macroH2A have a better prognosis.

2.3 Histone modifications

As noted, chromatin is made of DNA and its associated proteins which are in turn classified into histone and non-histone proteins. Non-histone proteins transiently interact with the DNA to regulate function after which they dropout or are removed. For example, non-histone proteins transiently interact with DNA during transcription, replication, and DNA repair mechanisms. Such proteins include polymerases, co-activators, co-repressors, chromatin remodeling complexes, structural proteins and histone like proteins (e.g. CENPS). On the other hand, histone proteins stably interact with DNA except during transcription, replication or DNA repair during which they are temporally displaced from the DNA as nucleosomes. Each histone can be divided into three segments: a basic N-terminal histone tail, a globular histone fold and a C-terminal tail. The conformation of chromatin is

dependent on posttranslational modifications at the tails of all histones which normally protrude from the nucleosome. Posttranslational modifications function by contouring the secondary structures of chromatin so as to allow or disallow accessibility to transcription and regulation sites. Posttranslational modification on histone can be classified as transient or stable. Transient posttranslational modifications include: phosphorylation, sumoylation and ubiquitination, and represent modifications that correlate with transient changes in gene regulation. Acetylation and methylation are fairly stable modifications that reflect the conformation of chromatin (e.g. closed/heterochromatin and open/euchromatin).

2.3.1 Histone acetylation

All histones are subjected to acetylation, and the process is catalyzed by histone acetyltransferase (HAT) complexes (Allfrey et al., 1964). Histone acetylation involves the transfer of acetyl groups from acetyl Coenzyme A to the imino group of lysine. HATs do not acetylate lysine moieties on histone randomly, a potential recognition motif (GKxxP) was revealed by crystal structure analysis (Rojas et al., 1999; Bannister et al., 2000). However, this motif is not a predictor of non-histone protein acetylation as a proteomic survey has identified different sets of preferentially acetylated amino acid stretches (Kim et al., 2006). Functionally, histone acetylation induces the so called open/euchromatin conformation via steric hinderance, changes in the positive charges of histones (Hong et al., 1993), and/or recruitment of regulatory proteins (Grant and Berger, 1999; Roth et al., 2001). The formation of open chromatin favors transcriptional activation. At the same time, the acetyl group of histones can be removed by histone deacetylase complexes (HDACs) which favors formation of closed chromatin and transcriptional repression (Yang and Seto, 2003). HATs are categorized into two groups based on their cellular localization. Type-A HATs are nuclear and acetylate histones and other chromatin-associated proteins. Type-B HATs are localized in the cytoplasm and have no direct influence on transcription. The latter are believed to function mainly to acetylate newly synthesized histones in the cytoplasm. Type-A HAT are further categorized into three groups: GNAT [GCN5 (general control of nuclear-5)-related N-acetyltransferase], p300/CBP [CREB (cAMP response element binding protein)-binding protein] and MYST [MOZ (Monocytic leukaemia zinc-finger protein), YBF2 (Yeast binding factor2)/SAS (something silencing), Tip60 (Tat interactive protein -60)] (Table 1). In addition some transcription factors and nuclear receptor coactivators have been shown to acetylate histones. For example, the nuclear receptor coactivator, Amplified in breast cancer-1 (AIB1), and transcription factor, TATA-box binding protein associated factor-250 (TAF250). Both of these proteins have been shown to acetylate histones H3 and H4.

The mechanism by which HAT complexes are recruited to the chromatin to acetylate histones is poorly understood. One possible mechanism involves the recruitment of HAT proteins as part of coactivator complexes. For example, p300/CBP HAT complexes are found to associate with polymerase II during transcription (Nakajima et al., 1997). Another plausible mechanism is through association with bromodomains containing proteins which recognize and bind to acetylated lysines (Dhalluin et al., 1999; Mujtaba et al., 2002). For example, the SWI/SNF complexes are recruited to the chromatin via their bromodomains (Hassan et al., 2002). In this case the HAT families of proteins are believed to complex with SWI/SNF complexes to acetylate histones.

The primary site for histone acetylation is the tail region which generally protrudes from the nucleosome. For example, core histones H3 and H4 can be acetylated at lysines 9, 14, 18, 23 and 5, 8, 12, 16 respectively (Roth et al., 2001) (Table 1). Histone acetylation can occur at

different sites giving rise to the possibility of functional crosstalk between different acetylation marks. In fact, communication is known to occur between the same histone marks (Wang et al., 2008), between marks within the same histone tail (Duan et al., 2008), or between marks in different histone tails (Nakanishi et al., 2008). These data suggest that a single acetylation mark does not determine the state of the chromatin. Indeed, the notion of a strictly closed and open chromatin conformation has been challenged by a recent study showing that up to 51 chromatin states are possible based on the level or combination of acetylation marks (Ernst et al., 2010). For example, an active H3K4me mark and a repressive H3K27me mark have been found to co-exist in embryonic stem cells, suggesting a chromatin state that is neither open nor closed (Bernstein et al., 2006; Mikkelsen et al., 2007).

HATs	Histone Substrates
GNAT family	
• GCN5	H2B, H3-K9/14/23/27, H4-K8/16
• PCAF	H3-K14, H4-K8
P300/CBP family	
• p300	H2A-K5, H2B-K12/15/20, H3-K14/18/23, H4-K5/8/12
• CBP	H2A-K5, H2B-K12/15/20, H3-K14/18/23, H4-K5/8
MYST family	
• Tip60	H2A-K5, H3-K14, H4-K5/8/12
• MOZ	H3, H4

Table 1. HATs families and substrate specifications

As noted earlier, acetylation is a reversible process and the removal of acetyl groups is catalyzed by a class of enzymes called histone deacetylases (HDACs). Histone deacetylation is correlated with transcriptional repression. In humans, 18 isoenzymes of HDACs have been identified to date, and grouped into four classes based on their homology to yeast HDACs (Table 2). Class I (HDAC1, 2, 3 and 8) are related to yeast *RPD3* gene and are mostly located in the nuclei, except HDACs 3 and 8 which can also be cytoplasmic. Class II (HDAC 4, 5, 6, 7, 9 and 10) are related to yeast *Hda1* gene and are primarily located in the cytoplasm, but can shuttle to nucleus. Class II HDACs are further divided into two subclasses, IIa (HDAC 4, 5, 7,9) and IIb (HDAC 6, 10), based on their sequence homology and domain organization. Class III, also known as the sirtuins (SIRTUINS (SIRT) 1–7), are related to the yeast *Sir2* gene, and are virtually unaffected by class I and II HDAC inhibitors. They are localized in the cytoplasm, mitochondria and nucleus (Table 2). Class IV (HDAC11) has a conserved domain similar to the catalytic region of Class I HDACs. Class IV is a fairly new class and needs further characterization. Class I, II and IV share similar structural organization and a common cofactor, Zn²⁺. Class III HDACs are structurally different from the rest and their active site is occupied by the nicotinamide adenine dinucleotide (NAD). Functionally, class II HDACs are regulated by class I HDACs and together class I and II are involved in transcriptional silencing and genomic organization during development. Class III HDACs are involved in maintenance of acetylation, as well as specific gene silencing (Denu et al., 2003).

HDAC classes	Cofactor	Localization
I <ul style="list-style-type: none">• HDAC 1 & 2• HDAC 3 & 8	Zn ²⁺ Zn ²⁺	Nucleus Nucleus/cytoplasm
IIa <ul style="list-style-type: none">• HDAC 4, 5, 7 & 9	Zn ²⁺	Nucleus/cytoplasm
IIb <ul style="list-style-type: none">• HDAC 6 & 10	Zn ²⁺	Nucleus/cytoplasm
III <ul style="list-style-type: none">• SIRT 1, & 2• SIRT 3• SIRT 4 & 5• SIRT 6 & 7	NAD ⁺ NAD ⁺ NAD ⁺ NAD ⁺	Nucleus/cytoplasm Mitochondria/Nucleus Mitochondria Nucleus
IV <ul style="list-style-type: none">• HDAC 11	Zn ²⁺	Nucleus

NAD: Nicotinamide adenine dinucleotide; SIRT: Sirtuin; Zn: Zinc

Table 2. Classes of HDACs, Cofactors and Subcellular Localization

Compared to normal cells, the interplay between acetylation and deacetylation is dysregulated in almost all cancers. HDACs have been shown to be overexpressed or mutated in many cancers (Zhu et al., 2004; Ropero et al., 2006). Under normal conditions, HDACs associate with and regulate transcription factors, tumor suupressor genes and oncogenes. HDAC1 has been shown to be in a complex with Rb and to suppress the transcriptional activation of E2F (Brehm et al., 1998). Also SIRT1 has been shown to regulate the inflammatory, stress, and survival responses of p53 (Vaziri et al., 2001). Thus, it stands to reason that loss of HDAC1 or SIRT1 could result in uncontrollable growth of cell leading to transformation. In accord with this interpretation, SIRT1 has been shown to interact with DNMT1 to affect DNA methylation patterns (Espada et al., 2007). In acute myelogenous leukemia (AML), fusion of eight-twenty-one zinc-finger nuclear protein (ETO) to AML, a transcription activator, converts the AML protein into a dominant transcriptional repressor. The AML-ETO fusion protein permanently binds to HDAC corepressor complexes and/or blocks the recruitment of coactivator complexes leading to transformation (Scandura et al., 2002). HDAC2 overexpression is a hallmark of familial-adenomatosis-polyposis-induced tumors (Zhu et al., 2004) and truncation of HDAC 2 is reported in sporadic tumors (Ropero et al., 2006). In addition, HDAC6 expression is correlated with better prognosis for breast cancer patients (Zhang et al., 2004), suggesting aberrant acetylation in breast tumors. In addition to aberrant levels of HDACs, several cancers also bear aberrant fusion proteins, mutations, or deletion of HATs and HAT releted genes (Bryan et al., 2002; Moore et al., 2004). For example, in cancer cells there is global reduction in monoacetylation at H4K16 (Fraga et al., 2005a), and HATs, such as p300 and CBP, are characterized as tumor suppressor genes since they can regulate the activity of oncoproteins, such as Jun, Fos, Myb and Rb (Yang et al., 2004). Likewise, p300 has been shown to be an interacting partner of the oncoprotein adenovirus E1A (Stein et al., 1999). CBP mutations have been shown to be involved in the initial steps of leukaemogenesis (Patrij et al., 1995). For the MYST family of HATs, mutations leading to loss of Tip60 acetyltransferase activity lead to apoptosis-resistant phenotypes and rampant cell proliferation (Ikura et al., 2000). In AML, a chimera

protein is created by fusion of CPB to MOZ protein creating a protein with both p300/CBP and MYST domains (Yang et al., 2004; Borrow et al., 1996). The CBP-MOZ chimera protein exhibits gain-of-function characteristics leading to hyperacetylation and aberrant transcriptional activation. This leads to global imbalance of histone acetylation in cancer cells. Table 3 summarizes predicted impacts of acetylation and deacetylation in cancer.

Gene	Histone Mark	Genetic Defect	Tumor Type	Function	Reference
Histone acetyltransferases (HATs)					
CBP (KAT3A)	H2AK5, H2BK12, H2BK15, H3K14, H3K18, H4K5, H4K8	Deletion	ALL; lung	Loss	Shigeno et al., 2004 & Kishimoto et al., 2005
p300 (KAT3B)	H2AK5, H2BK12, H2BK15	Deletion	cervix; ALL	Loss	Ohshima et al., 2001 & Shigeno et al., 2004
p300 (KAT3B)	H2AK5, H2BK12, H2BK15	Mutation	Breast; CRC	Loss	Gayther et al. 2000
p300 (KAT3B)	H2AK5, H2BK12, H2BK15	Translocation	AML	Loss	Ida et al., 1997 & Chaffanet et al., 2000
MOZ (KAT6A)	H3K14; H4K16	Translocation	AML	Loss	Chaffanet et al., 2000 & Panagopoulos et al., 2003
Histone deacetylases (HDACs)					
HDAC2	Many acetyl residues (except H4K16)	Mutation	MSI+	Loss	Ropero et al., 2006 & Hanigan et al., 2008

ALL: Acute Lymphoblastic Leukemia; AML: Acute Myeloid Leukemia; CRC: Colorectal cancer; MSI+: Colorectal cancer with microsatellite instability.

Table 3. Aberrant Acetylation and Deacetylation Marks in Cancer

2.3.2 Histone methylations

Histone methylation occurs at either arginine or lysine residues in the tails of all histones. Two classes of enzymes catalyze the addition of methyl groups to histones: those that catalyse the addition of methyl group to arginine residues, called protein arginine methyltransferase (RHMT), and those that catalyze the addition of methyl groups to lysine residues, called histone lysine methyltransferase (HKMTs). S-adenosyl methionine (SAM) donates the methyl group in both cases of histone methylation (Pluemsampant et al., 2008). Histone methylation at arginine residues occurs at mono- or di-methylated states, while methylation at lysine residues occurs at mono-, di, and tri-methylated states. A total of 24 arginine and lysine methylation sites has been identified in all the core histones so far. While DNA methylation generally represses transcription (except for gene body methylation), the functional impact of histone methylation is highly dependent on cellular context (Jenuwein and Allis, 2001). For example, histone methylation has been shown to cause both transcriptional activation and repression (Table 4). An emerging theme for these dual roles is explained by the fact that histone methylation creates motifs or domains that are recognized and bound by different proteins. The composition of these protein complexes

determines whether the modification results in gene activation or repression. Another explanation for this phenomenon is that histone modifier genes and regulatory genes have a tissue-specific expression pattern. The composition and recruitment of tissue specific proteins determines transcriptional states. Also, there is crosstalk between histone methylation and other epigenetic modifications. For example, several histone methyltransferases have been shown to direct DNA methylation (Tachibana et al., 2008; Zhao et al., 2009) by recruiting DNMTs. DNMT3L specifically interacts with H3 tails and induces recruitment of DNMT3a, however this interaction is strongly inhibited by H3K4me (Ooi et al., 2007). Thus, the transcription state of the chromatin is also an interplay between different epigenetic modifications. In addition, the domain composition of HMTs is a determining factor of the transcriptional state. Most HMTs contain a conserved SET (SUV39 (Suppressor of variegation 3-9), Enhancer or zeste, Trithorax) domain which in combination with other domains/complexes has the ability to confer either transcriptional activation or repression. SET domain containing proteins are divided into five families: SET1, SET2, SUV39, RIZ (retinoblastoma protein interacting zinc-finger) and SMYD3 (SET and MYND-domain containing protein 3) (Table 4). The mixed lineage leukemia (MLL), SMYD3, Nuclear receptor-binding SET domain protein-1 (NSD1) and CARM1 are HMTs that activate transcription. For example, MLL-specific methylation at H3K4 is followed by recruitment of bromo domain-containing trithorax complexes which result in formation of an open chromation (Milne et al., 2002). SMYD3 in complex with RNA polymerase II and HELZ, a helicase, is recruited to the promoters of target genes where it methylates H3K4 leading to

Enzymatic Activity	Histone/Lysine	Biological Impact on Transcription
Lysine Histone Methyltransferase (KHMT)		
SET 1 family		
• SET1	H3K4	Activation
• EZH2	H3K27	Repression
• MLL 1 & 2	H3K4	Activation
SET 2 family		
• NSD1	H2K36	Activation
SUV39 family		
• SUV39H 1 &2	H3K9	Repression
	H4K20	Repression
RIZ family		
• RIZ1	H3K9	Repression
SMYD3 family		
• SMYD3	H3K4	Activation
Arginine Histone Methyltransferase (RHMT)		
• CARM1	H3R2	Repression
• PRMT 5	H3R17	Activation
	H2A/H4	Repression

Note that only a few of the HMTs are cited to illustrate their role as transcriptional activation or repression.

Table 4. Histone Methylation at Specific Lysine and Arginine Residues

transcriptional activation. NSD1 methylation at H3K36 is responsible for activation of Hox genes (Wang et al., 2007). CARM1 methylation at H3R17 has also been shown to activate transcription by complexing with hormone receptor co-activator complexes (Hong et al., 2004). On the other hand, SUV39H1, EZH2, RIZ1 and PRMT5 participate in transcriptional repression. For example, EZH2 methylation at H3K27 leads to recruitment of chromo domain-containing polycomb complexes resulting in silencing of homeotic genes (Valk-Lingbeek et al., 2004). SUV39h1 trimethylation at H3K9 of the promoters of cell cycle control genes leads to recruitment of chromo domain-containing heterochromatin protein 1 (HP1) which in turn leads to transcriptional repression (Bannister et al., 2001; Lachner et al., 2001). RIZ1 methylation at H3K9 of the promoters of cell cycle control genes leads to apoptosis in breast cancer cells (He et al., 1998). Finally, methylation at H2A/H3 by PRMT negatively regulates cyclin E transcription resulting in cell cycle arrest (Fabbrizia et al., 2002). Table 4 summarizes the influence of histone methylation on transcription.

As described for histone acetylation, histone methylation is also reversible. Two classes of enzymes are responsible for removing methyl groups from histones: lysine-specific methyltransferase (KMAT) and arginine-specific methyltransferase (RMAT). KMAT includes amine oxidase domain-containing demethylases (Forneris et al., 2005) and Jumonji C (JmjC) domain containing demethylases (Tsukada et al., 2006) while RMAT includes peptidylarginine deiminase 4 (PAD4) (Cuthbert et al., 2004; Wang et al., 2004). PAD4 is the only RMAT known so far and it does not convert methylarginine to arginine, instead it converts methylarginine to citrulline. Citrullination is described as yet a unique histone modification. Several KMAT exist and the first one discovered is the lysine-specific

Gene Name	Histone Mark	Genetic Defect	Tumor Type	Function	Reference
Histone Methyltransferases (HMTs)					
DOT1L (KMT4)	H3K79	Aranslocation	AML	Loss	Okada et al., 2005
EZH2 (KMT6)	H3K27	Amplification	Prostate	Gain	Bracken et al., 2003
EZH2 (KMT6)	H3K27	Mutation	Lymphoma	Loss	Morin et al., 2010
NSD3	H3K4, H3K27	Amplification	Breast	Gain	Angrand et al., 2001
RIZ1 (KMT8)	H3K9	CpG hypermethylation	Breast, Liver	Loss	Du et al., 2001
SMYD2 (KMT3C)	H3K36	Amplification	ESCS	Gain	Komatsu et al., 2009 & Li et al., 2007
SUZ12 (HMT complex)	H3K9, H3K27	Translocation	ESS	Loss	Panagopoulos et al., 2008
Histone demethylase (HDMTs)					
LSD1	H3K4, H3K9	Amplification	Prostate, Lung, Bladder	Gain	Kahl et al., 2006 & Hayami et al., 2010
UTX	H3K4, H3K9	Mutation	Multiple cancers	Loss	Van Haaften et al., 2009
GASC	H3K9, H3K36	Amplification	Breast, Lung	Gain	Cloos et al., 2006 & Italiano et al., 2006 & Liu et al., 2009

Table 5. Aberrant histone methylation and demethylation in cancer

demethylase-1 (LSD1). LSD1 is a typical H3K4 demethylase, but can change its substrate specificity when in complex with different accessory proteins. For example, LSD1 in complex with androgen receptor is able to demethylate H3K9 (Mwtzger et al., 2005). LSD1 is also able to synergistically work with HDACs and HATs to affect transcription of target genes. For example, over expression of LSD1 in HEK293 cells leads to decreased H3K4 methylation which is in turn followed by H3 deacetylation and transcriptional repression (Lee et al., 2006). At the same time, inhibiting HDAC1 activity increases both H3 acetylation and H3K4 methylation (Lee et al., 2006).

In cancer, there is aberrant expression and composition of histone-modifier and -regulator genes. For example, silencing of the nuclear receptor SET domain protein I (NSD1) results in decreased H3K36 and H4K20 methylation, which is believed to play a role in tumors of the nervous system (Berdasco et al., 2009). CpG Island hypermethylation by the histone methyltransferase RIZ1, has been described in many cancers (Du et al., 2001). Suppressor of the zeste 12 homolog (SUZ12) which is a component of the PCR2/EED/EZH2 complex that methylates H3K9 and H3K27 is involved in cell proliferation and survival in tumors (Li et al., 2007). In leukemias, the presence of mixed lineage leukemia (MLL) fusion oncoproteins leads to aberrant patterns of H3K79 and H3K4 methylation and altered gene expression in these tumors (Krivtsov et al., 2008; Wang et al., 2009). Some histone demethylases have also been found to be overexpressed in prostate cancer and squamous carcinomas (Shi et al., 2007). For example, LSD1 overexpression is a predictive biomarker for prostate cancer (Kahl et al., 2006).

2.3.3 Histone phosphorylation

Histone phosphorylation is described as the addition of a phosphate group (PO_3^-) to histone. So far, a small number of kinases has been shown to phosphorylate histones, and these include protein kinase B (PKB/AKT), ribosomal S6 kinase-2 (Rsk-2), mitogen- and stress-activated protein kinases 1 and 2 (Msk1/2), mixed lineage triple kinase-alpha (MLTK- α), and aurora kinases. The most interesting of the histone kinases are the aurora kinases. In normal cells, aurora kinases are involved in chromosomal segregation, condensation and orientation (Katayama et al., 2003). For example, aurora-phosphorylates both H3S10 and H3S28 during mitosis and meiosis (Andrews et al., 2003). Aurora kinases are serine/threonine kinases that include auroras A, B and C. These proteins share similar carboxyl terminal catalytic domains, but divergent amino terminals of variable length. In cancer cells, aurora kinases are frequently overexpressed and their overexpression is implicated in oncogenic transformation, as evidenced by chromosomal instability and derangement of multiple tumor suppressor and oncoprotein-regulated pathways. The mechanism through which these kinases are activated is dependent on the microenvironment of the cell. These mechanisms include, but are not limited to, activation by mitogens, cytokines, stress, signaling pathways (e.g. Ras-mitogen-activated protein kinase pathway (MAPK)) and chemical and environmental toxicants.

Functionally, histone phosphorylation alone, or when synergistically-coupled to other histone modifications (e.g. acetylation and methylation), can either facilitate or repress transcription (Cheung et al., 2000). This dichotomy is explained by the fact that histone phosphorylation can create domains that are recognized and bound by transacting factors, the composition of which determines the transcriptional state. Also histone phosphorylation can facilitate or repress further acetylation or methylation thereby regulating the transcriptional state of chromatin. For example, phosphorylation at threonine 11 has been shown to hasten removal of repressive H3K9 methylation by recruitment of the histone

demethylase Jumonji C domain containing protein (JMJD2C) (Metzger et al., 2008). At this capacity, phosphorylation facilitates transcription by recruitment of histone demethylases. In addition, phosphorylation at H3 serine 10 inhibits recruitment of heterochromatin protein 1 (HP1) (Fischle et al., 2005; Hirota et al., 2005), which in turn prevents recruitment of DNMTs (DNMT1 and DNMT3a) thereby leaving chromatin in the open conformation and ready for transcription. At this level, phosphorylation prevents methylation of DNA by preventing recruitment of DNMTs. Phosphorylation can also facilitate transcription by allowing recruitment of histone acetyltransferase (HAT). For example, mutation at H3 serine 10 ablates recruitment of HATs (Chuang et al., 2000; Lo et al., 2000). Phosphorylation can also activate genes which in turn activate signaling pathways responsible for gene activation. Stimulation of inflammatory cytokine signaling activates I κ B kinase α which phosphorylates histone H3 at serine 10 in the promoters of multiple nuclear factor responsive genes (Anest et al., 2003; Yamamoto et al., 2003). These phosphorylations result in expression of several inflammatory responsive genes. Lastly, it is been shown that phosphorylation of core H3 at serine 10 (cH3S10) and threonine 11 (cHT11) occur during active transcription (Chuang et al., 2000; Nowak and Cores, 2000, 2004, Metzger et al., 2008), suggesting that the negative phosphate groups added to histones might neutralize the positive charges on DNA, thus causing chromatin to unwind and allow transcription to continue. Conversely, evidence for transcriptional repression due to histone phosphorylation is mounting. For example, during mitosis, H3S10 phosphorylation is associated with condensed chromosome (Goto et al., 1999). Aurora-B kinase-mediated phosphorylation of H3S28 is also associated with condensed chromosome. Constitutive phosphorylation of H1 through the Ras-MAPK pathway leads to chromatin condensation (Chadee et al., 1995). In addition, mammalian Sterile20-like 1 (Mst1) phosphorylation of H2B-14 is associated with condensed chromatin leading to apoptosis (Cheung et al., 2003).

Gene name	Histone Mark	Genetic Defect	Tumor Type	Function	Reference
Histone phosphorylation					
Jak2	H3Y41	Point mutation	Hematological tumors	Loss	(Dowson et al., 2009)
ATM/ATR	H2AXS139	Double stranded breaks	Melanomas	Loss	(Fernandez-Capetillo et al., 2004) and (Bassing, C.H. et al. 2003)
Aurora-kinase-B	H3S10	Chromosomal instability	Aneuploidy and Colorectal cancer	Loss	(Fischle, W. et al. 2005) and (Hirota, T. et al. 2005)
Mst1	H2BS14	Apoptosis resistance		Loss	(Hanahan and Weinberg, 2000) and (Ahn, S.H. et al. 2005)

Table 6. Aberrant Histone Phosphorylation in Cancer






Like other histone modifications, histone phosphorylation is counteracted by dephosphorylation. Phosphatases catalyze the removal of phosphate groups from histones. For example, phosphatase type 1 (PP1) interacts with aurora-B during mitosis as a feedback mechanism (Katayama et al., 2001). Also PP1 regulates Aurora-B and H3 phosphorylations

during cells division (Murnion et al., 2001). In cancer cells, the balance between phosphorylation and dephosphorylation is dysregulated. For example, histone phosphorylation has been shown to play a role in cancer through modulation of the DNA repair response, chromosome instability and apoptosis. Recently JAK2, a nonreceptor tyrosine kinase, has been shown to be activated by chromosomal translocation and point mutations in hematological malignancies (Dawson et al., 2009). Also, JAK2 has been shown to phosphorylate H3Y41 which prevents the recruitment of heterochromatin protein 1 α (HP1 α) leading to increased expression of genes in this region. Phosphorylation at serine 139 in the highly conserved C-terminal tail (-SQEY) of H2A.X has been shown to play an important role in DNA double-strand break (DSB) repair and tumor suppression (Fernandez-Capetillo, O. et al. 2004). In addition, H3S10 and H2BS14 phosphorylations play a role in chromosomal instability and apoptosis resistance, respectively which are both hallmarks of cancer (Fischle, W. et al. 2005; Hirota, T. et al. 2005; Hanahan and Weinberg, 2000; Ahn, S.H. et., 2005). Activation of MAPK pathway by environment carcinogens leads to phosphorylation of H3 which in turn results in the induction of immediate early genes. More specifically, ultraviolet light has been shown to activate MAPK pathways resulting in phosphorylation of H3S10 by p38 kinase.

2.3.4 Histone ubiquitination and sumoylation

Ubiquitination and sumoylation involve the transfer of a polypeptide to the histone tail. The polypeptide molecules for ubiquitination and sumoylation are ubiquitin and small ubiquitin related modifier (SUMO) (Takada et al., 2007), respectively. The enzymatic cascade responsible for ubiquitination and sumoylation are similar, with three classes of enzymes involved in both instances: E1 activating enzymes, E2 conjugating enzymes and E3 ligating enzymes. In the first step of this multistep cascade, E1 adds Ubiquitin/SUMO to the target substrate in an ATP dependent manner. E2 then transfers the Ubiquitin/SUMO to E3 which associates and ligates the Ubiquitin/SUMO to histones (Nathan et al., 2003). Histone ubiquitination involves mono-ubiquitination which is different from poly-ubiquitination in that it does not result in proteosomal degradation of the target histone. Depending on the lysines that are ubiquitinated, ubiquitination can result in transcriptional activation or repression (Table 7). For example, both H2A and H2B are targets of mono-ubiquitination, and mono-ubiquitination has been shown to be a precursor to histone methylation (Gerber and Shilatifard, 2003; Hampsey and Reinberg, 2003; Osley, 2004; Margueron et al., 2005). In particular, mono-ubiquitination at H2B lysine 120 (H2BK120) by E3 ligase (RNF20/RNF40) initiates methylation at H3 lysine 4 (H3K4) resulting in recruitment of homeobox genes (Zhu et al., 2005) and transcriptional activation. Conversely mono-ubiquitination at H3 lysine 119 (H3K119) by Bmi1/Ring1A induces transcriptional repression (Wand et al., 2004).

It is not completely clear what role sumoylation plays on transcriptional regulation, however sumoylation has recently been shown to cause transcriptional repression (Shiio and Eisenman, 2003; Girdwood et al., 2003) (Table 1). For example, H4 sumoylation is associated with recruitment of HP1 and HDAC which is known to repress transcription. A number of oncogenes and tumor suppressor genes, including PML, Mdm2, c-Myb, c-Jun, Rb and p53, undergo SUMOylation (Muller et al., 1998; Bushmann et al., 2000; Bies et al., 2002; Schmidt et al., 2002; Huang et al., 2004; Besten et al., 2005; Ghioni et al., 2005; Ghost et al., 2005). SUMOylation of Mdm2 increases its E3 activity toward p53 tumor suppressor. SUMO negatively regulates c-Jun activity and thus restricts its oncogenic capacity. SUMOylation of

Histone Modification	Modifying Enzyme	Type of Modification	De-modifying Enzyme	Impact on transcription
Phosphorylation	Kinase e.g. JAK	Transient	Phosphatase	
Sumoylation	E3 Ligase	Transient	SUMO Protease e.g. SENP1	
Ubiquitination	E3 Ligase	Transient	De-ubiquitinase	
Methylation	HMT/PRMT	Stable	PADI, JMJD	
Acetylation	HAT	Stable	HDAC	

Red arrows indicate transcriptional activation, while black arrows indicate transcriptional repression.

Table 7. Summary, Impact of Posttranslational Modification of Histones on Transcription

c-Myb increases its stability, but negatively regulates its transactivation function of. Increasing evidence supports the notion that protein SUMOylation is important during the course of tumorigenesis and oncogenesis, and altered in human cancers, however further work is needed to determine the impact of sumoylation on cancer.

In summary, epigenetic modifications are not stand alone processes. Instead, considerable crosstalk exists among the different types of epigenetic marks. In accord with this principle, epigenetic marks by themselves, or synergistically with other epigenetic marks, function to either repress or activate transcription (Table 7). Of relevance to epigenomic regulation is that all epigenetic modifications identified to date have been shown to be reversible. Genes regulated by epigenetic modification remain intact and can therefore be returned to their original state. The reversibility of epigenetic mechanisms makes them highly susceptible to pharmacological intervention and therefore, ideal targets for cancer therapeutics.

3. Epigenetic therapies

Genetic mutations and gross structural defects permanently activate or inactivate genes; however genes modified by aberrant epigenetic modification remain structurally intact and subject to reversal of aberrant epigenetic modifications that can restore their original state. This phenomenon has made epigenetic modifications an ideal target for the treatment of many diseases, including cancer. As discussed in previous sections, cancers are plague with aberrant epigenetic modifications which have been shown to contribute to initiation and transformation. In fact, several exogenous chemicals used to treat cancers have been shown to cause unintended epigenetic modifications which in many cases have led to exacerbation of tumor progression. These factors, combined and our understanding of epigenetic modifying enzymes, pathways and accessory proteins pivotal to epigenetic modifications, have lead to the development of therapies targeting DNA methylation and DNMTs, histone modifications and histone modifying enzymes (i.e HAT, HDAC, kinases, HMT, SUMO ligase, ubiquitin ligase, etc.). Indeed, therapies targeting chromatin remodeling complexes have attracted significant interest in recent years as a means for cancer prevention, either alone or in combination with conventional cancer treatments.

3.1 DNA Methyltransferase inhibitors (DNMTis)

Inhibitors of DNA methylation (DNMTis) cause reactivation of silenced genes, inhibition of cell proliferation, apoptosis and enhancement of sensitivity to other cancer drugs. DNMTis can be grouped into nucleoside DNMTis and non-nucleoside DNMTis, based on their structure and mode of action. Nucleoside DNMTis are analogues or derivatives of the nucleoside cytidine and they include 5-azacytidine (5-Aza-CR), 5-Aza-2-deoxycytidine (5-Aza-CdR), zebularine, cytarabine and 5-Fluoro-2-deoxycytidine. The cytidine analogues (5-Aza-CR and 5-Aza-CdR) have been approved by FDA for the treatment of myeloid malignancies in the USA. The anticancer activity of these drugs is believed to be mediated by two mechanisms: (1) cytotoxicity which stems from incorporation of these drugs into DNA and/or RNA, and (2) reactivation of tumor suppressor genes by demethylation of their promoter regions (Jones and Liang, 2009). These drugs do not demethylate DNA per se, but rather with continued replication, cytidines are replaced by the cytidine analogues resulting in serial dilution of methylable cytidines. In addition, DNMTs are trapped in covalent adducts with DNA through the incorporated cytidine analogues. 5-Aza-CR and 5-Aza-CdR are taken into the cell through the concentrated nucleoside transporter 1 (hCNT1) (Rius et al., 2009). Once inside the cell, 5-Aza-CR is phosphorylated by uridine-cytidine and 5-Aza-CdR by diphosphate kinase (Stresemann et al., 2008; Issa et al., 2009) which in turn convert them into active triphosphates (i.e. 5-Aza-CTP and 5-Aza-dCTP). 5-Aza-CTP is incorporated into the DNA resulting in the formation of covalent adducts between DNMTs and DNA (Santi et al., 1984). This traps the DNMTs and prevents further methylation. In other studies, 5-Aza-dCTP was shown to be incorporated into RNA which interferes with ribosomal biogenesis and protein synthesis (Momparlar et al., 1984; Stresemann et al., 2008). In accord with these findings, Ghoshal et al. 2005 and Kuo et al. 2007 have both shown that 5-Aza-CTP and 5-Aza-dCTP hypomethylate the genome through passive dilution of cytidine and not through active demethylation. Because of the cytotoxicity and instability of 5-Aza-CR and 5-Aza-CdR, DNMTis cannot be continually given to patients. For this reason, zebularine has been developed as an alternative. Although this drug works in a manner similar to 5-Aza-CR and 5-Aza-CdR, it is more stable and less toxic than 5-Aza-CR and 5-Aza-CdR DNMTis (Zhou et al., 2002; Cheng et al., 2003). In line with these findings, zebularine has been shown to reactivate tumor suppressor genes (Flotho et al., 2009; Billam et al., 2010), enhance tumor cells' chemotherapy and radiation sensitivity (Dote et al., 2005), exert angiostatic and antimitogenic activities (Balch et al., 2005; Hellebrekers et al., 2006) and to be stable enough for oral administration (Zhou et al., 2002; Cheng et al., 2003). In addition, at low doses, zebularine can be given to patients continuously without the overt cytotoxicity associated with 5-Aza-CR and 5-Aza-CdR. Another cytidine analogue, 5-fluoro-2-deoxycytidine (FdCyd) has been shown to cause demethylation in human breast and lung cancer cells (Beumer et al., 2008). In the case of FdCyd, the hydrogen atom at carbon-5 (C5) which is the methyl acceptor during the methylation reaction is replaced by a fluorine atom. When FdCyd is incorporated into DNA, the β -elimination step in which DNMT transfers the methyl group to the cytidine is inhibited. At the same time, the fluorine atom traps the DNMT to prevent elimination of the FdCyd moiety (Jones et al., 1980; Reither et al., 2003). FdCyd is currently in Phase I clinical trials for the treatment of breast and other solid tumors (Gowher et al., 2004) (Table 8). Moreover, FdCyd, in combination with other epigenetic drugs (i.e. tetrahydrouridine and dihydro-5-azacytidine (DHAC), is being evaluated in clinical studies for the treatment of malignant mesothelioma (Kratzke et al., 2008).

Although nucleoside DNMTis have proven effective for the treatment of cancers, their cytotoxicity remains a significant limitation. To address this shortcoming, non-nucleoside

DNMTis are being evaluated. Non-nucleoside DNMTis include procaine, L-tryptophan derivatives, RG108, hydralazine, MG98, procainamide, and epigallocatechin-3-gallate (EGCG). Procaine is a local anesthetic drug that can also function as a DNMTi. For example, procaine has been shown to cause global demethylation and reactivation of tumor suppressor genes in human breast cancer cells (Jin et al., 2009). Unlike the nucleoside analogues, procaine competes with DNMTs for binding to CpG rich regions (Jin et al., 2001). Procainamide and hydralazine are antiarrhythmic drugs that can also function as DNMTis, and both agents have been shown to inhibit DNA methylation through interactions between the nitrogen atom of procainamide and hydralazine with the lys-162 and Arg-240 moities in the catalytic site of DNMTs (Song et al., 2009; Singh et al., 2009; Mund et al., 2006). In accord with these findings, procainamide has been shown to specifically inhibit DNMT1 (Lee et al., 2005). RG108 is a small molecule inhibitor of DNMTs that inhibits free DNMTs (Brueckner et al., 2005). This drug works by blocking the catalytic pocket of DNMTS without the formation of covalent adducts that cause cytotoxicity (Stressmann et al., 2006). Studies have also shown RG108 to cause demethylation and reactivation of tumor suppressor genes without affecting the methylation level of microsatellite regions in lung cancer cells (Suzuki et al., 2010), suggesting a specificity level in RG108 that has not been seen in other DNMTis. Table 8

DNMTi	Phase	Type of cancer	Clinical trial finding	Reference
NON-NUCLEOSIDE ANOLOGUES				
MG98	N/A	Cervical cancer	26% SD	Garzon et al., 2009
EGCG	Phase I		N/A	Brueckner et al., 2004
Procaine	Phase I	Solid Tumor	N/A	Villar-Garea et al., 2003
Procanamide	N/A	Colon cancer	N/A	Segura-Pacheco et al., 2003
Hydralazine	N/A	Cervical cancer	N/A	Song et al., 2009
RG108	N/A	Colon cancer	N/A	Suzuki et al., 2010
COMBINATION THERAPIES				
Aza-CR+Sodium Phenylbutyrate	Preclinical	Solid Tumors	50 24.2% CR; PR 11.2%	Soriano et al., 2007
Aza-CR + Valproic Acid	Phase II	MDS	62 30.7% CR; PR, 15.4%	Blum et al., 2011
Aza-CR + Lenalidomide	Phase I	MDS	44% CR, 17% HI, and 67% ORR	Jabbour et al., 2009
Aza-CR + Cytarabine	Phase I	MDS/AML	N/A	Plummer et al., 2009

DNMTi	Phase	Cancer Type	Clinical Trial Findings	Reference
NUCLEOSIDE ANOLOGUES				
5-Aza-CR	Phase II	MDS	7% CR, 16% PR, 37% HI	Yoo et al., 2008
		MDS	17% CR, 12% PR, 42% SD	Silverman et al., 2006
		MDS	10.8% CR, 9.5% PR, 20.3% HI	Santini et al., 2009
		MDS/AML	15.6% CR, 25% HI, 34.4% SD	Martin et al., 2009
5-Aza-CdR	Phase II	SM	N/A	Winqvist et al., 2006
		MDS	9% CR, 13% HI, 17% ORR	Muller-Thomas et al., 2009
		MDS	34% CR	Wijermans et al., 2008
		MDS	17% CR, 18% HI, 32% ORR	Kantarjian et al., 2007
		MDS/AML	26% SD	Schrump et al., 2006
Zebularine	Phase II	MDS	13.4% CR, and 7.5% PR	Gore et al., 2006
5-FdCyd	N/A	Breast and lung	N/A	Relither et al., 2003

AML: Acute Myeloid Leukemia; CR: Complete Remission; HI: Hematologic Improvement; MDS: Myelodysplastic Syndrome; PR: Partial response; ORR: Overall Response Rate; CR: Conventional Care Regimens; N/A: Data not available; SD: Stable Disease. Note that only few DNMTis are shown here to illustrate their role in the treatment of cancers.

Table 8. DNMTis and Their Impact in Cancer

summarizes the DNMTis in clinical trial and their efficacy for cancer treatment either alone or in combination with other regimens.

3.2 Histone acetyltransferase inhibitors (HATis)

HAT inhibitors can be classified into synthetic peptide CoA base bisubstrate HATis, natural product HATis and small molecule HATis (Table 9). The synthetic bisubstrate HATis were the first to be identified based on the observation that polyamine-CoA conjugates can inhibit HAT activity in cell extracts (Cullis et al., 1982). In particular, H3-CoA-20 and Lys-CoA specifically inhibit pCAF and p300 (Lau et al., 2000) rather weakly. Introduction of a phenyl or methyl group between lysine and CoA improves the inhibition fourfold (Sagar et al., 2004). Most of the synthetic bisubstrate HATis work by mimicking the acetyl CoA-lysine intermediate complex in the HAT reactions. Crystal structure information between GCN5 and these HATis shows that GCN5 interacts with the pyrophosphate moiety, the pantothenic moiety and the phosphate group of CoA. The major deficiency for this class of HATis is their impermeability to cells. Unfortunately, most of the naturally occurring HATis also suffer from a similar problem. For example, anacardic acid isolated from the shell of cashewnuts displays permeability restriction in vitro. Nevertheless, garcinol and isogarcinol were both shown to inhibit p300 and pCAF (Balasubramnyam et al., 2004; Mantelingu et al., 2007). The derivative of isogarcinol, LTK14, was shown to selectively inhibit p300, but not

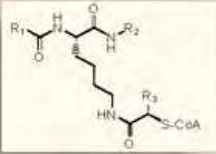
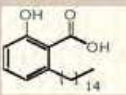
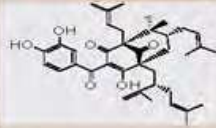
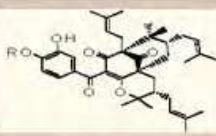
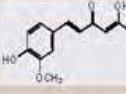

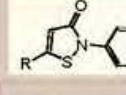
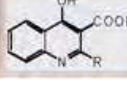
HATi compound	Parental and Derivative compounds	Specificity	Reference
SYNTHETIC HATis			
	Lys-CoA: (R1 = CH ₃ ; R2 = R3 = H)	p300	Lau et al., 2000
	H3-CoA-20: (R1 = G-G-T-S-K-R-A-T-Q-K-T-R-A-NH-COCH ₃ ; R2 = A-P-R-K-Q-L; R3 = H)	PCAF	Lau et al., 2000
	H3-(Me)-CoA: (R1 = CH ₃ CO-NH-A-R-T-A-R-K-S-T-G-G; R2 = A-P-R-K-Q-L; R3 = Me)	p300	Sagar et al., 2004
	Lys-Phe-Coa: (R1 = Phe, R2 = R3 = H)	p300	Sagar et al., 2004
	Lys-CoA-3'dephospho: R1 = (R1 = Phe, R2 = R3 = H)	p300	Sagar et al., 2004
NATURAL HATis			
	Anacardic Acid	p300, PCAF	Varier et al., 2004
	Garcinol	p300, pCAF	Balasubramanyam et al., 2004a
	Isogarcinol: (R = H)	p300 pCAF	Mantelingu et al., 2007
	LTK14: (R = CH ₃)	p300	
	Curcumin	p300 CBP	Balasubramanyam et al., 2004b
SMALL MOLECULES HATis			
	γ-Butyrolactone	hGCN5	Biel et al., 2004
	Isothiazolones: (R = H, Cl; R1 = NO ₂ , Cl, CF ₃ , OCH ₃ , COOEt)	p300 PCAF	Mantelingu et al., 2007 & Stimson et al., 2005
	Quinolines	N/A	Mai et al., 2006

Table 9. HAT Inhibitors and Their Selectivity

pCAF (Mantelingu et al., 2007). The best characterized of the naturally occurring HATis is Curcumin, which is isolated from the *Curcuma longa* rhizome. Curcumin has shown high efficacy in the prevention and treatment of colorectal, prostate, kidney, lung, ovarian, breast, cervical and liver cancers (Balasubramnyam et al., 2004). The last group of HATis includes a number of small molecules designed to overcome the challenges in permeability of the first two groups. These include γ-butyrolactone MB-3, quinoline and isothiazolone and their

derivatives. Although in their infancy, isothiazolone has been shown to inhibit the enzymatic activity of both pCAF and p300 leading to reduction in cell proliferation of human ovarian and colon cancer cell lines (Stimson et al., 2005). γ -butyrolactone MB-3 inhibits GCN5 and contains an $\alpha\beta$ -unsaturated carbonyl group that is prone to covalently bind to the thiol group in the active site of GCN5 (Biel et al., 2004).

3.3 Histone deacetylase inhibitors (HDACis)

HDAC inhibitors (HDACis) have been classified into seven categories based on their chemical structures and mode of inhibition: short chain fatty acids, benzamides, cyclic peptides, electrophilic ketones, hydroxamine-acid-derived compounds (Espino et al., 2005; Rasheed et al., 2007), miscellaneous compounds (e.g. Depudecin and MGCD-0103) and sirtuin inhibitors (Table 10). Sirtuin or class III HDACis can be further classified structurally, but for simplicity, they are classified here into a single group. For details on class III HDACi subgroups, the reader is referred to Schemies et al., 2009. Class I, II and IV HDACis share a common metal binding domain that serves to block Zn^{+} chelation at the active site (Miller et al., 2003). Because of the presence of a different co-factor (nicotinamide (NAD)) at the active site of class III HDACs, zinc-dependent HDACis are ineffective against them. Class III

HDACi Class	Chemical Compound
Hydroxamic acid-derived compounds	TSA (trichostatin A), SAHA (suberoylanilide hydroxamic acid or Vorinostat) CBHA (<i>m</i> -carboxycinnamic acid bis-hydroxamide), ABHA (azelaic bis-hydroxamic acid), LAQ-824, LBH-589, oxamflatin, PXD-101, scriptaid, pyroxamide, SK-7041, SK-7068 and tubacin.
Cyclic peptides	Romidepsin (depsipeptide, FK-228/FR-901228), apicidin, CHAPS (cyclic hydroxamic acid-containing peptides) and trapoxin.
Short-chain fatty acids	Valproic acid, phenylbutyrate, phenylacetate and AN-9.
Benzamides	MS-275 and CI-994.
Ketones	Trifluoromethyl ketone.
Miscellaneous	Depudecin and MGCD-0103.
Sirtuin inhibitors	Nicotinamide (NAD), 2-Anilino-benzamide, Sirtinol, Dihydropyridine, Cambinol, etc.

Table 10. Histone Deacetylase Inhibitor Subgroups

HDACis are inhibited by nicotinamide, NAD⁺ analogues, indoles, hydroxynaphthaldehyde derivatives, Splitomicins, Suramins and kinase inhibitors (Schemies et al., 2009). NAD works by specifically blocking the entry of nicotinamide adenine dinucleotide into the active site of class III HDACs. The modes of action of other satiuin inhibitors are still unknown. While zinc-dependent HDACis are established anticancer drugs, and two inhibitors (Vorinostat (SAHA), Romidepsin) have been approved for cancer treatment in the United States (Johnstone et al., 2002), much less is known about the biological consequences of sirtuin inhibitors (North et al., 2004; Wesphal et al., 2007; Fatkins et al., 2008). In fact, sirtuin inhibitors shown to be effective in lower organisms, do not work on human subtypes (Biel et al., 2005; Schafer et al., 2005). In general, HDACis have shown to induce cell cycle arrest and apoptosis in G1 or G2/M. For example, in response to HDACis, p21 gene is consistently upregulated in a p53-independent manner and p21 expression is correlated with cell cycle G1 arrest (Gui et al., 2004; Vrana et al., 1999). The upregulation of p21 gene is correlated with increased acetylation of histones H3 and H4 near the p21 promoter (Hirsch et al., 2004). In addition, HDACis, such as butyrate and trichostatin A, have been shown to stabilize p21 mRNA (Hirsch et al., 2004). Moreover, HDAC inhibition represses cyclins A and D, and activates p16 and p27 to induce cell cycle arrest (Sandor et al., 2000; Wharton et al., 2000). In other studies, HDACis upregulate the expression of pro-apoptotic genes (i.e., TRAIL, DR5, Bax, Apaf-1, Bmf, Bim and TP2) and/or downregulate the expression of anti-apoptotic genes (i.e., Bcl-2, Mcl1, and XIAP) (see review by Bolden et al., 2006). The biggest advantage for many HDACis is that they can induce their effect in the nano/micromolar range, as seen for SAHA and butyric acid, respectively (Espino et al., 2005; Kelly et al., 2003). Moreover,

Class	HDACs	Relevant HDACis
I	HDACs 1 and 2	SK-7041, SK-7068, MS-275, VPA, romidepsin butyrate, trapoxin, SAHA, TSA, PXD-101, LBH-589, LAQ-824 and MGCD-0103
	HDAC 3	MS-275, VPA, butyrate, trapoxin, SAHA, TSA, PXD-101, LBH-589, LAQ-824 and MGCD-0103
	HDAC 8	VPA, butyrate, trapoxin, SAHA, TSA, PXD-101, LBH-589 and LAQ-824
IIa	HDAC 4	Romidepsin, VPA, butyrate, trapoxin, SAHA, TSA, PXD-101, LBH-589 and LAQ-824
	HDACs 5, 7 and 9	VPA, butyrate, trapoxin, SAHA, TSA, PXD-101, LBH-589 and LAQ-824
IIb	HDAC 6	Romidepsin, tubacin, SAHA, TSA, PXD-101, LBH-589 and LAQ-824
	HDCA 10	Tubacin, SAHA, TSA, PXD-101, LBH-589 and LAQ-824
III	SIRT-1, -2, -3, -4, -5, -6 and -7	Nicotinamide (NAD), 2-Anillno-benzamide, Sirtinol, Dihydropyridine, Cambinol, etc.
IV	HDAC 11	SAHA, TSA, PXD-101, LBH-589, LAQ-824 and MGCD-0103

HDAC: Histone deacetylase; SAHA: Suberoylanilide hydroxamic acid; SIRT: Sirtuin; TSA: Trichostatin A; VPA: Valproic acid.

Table 11. HDAC Classes and Relevant Inhibitors

HDACis have been shown to suppress angiogenesis and to activate and enhance the host immune system in cancer patients (Bhalla et al., 2005; Dokmanovic et al., 2005; Bolden et al., 2006).

At the clinical level, HDACis function synergistically with a host of structurally and functionally diverse cancer drugs, chemotherapeutic agents and biologically active polypeptides. In this manner, HDACis can increase the efficacy of other drugs by increasing target susceptibility. For example, in breast cancer therapy, the effectiveness of topoisomerase II inhibitors can be increased by pretreatment with SAHA (Marchion et al., 2004). In addition, HDACis have been used in combination with DNA demethylating agents in an attempt to reactivate silenced genes involved in tumor suppression. For example, three Phase I/II trials combining 5-Aza-CR or decitabine with HDAC inhibitors (phenyl butyrate or valproic acid) in patients with AML and MDS showed both tolerability and promising efficacy (Gore et al., 2006; Maslak et al., 2006; Garcia-Manero et al., 2006). Of a total of 93 patients who were treated, 14 showed complete remissions (CR), two showed partial complete remission (pCR), four showed partial response (PRs) and 6 showed hematologic improvements (HI) (Table 12). These studies combined have an overall response rate of 28%. In another phase I clinical study, the efficacy of CI-994 and various chemotherapeutic agents was examined in 104 patients. The results from these studies showed that CI-994 at doses of 4-10mg/m²/day can be safely administered to patients for 7-21 days in a 3-4 week dosing regimen (Nemunaitis et al., 2003; Undevia et al., 2004; Paur et al., 2004). In this study, two patients with esophageal and bladder cancer showed complete remission (CR) and five (three with non- small lung cancer and two with colorectal cancer) demonstrated partial remission (PR). On a related note, some leukemia and breast cancers plagued with the expression of fusion proteins (RAR-PML, RAR-PLZF or AML-ETO chimeras) that inhibit differentiation have shown improvements when HDACis in combination therapy with transretinoic acid (ATRA) is used to inhibit the function of these fusion proteins (Johnstone et al., 2003) (Table 12). Another emerging area of combination therapy is the use of HDACis with tyrosine kinase inhibitors in cancers that overexpress antiapoptotic genes. For example, SAHA, LBH-589, LAQ-824 and romidepsin have demonstrated synergistic apoptotic activity in combination with imatinib and other tyrosine kinase inhibitors, such as AMN-107 in imatinib-sensitive, as well as imatinib-resistant *bcr-abl* leukemic cells (Nimmanapalli et al., 2003a, 2003b; Yu et al., 2003; Kawano et al., 2004; Fiskus et al., 2006).

HDACis	Phase	Tumor type	Patient number	Responses	Reference
Single Therapy					
Phenyl butyrate	I	Solid	75	CR(1) and SD (9)	Carducci et al., 2001; Gilbert et al., 2001; Phuphanich et al., 2005
Valporic acid	I/II	AML/MDS	18	OR(8) and PR (1)	Kuendgen et al., 2004
SAHA	I	Mesothelioma	37	PR (2)	Kelly et al., 2003
	I	Solid	13	TR (4)	Krug et al., 2006
Romsidepsin	II	CTCL	28	CR(2), PR(8), SD(16)	Whittaker et al., 2006

Combination Therapy	Phase	Tumor Type	Patient number	Response	Reference
Phenyl butyrate (+)					
5-Aza	I/II	AML/MDS	29	CR (4), PR (1), HI (6)	Gore et al., 2006
5-Aza	II	AML/MDS	10	PR (3) , SD (2)	Maslak et al., 2006
ATRA	I	APML	5	CR (1)	Zhou et al., 2002
Valproic acid (+)					
Decitabine	I/II	AML/MDS	54	CR (10) and CRp (2)	Garcia-Manero et al., 2006
ATRA	II	AML	11	CR (1) and CRi (2)	Raffoux et al., 2005
ATRA	II	AML/MDS	20	HI (6/11 evaluable)	Pilatrino et al., 2005
ATRA	II	AML	30	CR (1), CRi (1), PR (1) and SD (20)	Kuendgen et al., 2006
SAHA (+)					
Carboplatin + paclitaxel	I	Colorectal	9	PR (4) and SD (2)	Ramalingam et al., 2006
CI-994 (+)					
Capecitabine	I	Solid	4	PR (1) and SD (19)	Undenvia et al., 2004
Carboplatin + paclitaxel	I	Pancreatic	30	CR (2) and PR (5)	Pauer et al., 2004

5-AZA: 5-Azacytidine; AML: Acute myeloid leukemia; APML: ATRA: All-trans-retinoic acid; CR: Complete response; CRi: Morphologic complete remission with incomplete count recovery; CRp: Complete response without complete platelet recovery; HDACi: Histone deacetylase inhibitors; MDS: Myelodysplastic syndrome; PR: Partial response; SAHA: Suberoylanilide hydroxamic acid; SD: Stable disease.

Table 12. HDACis in Clinical Trial and Combination Therapies

4. Concluding remarks

Only a handful of studies have been published examining the usefulness of drugs targeting ubiquitination, sumolyation and phosphorylation of histone as a means to combat the proliferative and differentiation deficits seen in cancer. The above discussion was not intended to provide complete coverage to a fast emerging field at rather as a means to highlight the most promising therapies investigated to date employing epigenetic-based approaches. Although these early successes establish the promise of epigenetic-based chemotherapeutic regimens in the treatment of various cancers, the degree to which gene-specific epigenetic modifications can be achieved, or the extent to which targeted therapies can be developed using epigenetic approaches remains to be fully investigated. Undeniably, the ultimate benefit to be realized from such strategies is based on the fact that the epigenetic modifications in cancer cells are reversible and subject to environmental control. Clearly, the jury is still out!

5. References

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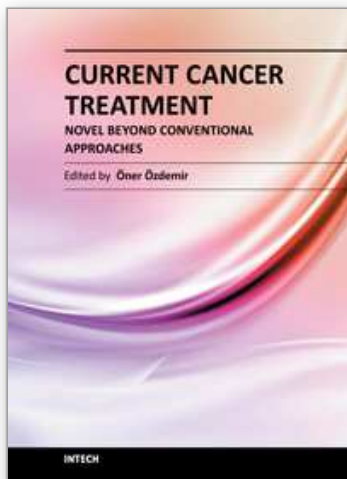
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Currently there have been many armamentaria to be used in cancer treatment. This indeed indicates that the final treatment has not yet been found. It seems this will take a long period of time to achieve. Thus, cancer treatment in general still seems to need new and more effective approaches. The book "Current Cancer Treatment - Novel Beyond Conventional Approaches", consisting of 33 chapters, will help get us physicians as well as patients enlightened with new research and developments in this area. This book is a valuable contribution to this area mentioning various modalities in cancer treatment such as some rare classic treatment approaches: treatment of metastatic liver disease of colorectal origin, radiation treatment of skull and spine chordoma, changing the face of adjuvant therapy for early breast cancer; new therapeutic approaches of old techniques: laser-driven radiation therapy, laser photo-chemotherapy, new approaches targeting androgen receptor and many more emerging techniques.

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