12

DNA Methylation and Histone Deacetylation: Interplay and Combined Therapy in Cancer

Yi Qiu, Daniel Shabashvili, Xuehui Li,
Priya K. Gopalan, Min Chen and Maria Zajac-Kaye
Department of Anatomy and Cell Biology
and Department of Medicine,
College of Medicine, University of Florida,
Gainesville, Florida,
USA

1. Introduction

In mammalian cells, DNA can be modified by methylation of cytosine residues in CpG dinucleotides, and the N-terminal tails of histone proteins are subject to a wide range of different modifications, including acetylation, methylation, phosphorylation and ubiquitylation. All of these chemical changes have a substantial influence on chromatin structure and gene expression. These epigenetic modification patterns can be regarded as heritable marks over many cell generations. Importantly, patterns and levels of DNA methylation and histone acetylation/deacetylation are profoundly altered in human cancers. Inhibitors of DNA methytransferases (DNMTs) and histone deacetylases (HDACs) have been shown to inhibit tumor growth by reactivating epigenetically silenced tumor suppressor genes. Although DNA methylation and histone deacetylation are carried out by different chemical reactions and require different sets of enzymes, it seems that there is a biological relationship between the two systems in modulating gene repression programming. Accumulating evidence also suggests that this epigenetic cross-talk may be involved in gene transcription and aberrant gene silencing in tumors. Thus, combined therapy with both DNMT and HDAC inhibitors can be a promising approach for cancer treatment.

2. Epigenetic gene silencing through DNA methylation and histone deacetylation

2.1 DNA methylation in regulating gene transcription

DNA methylation is a covalent chemical modification of DNA occurring at cytosine residues in CpG dinucleotides. Approximately 70–80% of cytosine in CpG dyads is methylated on both strands in human somatic cells(Chen & Riggs, 2011). DNA methylation is a stable epigenetic mark that is linked to the maintenance of chromatin in a silent state, therefore regulating chromatin structure and gene expression involved in processes such as X chromosome inactivation, genomic imprinting, embryogenesis, gametogenesis, and

silencing of repetitive DNA elements. Deregulation of DNA methylation directly affects mammalian development and development of cancer (Gopalakrishnan et al., 2008).

The mammalian DNA methyltransferases (DNMTs) are enzymes that catalyze the transfer of a methyl group from S-adenosyl-L-methionine to cytosine. Among the three enzymatically active DNMTs, DNMT1 is thought to function as the major maintenance methyltransferase(Chen & Riggs, 2011). This enzyme maintains DNA methylation at hemi-methylated DNA after DNA replication (Pradhan et al., 1999), and it is responsible for copying pre-existing methylation patterns to the newly synthesized strand (Chen & Li, 2004). DNMT3A and DNMT3B are *de novo* methyltransferases active on unmethylated DNA. Both of them are responsible for establishing new DNA methylation patterns during early development (Okano et al., 1999) as well as maintaining these patterns during mitosis (Chen et al., 2003). DNMT3L is homologous to DNMT3A and DNMT3B within the N-terminal regulatory region and is highly expressed in germ cells. Although catalytically inactive, DNMT3L regulates DNMT3A and DNMT3B by stimulating their catalytic activity (Chen et al., 2011).

Our knowledge about the function of DNA methylation in mammals comes mainly from DNMTs transgenic mice studies(Chen & Li, 2004). Studies of the zygotic functions of DNMTs have shown that the establishment of embryonic methylation patterns requires both *de novo* and maintenance methyltransferase activities, and that the maintenance of DNA methylation above a threshold level is essential for embryonic development (Lei et al., 1996). Complete elimination of DNMT1 function results in embryonic lethality around E9.5, with extensive loss of global DNA methylation (Li et al., 1992). DNMT3B is also essential for embryogenesis. DNMT3B-deficient embryos show growth impairment and multiple developmental defects after E9.5 and die after E12.5. DNMT3A mutant mice die around 4 weeks of age. DNMT3A/DNMT3B-double knockout embryos die around E9.5, similar to DNMT1-null mutants (Okano et al., 1999). Loss of DNA methylation does not affect ES proliferation and viability, and the effect of demethylation only becomes apparent during or after gastrulation when the pluripotent embryonic cells begin to differentiate (Li et al., 1992,Okano et al., 1999). Conditional disruption of DNMT1 in mouse embryonic fibroblasts (MEFs) results in severe demethylation and cell death, and DNMT3B deficient MEFs show moderate demethylation, chromosomal instability, and abnormal proliferation (Jackson-Grusby et al., 2001,Farthing et al., 2008,Dodge et al., 2005). These findings suggest that DNA methylation is essential for cellular differentiation and normal functioning of differentiated cells.

Development: In mammals, DNA methylation patterns undergo dramatic changes during early development. During preimplantation development, both paternal and maternal genomes undergo a wave of demethylation, which erases the methylation marks inherited from the gametes. Within 3-6 hours after fertilization, the maternal genome is rapidly methylated through *de novo* DNA methylation, while the paternal genome is actively demethylated before the first replication event occurs (Mayer et al., 2000,Oswald et al., 2000). This is followed in the maternal genome by a loss of DNA methylation gradually over several DNA replication cycles (Mayer et al., 2000,Rougier et al., 1998). Replication-dependent demethylation is caused by the exclusion of maintenance methyltransferase DNMT1 from the nucleus of embryos (Carlson et al., 1992,Cardoso & Leonhardt, 1999). As a result, maternal and parental genomes become almost equally low in DNA methylation by the eight cell stage of development (Mayer et al., 2000). At morula stage, there is an increase

in *de novo* methylation. This methylation occurs through the activity of *de novo* methyltransferases DNMT3A and 3B (Okano et al., 1999). Consistent with these data, DNMT1-deficient or DNMT3A/DNMT3B-double mutant ES cells show severe differentiation defects (Tucker et al., 1996).

Genomic imprinting: Another developmental stage that exhibits substantial *de novo* DNA methylation in mammals is gametogenesis. DNA methylation in both male and female germ cells plays a critical role in the establishment of genomic imprinting. Genomic imprinting is an epigenetic process that marks alleles according to their parental origin and results in monoallelic expression of a small subset of genes (Reik & Walter, 2001). Genetic studies demonstrate that DNMT3A and DNMT3L are essential for setting up DNA methylation imprints in germ cells (Kaneda et al., 2004,Bourc'his et al., 2001). Although DNMT3L has no enzymatic activity, it has been shown to interact with DNMT3A and stimulate its activity (Suetake et al., 2004). Active transcription across imprinting control regions also appears to be required for the establishment of DNA methylation imprints in female germ cells (Chotalia et al., 2009). It is possible that the removal of H3K4 methylation and active transcription at imprinted loci create or maintain a chromatin state that facilitates access of the DNMT3A-DNMT3L complex to these loci (Ooi et al., 2007).

Differentiation: Differentiation is an epigenetic process associated with selective temporal activation of lineage-specific genes and regulated silencing of pluripotency genes. Pluripotency genes are those that are important for maintaining the unrestricted developmental potential of the early embryo and ES cells. Undifferentiated ES cells do not show global differences in DNA methylation levels compared to somatic cells (Mohn et al., 2008). However, changes in DNA methylation at promoter regions were observed (Meissner et al., 2008). Among those promoter regions that gain DNA methylation upon differentiation of ES cells in culture are pluripotent genes and germ line-specific genes (Farthing et al., 2008,Mohn et al., 2008). *De novo* DNA methylation and silencing of the pluripotency genes contributes to loss of pluripotency in differentiated cells (Farthing et al., 2008). Oct4 and Nanog are two such genes which are essential for pluripotency and early development and become methylated after differentiation (Hattori et al., 2004,Hattori et al., 2007,You et al., 2011). Thus, *de novo* methylation at promoter regions during cellular differentiation may in part control the loss of pluripotency by silencing stem cell-specific genes.

Once cells have passed through the early embryonic stage, differentiation toward a specific lineage pathway occurs. These somatic tissues then contain specific gene expression patterns unique for that terminally differentiated cell type. Although DNMT1 is dispensable for ES cell maintenance, it is required for maintaining the somatic progenitor state through cell divisions. Depletion of the maintenance DNA methyltransferase DNMT1 in epidermal progenitors leads to premature differentiation. Genome-wide DNA methylation analysis showed that some epidermal differentiation gene promoters were methylated in self-renewing progenitor cells but were subsequently demethylated during differentiation (Sen et al., 2010). The correlation between gene expression and DNA methylation was investigated in diverse tissues and suggests that DNA methylation is critical for regulating the expression of some tissue specific genes (Shen et al., 2007,Illingworth et al., 2008). In the case of neuronal development, this methylation involves DNMT3A, which is expressed in postnatal neural stem cells and is required for neurogenesis (Mohn et al., 2008,Wu et al., 2010).

2.2 Histone deacetylation in regulating gene repression

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from the ε-amino groups of lysine residues. The reversible acetylation of histones and non-histone proteins by histone acetyltransferases (HATs) and deacetylases plays a critical role in transcriptional regulation and many other cellular processes in eukaryotic cells. Acetylation of histones is commonly associated with the transcriptional activation of genes, and is thought to be responsible for the formation of a local "open chromatin" structure required for the binding of multiple transcription factors (Sterner & Berger, 2000). In contrast, the removal of acetyl groups by HDACs frequently accompanies the suppression of gene activity (Ng & Bird, 2000). However, non-histone protein lysine acetylation plays a diverse role in the regulation of all aspects of cellular processes (Glozak et al., 2005).

Classification: Mammalian HDACs are classified into four classes (I, II, III and IV) based on the sequence homology of the yeast histone deacetylases Rpd3 (reduced potassium dependency), Hda1 (histone deacetylase1), and Sir2 (silent information regulator 2), respectively. Class I HDACs include HDAC1, 2, 3 and 8. Class II HDACs contain HDAC4, 5, 6, 7, 9 and 10. Class III enzymes, however, require the coenzyme NAD+ as a cofactor. HDAC11 belongs to the class IV family (Reviewed in (Yang & Seto, 2008)). Although the precise cellular functions of the different HDAC enzymes are still poorly understood, evidence suggests that different members of the HDAC family have distinct functions (Cho et al., 2005,Foglietti et al., 2006).

Post-translational modifications: All class I HDACs can be phosphorylated. Phosphorylation of HDAC1, 2 and 3 increases deacetylase activities while phosphorylation of HDAC8 inhibits deacetylase activity (Sengupta & Seto, 2004). HDAC1 can also be acetylated at the catalytic core domain and the C-terminal region, resulting in dramatic reduction of enzymatic activity (Qiu et al., 2006). Other modifications of HDAC1 include sumoylation and ubiquitylation, which also influence deacetylase activity and protein stability (David et al., 2002,Oh et al., 2009). Class II HDACs can also be phosphorylated. Phosphorylation does not appear to regulate enzymatic activity of class II HDACs; instead, it modulates their subcellular localization (Yang & Gregoire, 2005). Class III deacetylases can also be phosphorylated. One report showed that phosphorylation of SIRT1 enhances deacetylation of p53 (Guo et al., 2010).

Function and regulation of Class I histone deacetylases: Class I HDACs are ubiquitously expressed nuclear proteins. Although they share a high level of sequence homology and common substrates, each of the enzymes has a unique role in cell function and cannot compensate for the other enzymes' functions, as deletion of each class I deacetylase leads to lethality (Reviewed in (Haberland et al., 2009)). HDACs can be recruited to genes by sequence-specific or non sequence-specific DNA-binding factors. Class I deacetylases are found to associate with a variety of proteins, such as transcription factors, coactivators, corepressors, chromatin remodeling proteins, etc, adding to the complexity of HDACs functions (Yang & Seto, 2007). Class I HDACs have been widely implicated in gene repression through the hypoacetylation of localized chromatin domains (Strahl & Allis, 2000). It is a traditionally held view that HDACs are associated with repressed promoters and are replaced by coactivators during gene activation (Berger, 2007). However, recent advancements in methodology allow studying the localization of HDACs or HATs on a

genome-wide scale. The surprising result is that HATs and HDACs are positively correlated with gene expression. Levels of HDAC1,2 and 3 are high in active genes and absent from silenced genes (Wang et al., 2009). The emerging new model for the role of HDACs is that they can counteract histone acetyltransferase to maintain transcription activation within a certain level, and they can regulate gene activation as deacetylation resets chromatin for subsequent rounds of transcription (Perissi et al., 2010). This model is also supported by data that shows HDAC complexes in yeast, Rpd3s, can interact with active chromatin in order to prevent transcription initiation from cryptic sites (Li et al., 2007). In some cases, HDAC can even function as coactivator (Qiu et al., 2006). Besides regulation of gene transcription, class I HDACs have been shown to also be involved in many other cellular processes, especially events that are linked to oncogenesis, such as DNA repair, recombination, and replication, cell cycle check point control, and other signaling regulators (Spange et al., 2009).

Class I HDACs, except HDAC8, are often found in multiprotein complexes and their activity is regulated through associated complexes (reviewed in (Sengupta & Seto, 2004). HDAC1 and 2 are found to coexist in at least three evolutionally conserved, distinct protein complexes: the Sin3, the CoREST and the NuRD/Mi2 complexes (Grozinger & Schreiber, 2002,Yang & Seto, 2008). All complexes are recruited to target genes through interactions with DNA binding transcription factors (Yang & Seto, 2007). Sin3 contains the conserved basic structure of multiple paired amphipathic helix (PAH) domains for protein-protein interaction. Mammals have two isoforms, Sin3A and Sin3B, which provide more diverse protein complexes for gene regulation. Sin3 does not bind to DNA and has no known enzymatic activity of its own. It is suggested that it functions through its ability to interact with other proteins. The CoREST complex is a multi-subunit complex containing the lysine demethylase LSD1, corepressors CtBP, BHC80, CoREST, HDAC1 and HDAC2 (Lee et al., 2005,Shi et al., 2003). Thus, this complex is capable of deacetylating as well as demethylating nucleosomes. Mi2 belongs to the CHD (chromo-helicase DNA-binding) protein family (Woodage et al., 1997). The NuRD complex includes the ATPase/helicase Mi-2, HDAC1 / HDAC2, MTA2 (Metastasis-associated) proteins, MBD3 (methyl CpG-binding domain-1), RbAp46/48 (Wade et al., 1998,Zhang et al., 1998). Interestingly, recent reports suggest that LSD1, a lysine specific demethylase, is also recruited to the NuRD complex through its interaction with MTA2 (Wang et al., 2009). Thus, the NuRD complex may regulate gene transcription through a combination of deacetylation, demethylation and remodeling. The NuRD complex is also part of a larger protein complex, MeCP1 (methyl CpG-binding protein 1) complex (Fatemi & Wade, 2006). The MeCP1 complex contains the additional component MBD2, a CpG methyl-binding protein. Therefore, NuRD/MeCP1 is targeted to methylated CpG sites leading to histone deacetylation and repression at the gene promoter (Nan et al., 1998).

HDAC3 forms a stable complex with nuclear receptor corepressor (N-CoR) and silencing mediator of retinoic and thyroid receptors (SMRT) (Wen et al., 2000,Guenther et al., 2001,Guenther et al., 2000). Both N-CoR and SMRT had been discovered as interacting partners of unliganded nuclear receptors, such as TR and RAR and mediators of their repressive functions. The interaction between HDAC3 and SMRT/N-CoR seems to be essential for HDAC3 deacetylase activity (Guenther et al., 2001).

HDAC8 was identified as a ubiquitously distributed nuclear enzyme (Hu et al., 2000). However, it is also found in the cytosol of smooth muscle cells where it associates with α-

actin cytoskeleton (Waltregny et al., 2004). A gene deletion study also shows that HDAC8 plays an important role in skull formation (Haberland et al., 2009). HDAC8 is phosphorylated by cyclic AMP-dependent protein kinase A (PKA) *in vitro* and *in vivo*. Induction of phosphorylation decreases HDAC8's enzymatic activity. Remarkably, inhibition of HDAC8 activity by hyperphosphorylation leads to hyperacetylation of histones H3 and H4, suggesting that PKA-mediated phosphorylation of HDAC8 plays a central role in the overall acetylation status of histones (Lee et al., 2004).

Functions of Class II deacetylases: class IIa mammalian HDACs mainly function as transcriptional corepressor through their deacetylase domain and other repression domains (Yang & Gregoire, 2005). Class IIa HDACs share conserved motifs, such as MEF-2 binding, 14-3-3 binding and nuclear localization signal at N-terminal region which are important for the function and regulation of class IIa members (Yang & Gregoire, 2005). These HDACs possess intrinsic nuclear import and export signals for nucleo-cytoplasmic trafficking (McKinsey et al., 2000). MEF2 and 14-3-3 are major HDAC4 binding partners. While MEF2 promotes nuclear localization of class IIa HDACs (Miska et al., 1999,Wang & Yang, 2001), 14-3-3 proteins stimulate cytoplasmic retention (Grozinger & Schreiber, 2000). Class IIa HDACs have low deacetylase activity on their own, it is suggested that HDAC3 is needed for class IIa HDACs to exert full deacetylase activity (Fischle et al., 2002).

A class IIb member, HDAC6 possesses two deacetylase domains and a zinc finger motif (Zn-UBP, ubiquitin carboxyl-terminal hydrolase-like zinc finger) (Seigneurin-Berny et al., 2001). HDAC6 deacetylates α-tubulin, Cortactin and HSP90 to regulate cell motility, cilium assembly, cell adhesion, the immune synapse, macropinocytosis, maturation of the glucocorticoid receptor (GR) and activation of some protein kinases (Yang & Seto, 2008) . Depending on its availability in the nucleus, HDAC6 is also able to deacetylate histones (Yoshida et al., 2004). In addition to its deacetylase domains, HDAC6 possesses a Zn-UBP finger that binds to ubiquitin and is involved in ubiquitin-dependent aggresome formation and cellular clearance of misfolded proteins (Rodriguez-Gonzalez et al., 2008,Yang & Seto, 2008). Both deacetylase and ubiquitin-binding activities of HDAC6 are required for these processes. Therefore, HDAC6 regulates various processes in the cytoplasm. Several lines of evidence suggest that HDAC6 also plays a role in the nucleus. It interacts with several nuclear proteins, including HDAC11 (Gao et al., 2002), sumoylated p300 (Girdwood et al., 2003), transcriptional corepressors such as ETO2 and L-CoR, and sequence specific transcription factors such as NF-kB and Runx2 (Yang & Gregoire, 2005). HDAC10 is also a class IIb histone deacetylase. The N-terminal half of HDAC10 is more similar to the first catalytic domain of HDAC6 than to the second (Guardiola & Yao, 2002). The C-terminal half of HDAC10 is leucine rich and shows limited sequence similarity to the second deacetylase domain of HDAC6 (Guardiola & Yao, 2002,Yang & Seto, 2008), but its function remains elusive.

Class IV deacetylase, HDAC11, is a highly conserved deacetylase. It shows sequence similarity to class I and II HDACs. Little is known about its function or regulation (Gao et al., 2002).

Class III deacetylases and function: Mammals have seven Sir2 homologs (sirtuins, SIRT1–7). These proteins have a highly conserved NAD-dependent sirtuin core domain, first identified in the founding yeast SIR2 protein. Mammalian sirtuins have diverse cellular locations,

multiple substrates, and affect a broad range of cellular functions (Haigis & Guarente, 2006). Three mammalian sirtuins (SIRT1, SIRT6, and SIRT7) are localized to the nucleus. SIRT1 is most extensively studied, has more than a dozen known substrates (Haigis & Guarente, 2006). SIRT1 regulates histone acetylation levels (mainly K16-H4 and K9-H3 positions) (Vaquero et al., 2004,Pruitt et al., 2006), and the acetylation of transcription factors such as p53 (Vaziri et al., 2001), p300 histone acethyltransferase (Bouras et al., 2005), E2F1 (Wang et al., 2006), the DNA repair ku70 (Cohen et al., 2004), NF-KB (Yeung et al., 2004), and the androgen receptor (Fu et al., 2006). It is also responsible for tissue metabolism, cellular oxidative stress and DNA repair (Finkel et al., 2009, Uhl et al., 2010). SIRT6 and SIRT7 may also be important regulators of DNA damage and metabolism (Finkel et al., 2009). SIRT2 is a predominantly cytoplasmic protein. It colocalizes with tubulin, and can deacetylate a number of substrates *in vitro*, including α tubulin and histones (North et al., 2003). SIRT2 may be important in regulating mammalian cell cycle (Dryden et al., 2003). SIRT3-5 are localized at the mitochondria and are important for micochondria energy usage (Pereira et al., 2011).

3. Connections between DNA methylation and histone deacetylation for gene silencing

Alteration of DNA methylation affects histone acetylation, or vice versa: Both DNA methylation and hypoacetylation of histones H3 and H4 are frequently associated with silent genes(Dobosy & Selker, 2001). For example, in DNMT1 knockout cancer cells there is an increase in the amount of acetylated forms of histone H3 and a decrease in that of the methylated forms of histone H3. These changes are associated with a loss of interaction of HDACs and the heterochromatin protein HP1 with histone H3. These data strongly indicate that histone hyperacetylation is not always a result of a loss of HDAC activity, but that it could be due to a loss of HDACs targeted to specific DNA sequences. One possible explanation is that changes in DNA methylation also cause histone modification due to direct interactions between the enzymes regulating different epigenetic modifications (Espada et al., 2004). The evidence supporting the communication between DNA methylation and histone deacetylation has been demonstrated from inhibitor studies. HDAC inhibitors do not only change acetylation of histones, but also induce DNA demethylation. Using DNA demethylating agents, like 5-aza-2'-deoxycytidine, in combination with HDAC inhibitors changes the status of DNA methylation, reactivates gene transcription, and inhibits cancer cell growth (reviewed in (Dobosy & Selker, 2001,Hellebrekers et al., 2007)). Recent studies show that histone deacetylase inhibitor Trichostatin A (TSA) treatment reduces global DNA methylation and DNMT1 protein level, alters DNMT1 nuclear dynamics and interactions with chromatin. The mechanisms underlying these effects are apparently distinct from the mechanisms of action of the DNMT inhibitor 5-Azacytidine (Arzenani et al., 2011). Therefore, communication between histone deacetylation and DNA methylation is likely to be a dynamic process in the regulation of gene silencing.

Connections through methyl-CpG-binding proteins: Early studies show that DNA methylation can lead to transcriptional repression through interaction with methyl-CpG-binding proteins (Nan et al., 1997). Methyl-CpG-binding proteins are a group of proteins that bind to methylated CpG sites through their mentyl-CpG-binding domain (MBD). The founding member of the MBD family was MeCP2 (Lewis et al., 1992). It is a multidomain protein and

is associated with and localized to densely methylated chromatin regions. This protein also contains a transcriptional repression domain (TRD). Subsequently, MBD1 to MBD4 were all discovered as EST clones with sequence similarity to the MBD motif of MeCP2 (Hendrich & Bird, 1998). All these proteins, except MBD3, contain both MBD domain and TRD domain, and recognize methylated DNA. The identification of methyl-CpG binding proteins (MBDs) leads to insights into the communication between DNA methylation and HDACs as all MBDs are components of co-repressor complexes that contain histone deacetylases (Fatemi & Wade, 2006).

Transcription repression mediated through MeCP2 requires both MBD and TRD domains. It was then demonstrated that MeCP2 can interact with transcription repressor SIN3. Furthermore, the region of interaction with Sin3 on MeCP2 significantly overlapped the TRD domain and tethering of the TRD resulted in repression that is sensitive to inhibitors of histone deacetylase. Thus, recruitment of the Sin3 repressive complex containing HDAC1 and HDAC2 to the methylation sites in the gene promoters results in a deacetylated repressive chromatin structure (Jones et al., 1998,Nan et al., 1997) . In neural progenitor/ES cells, genes are regulated by the REST corepressor complex which contain CoREST and histone deacetylases. MeCP2/Sin3 corepressor complexes can interact with REST repressor complex in the promoter region, resulting in gene inactivation (Ballas et al., 2005). It is noteworthy that MeCP2 can mediate transcriptional repression in both HDAC-dependent and –independent manners (Kaludov & Wolffe, 2000). In addition, a recent study suggests that MeCP2 can also function as an activator of gene transcription by recruiting transcriptional activator CREB1 to an activated gene promoter (Chahrour et al., 2008).

MeCP1 complex was purified using methylated DNA oligoes (Meehan et al., 1989). Later studies demonstrated that MeCP1 complex contains MBD2, HDAC1, HDAC2, and RbAp48 (Ng et al., 1999). Exogenous MBD2 represses transcription and this repression can be relieved by the deacetylase inhibitor trichostatin A (Ng et al., 1999). Interestingly, MBD2 is also capable of recruiting Mi-2/NuRD complex to methylated DNA through its heterodimerization with MBD3 (Feng & Zhang, 2001,Hendrich et al., 2001). This suggests an interplay of DNA methylation, nucleosome remodeling and histone deacetylation in gene silencing. In addition, activity of MBD2 is modulated through association with cofactors. A recent study indicates that methyl-CpG-binding protein 3-like-2 (MBD3L2) may function as a transcriptional modulator in MBD2-MeCP1-NuRD-mediated methylation silencing (Jin et al., 2005). MBD3L2 can convert inactivated genes to activated genes by displacing MBD2 present in the NuRD complex, which sequesters the MeCP1-NuRD complex away from methylated DNA. In addition, MBD*in* (Lembo et al., 2003) and transforming-acid-coiled-coil 3 (TACC3) can interact with MBD2 and reactivate MBD2-repressed genes through different regulation mechanisms. The recruitment of MBD*in* to MBD2 disrupts the association of MBD2 to its repressor complex (Lembo et al., 2003). Unlike MBD*in*, TACC3 can reactivate methylated genes by forming a complex with MBD2 and histone acetyltransferase PCAF (Angrisano et al., 2006).

MBD1 represses transcription of methylated reporter constructs. The repression requires both the TRD and MBD motifs and is sensitive to HDAC inhibitors (Ng et al., 2000). However, it remains undetermined which HDACs associate with the MBD1 complex.

Connections through DNMT-HDAC interaction: DNMTs, including DNMT1 and DNMT3s, can directly interact with HDAC1 and HDAC2 to repress gene transcription *in vitro* and *in vivo* (Geiman & Robertson, 2002). A study shows that repression of gene transcription by direct interaction between DNMT3A and HDAC1/2 can be abolished by sumoylation of DNMT3A, but not the other interaction partner, DNMT3B (Ling et al., 2004), which suggests a specific regulation of DNMT3A-mediated gene silencing. Notably, DNMT3L, which lacks the catalytic domain, has been shown to bind to HDAC1 and regulate DNA methylation independent of methylating activity (Deplus et al., 2002).

Another connection between DNA methyl transferase and deacetylase is that DNMT1 is stabilized by HDAC1 and the deubiquitinase HAUSP (herpes virus-associated ubiquitin-specific protease). In human colon cancers, the abundance of DNMT1 is correlated with that of HAUSP. HAUSP knockdown rendered colon cancer cells more sensitive to killing by HDAC inhibitors both in tissue culture and in tumor xenograft models (Du et al., 2010). HDACs may also be involved in regulating DNMT1 gene expression as HDAC inhibitor TSA decreased DNMT1 mRNA stability and reduced its transcript's half-life (Januchowski et al., 2007).

DNA methylation and Histone deacetylation, who comes first? Although it is well accepted that cross-talk between DNA methylation and histone deacetylation plays a pivotal role in gene silencing, it is still unclear which event is the dominant epigenetic determinant in this communication process. Some evidence suggests that DNA methylation may be the primary event to trigger histone deacetylation and lead to gene silencing (Irvine et al., 2002,Jones et al., 1998,Kaludov & Wolffe, 2000,Kudo, 1998,Nan et al., 1998,Rountree et al., 2000). Controversially, loss of histone acetylation may guide the local DNA hypermetylation and initiate transcriptional repression. Felsenfeld's group has shown that chicken β-globin insulator recruits HAT to establish a high level of histone acetylation which prevents promoter CpG methylation by blocking binding of the transcriptional repressor complex to the promoters (Mutskov et al., 2002). A global methylation study shows that histone hypoacetylation caused by TSA induces a significant decrease in global methylation (Ou et al., 2007). In addition, inhibition of HDAC1 by sodium butyrate induces promoter demethylation and reactivation of RARbeta2 in colon cancer cells (Spurling et al., 2008). A recent study in *Neurospora crassa* found that specific deacetylation of histone H2B and H3 is required for DNA methylation and heterochromatin formation (Smith et al., 2010). These results indicate that histone deacetylation status is crucial to sustaining DNA methylation of the promoters and gene silencing.

4. Aberrant DNA methylation and histone deacetylation in cancer

4.1 Aberrant DNA methylation and DNMTs recruitment in cancer

Altered methylation patterns are found in the majority of tumor types. Methylation changes generally occur early in cancer development, which supports the hypothesis that epigenetic changes precede cancer development (Linhart et al., 2007,Belinsky et al., 1998,Derks et al., 2006,Palmisano et al., 2000). A hallmark of many cancers is global hypomethylation and regional hypermethylation of CpG islands. This local hypermethylation in cancer is usually found on CpG islands associated with promoters of tumor suppressors or other genes involved in cell cycle regulation, leading to inactivation of these genes. Promoter

hypermethylation also facilitates mutations in these regions as methylated cytosine residues are spontaneously deaminated to thymine residues, causing mutational silencing of the genes (Sulewska et al., 2007,Fryxell & Zuckerkandl, 2000,Rideout et al., 1990). This hypothesis explains the high incidence of CpG to TpG transition mutations observed in the promoters of tumor suppressors, for example, the p53 tumor suppressor gene (Rideout et al., 1990).

The effects of global hypomethylation are more varied and not well understood. In mouse models, hypomethylation has been shown to induce genomic instability and tumorigenesis (Gaudet et al., 2003,Eden et al., 2003). It has been suggested that global hypomethylation can induce reexpression of normally silenced genes, some of which may be oncogenic. Genes reactivated by global hypomethylation can include silenced oncogenes, imprinted genes, genes on the inactivated X-chromosome (Sharp et al., 2011), endogenous retroviruses and transposons (Yoder et al., 1997), as well as silenced drug resistance genes (Chekhun et al., 2007). For example, a cell-cell adhesion glycoprotein P-cadherin is often overexpressed in breast cancer, but not in normal breast tissue. The aberrant expression of P-cadherin in breast cancer is regulated by gene promoter hypomethylation. (Paredes et al., 2005). A similar mechanism regulates overexpression of cyclin D2 in gastric and ovarian cancer (Sakuma et al., 2007,Oshimo et al., 2003), and MAGE in melanomas (De Smet et al., 2004). Global hypomethylation also induces chromosomal instability by a mechanism that is not well understood; one possible cause is a large number of derepressed transposons and retroviruses created by this hypomethylated state (Florl et al., 1999,Howard et al., 2008).

Tumor heterogeneity is a major barrier to effective cancer diagnosis and treatment. Recent studies suggest that methylation patterns can be different in different cancer types and tumor stages (Wermann et al., 2010). Epigenetic analysis of large gene panels and genome-wide screening of DNA methylation levels discovered that overall methylation patterns can be used as biomarkers for cancer risk and/or tumor type (Kondo & Issa, 2010,Figueroa et al., 2010,Hawes et al., 2010,Worthley et al., 2010). Cancer-specific differentially methylated regions (cDMRs) were identified; methylation variation within these cDMRs distinguishes various cancers from normal tissue, with intermediate variation in adenomas (Hansen et al., 2011). Whole-genome bisulfite sequencing shows these variable cDMRs are related to loss of sharply delimited methylation boundaries at CpG islands. It suggests that the loss of epigenetic stability of well-defined genomic domains underlies increased methylation variability in cancer and may contribute to tumor heterogeneity. The distinct methylation patterns can be used not only to differentiate carcinoma from other tumor types, but also to predict tumor progression stage, with potential clinical applications in diagnosis and prognosis (Hernandez-Vargas et al., 2010).

Exact nature of the defect in the cellular methylation machinery in tumor cells remains unknown. It is proposed that inappropriate DNMT expression pattern or timing during the cell cycle could disrupt the regulation of DNA methylation patterns as DNMT1, 3A, and 3B are expressed differentially during the cell cycle (Robertson, 2001). Global hypomethylation in cancer cells may also be due to upregulation of DNA demethylase system (Rai et al., 2010). Increased expression of DNMTs can result in hypermethylation of CG islands in cancer cells and may play important roles in malignant progression of cancer, leading to aberrant methylation in many important tumor suppressor genes. In fact, it had been shown that DNMT overexpression is an early and significant event in urothelial, hepatic, gastric, pancreatic, lung, breast, and uterine cervix carcinogenesis (Daniel et al., 2011).

Although the DNMT1 and DNMT3 family of proteins have been considered either maintenance or *de novo* methyltransferases, respectively, it is likely that all three DNMTs possess both functions *in vivo*, particularly during carcinogenesis (Robertson, 2001). DNMT1 has been shown to be essential for the survival and proliferation of human cancer cells (Chen et al., 2007). Increased DNMT1 protein expression correlates significantly with frequent DNA hypermethylation of multiple CpG islands, poorer tumor differentiation and malignant progression (Etoh et al., 2004,Nakagawa et al., 2005,Saito et al., 2003). In bladder cancer, progressive increase of expression of DNMT1 protein occurs during the precancerous stages (Nakagawa et al., 2003). Depletion of DNMT1 resulted in lower cellular maintenance methyltransferase activity, global and gene-specific demethylation and re-expression of tumor-suppressor genes in human cancer cells. Specific depletion of DNMT1 but not DNMT3A or DNMT3B markedly potentiated the ability of 5-aza-2'-deoxycytidine to reactivate silenced tumor-suppressor genes, indicating that inhibition of DNMT1 function is the principal means by which 5-aza-2'-deoxycytidine reactivates genes. These results indicate that DNMT1 is necessary and sufficient to maintain global methylation and aberrant CpG island methylation in human cancer cells (Robert et al., 2003).

DNMT3B depletion reactivated methylation-silenced gene expression but did not induce global or juxtacentromeric satellite demethylation as did specific depletion of DNMT1, indicating that DNMT3B has significant site selectivity that is distinct from DNMT1 (Beaulieu et al., 2002). It is shown that DNMT3B1 but not DNMT3A1 efficiently methylates the same set of genes in tumors and in nontumor tissues, demonstrating that *de novo* methyltransferases can initiate methylation and silencing of specific genes in phenotypically normal cells. This suggests that DNA methylation patterns in cancer are the result of specific targeting of at least some tumor suppressor genes, such as Sfrp2, Sfrp4, and Sfrp5, rather than of random, stochastic methylation followed by clonal selection due to a proliferative advantage caused by tumor suppressor gene silencing (Linhart et al., 2007).

4.2 HDACs and cancer

One common theme in cancer cells is elevated HDAC expression and global hypoacetylation. Loss of acetylated Lys16 (K16-H4) and trimethylated Lys20 (K20-H4) of histone H4 may be a common event in human cancer (Fraga et al., 2005), while other studies also show that the decrease in histone acetylation is not only involved in tumorogenesis, but also in tumor invasion and metastasis (Yasui et al., 2003).

It has become increasingly clear that class I HDAC enzymes are clinically relevant to cancer therapy (Haberland et al., 2009,Ropero & Esteller, 2007). Increased HDAC1 expression levels have been reported to in a variety of cancers, such as gastric (Choi et al., 2001), prostate (Halkidou et al., 2004), colon (Wilson et al., 2006) and breast (Zhang et al., 2005) carcinomas. Overexpression of HDAC2 has been found in cervical (Huang et al., 2005), gastric (Song et al., 2005), and colorectal carcinoma (Ashktorab et al., 2009). Other studies have reported high levels of HDAC3 (Wilson et al., 2006) expression in colon cancer specimens. HDAC8 has been found to be associated with various types of leukemia (Balasubramanian et al., 2008). HDAC1, 6 and 8 could also be important for breast cancer invasion (Park et al., 2011). These observations suggest that transcriptional repression of tumor-suppressor genes by overexpression or aberrant recruitment of HDACs to their promoter regions could be a common phenomenon in tumor onset and progression. It is now known that HDACs have

been associated with the deregulation of a number of well-characterized cellular oncogenes and tumor-suppressor genes. For example, Class I HDACs promote cell proliferation by inhibiting p21 and p27 promoter activity (Lagger et al., 2002,Wilson et al., 2006). In some tumors, p21(WAF1/cip1) is epigenetically inactivated by hypoacetylation of the promoter, and treatment with HDAC inhibitors leads to inhibition of tumor cell growth and an increase in both acetylation of the promoter and gene expression (Gui et al., 2004). The transcription factor Snail recruits HDAC1, HDAC2, and the corepressor complex mSin3A to the E-cadherin promoter to repress its expression (Peinado et al., 2004). Downregulation or loss of function of E-cadherin has been implicated in the acquisition of invasive potential by carcinomas (Hajra & Fearon, 2002), and so aberrant recruitment of HDACs to this promoter may have a crucial role in tumor invasion and metastasis. The role of HDACs in cancer is not restricted to their contribution to histone deacetylation, but also includes their role in deacetylation of non-histone proteins. For example, HDAC1 interacts with the tumor suppressor p53 and deacetylates it *in vivo* and *in vitro* (Juan et al., 2000,Luo et al., 2000). p53 is phosphorylated and acetylated under stress conditions. Since lysine residues acetylated in p53 overlap with those that are ubiquitinated, p53 acetylation serves to promote protein stability and activation, inducing checkpoints in the cell-division cycle, permanent cell-division arrest, and cell death. Aberrant recruitment of HDACs to specific promoters through the interaction with fusion proteins that result from chromosomal translocations in hematological malignancies has also been intensively studied. In acute promyelocytic leukemia, leukemic fusion between the PML (promyelocytic leukemia) gene and the retinoic acid receptor (RAR) gene suppresses transcription through recruitment of HDACs. Thus, cancer cells are unable to undergo differentiation, leading to excessive proliferation (Lin et al., 2001,He et al., 2001). Similar phenomena have been described for the RARα-PLZF (promyelocytic leukemia zinc finger protein) fusion, the AML1 (acute myelocytic leukemia protein1)-ETO fusion, and for the myc/Mad/Max signaling pathway involved in solid malignancies (Minucci et al., 2001,Ferrara et al., 2001,Kitamura et al., 2000,David et al., 1998).

Class II HDACs have also been shown to associate with various cancer types. Inhibition of class II HDACs induces p21 expression in breast cancer cell lines, suggesting that class II HDAC subfamily may exert specific roles in breast cancer progression (Duong et al., 2008). HDAC4 inhibits p21 gene expression through interaction with Sp1 at p21 proximal promoter. Induction of p21 mediated by silencing of HDAC4 arrested cancer cell growth *in vitro* and inhibited tumor growth in an *in vivo* human glioblastoma model (Mottet et al., 2009). HDAC4 also interacts with PLZF and represses PLZF-RARα fusion protein activity (Chauchereau et al., 2004,Yuki et al., 2004). In the prostate cancer model, HDAC4 is recruited to the nuclei of cancer cells, where it may exert an inhibitory effect on differentiation and contribute to the development of the aggressive phenotype during late stage of prostate cancer (Halkidou et al., 2004). HDAC5 and HDAC9 are significantly upregulated in high-risk medulloblastoma in comparison with low-risk medulloblastoma, and their expression is associated with poor survival (Milde et al., 2010). Higher expression of HDAC7 and HDAC9 is associated with pancreatic adenocarcinomas and poor prognosis in childhood ALL (Ouaissi et al., 2008, Moreno et al., 2010).

Class IIb deacetylase HDAC6 is linked to breast cancer. HDAC6 is expressed at significantly higher levels in breast cancer patients with small tumors and low histologic grade, and in estrogen receptor α- and progesterone receptor-positive tumors. Furthermore, patients with high levels of HDAC6 mRNA tended to be more responsive to endocrine treatment than

those with low levels, indicating that HDAC6 may be an early prognosis marker (Zhang et al., 2004,Saji et al., 2005). Overexpression of HDAC6 in MCF-7 breast cancer cells increased cell motility, suggesting a role for HDAC6 in metastases (Saji et al., 2005). HDAC6 has additional functions in integrating signaling and cytoskeleton remodeling. It is shown that cortactin, a genuine substrate of HDAC6, is overexpressed in several carcinomas (Zhang et al., 2007,Luxton & Gundersen, 2007). Therefore, HDAC6 could be a viable target for cancer therapy. There is emerging evidence that inhibiting HDAC6 mediated aggresome pathway leads to the accumulation of misfolded proteins and apoptosis in tumor cells through autophagy (Rodriguez-Gonzalez et al., 2008).

Class III deacetylases, the NAD+ dependent SIRT proteins, are also connected to cancer. For instance, SIRT1 is upregulated in human lung cancer, prostate cancer and leukemia and has been found to be downregulated in colon tumors (Reviewed in (Ropero & Esteller, 2007)). SIRT1 is also responsible for the loss of the acetylation levels of K16-H4 and K9-H3, which is common in human cancer at early cancer development (Fraga et al., 2005). Upregulation of SIRT1 expression in human cancer can also induce deregulation of key proteins, such as p53 and E2F (Chen et al., 2005,Wang et al., 2006).

5. Epigenetic agents and combinatorial therapies in cancer treatment

5.1 DNA methylation inhibitors

The most common DNMT inhibitors in clinical use, 5-azacytidine (5-aza) and 5-aza-2'-deoxycytidine (decitabine), were synthesized almost 50 years ago (Sorm et al., 1964). 5-aza is a cytidine analog. It was originally developed as a nucleoside antimetabolite that could be incorporated into nucleic acids to induce chromosome breakage and mutations, and inhibit protein synthesis by interfering with tRNA and rRNA function (Viegas-Pequignot & Dutrillaux, 1976,Cihak, 1974,Karon & Benedict, 1972). Subsequently it was shown that 5-aza incorporates into nucleic acids and covalently binds to DNMTs, leading to a rapid loss of methylation as a result of DNMT depletion (reviewed in (Christman, 2002)). Decitabine is a very structurally similar compound that was suggested as a less toxic and more specific alternative to 5-aza, as it is not integrated into RNA (Vesely & Cihak, 1977,Bouchard & Momparler, 1983)(reviewed in (Christman, 2002). In the last twenty years, 5-aza and decitabine have been extensively tested in the clinic and approved by the FDA for treatment of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML), respectively (section 5.3). However, both 5-aza and decitabine are toxic and highly unstable in aqueous solutions (reviewed in (Stresemann & Lyko, 2008). This makes them difficult to use in clinical settings, especially in solid tumors, so there is a need for other DNMT inhibitors with more favorable properties. Several new DNMT inhibitors have been designed in the last two decades, but most have minimal efficacy *in vivo*, with the exception of zebularine (reviewed in (Brueckner et al., 2007)). Zebularine is also a cytidine analog, but it is more stable and less toxic than 5-aza and decitabine. Zebularine was originally developed as a cytidine deaminase inhibitor, but was later shown to potently inhibit DNMT activity (Zhou et al., 2002) and cancer cell growth (Cheng et al., 2004,Balch et al., 2005) (reviewed in (Yoo et al., 2004). The mechanism of action is largely the same as 5-aza and decitabine, and although its IC50 is higher, lower toxicity and longer biological half-life makes it an attractive candidate for pre-clinical testing and future clinical trials.

5.2 Histone deacetylase inhibitors

HDAC inhibitors were discovered in the 1970s, when it was shown that treatment of cells with sodium butyrate led to hyperacetylation of histones (Candido et al., 1978). Sodium butyrate was found to be difficult to use clinically due to its poor pharmacological properties (Miller et al., 1987). In the following two decades, several more promising antitumor agents that inhibit HDACs were discovered. These include valproic acid (VPA), suberoylanilide hydroxamic acid (SAHA, trade name Vorinostat), Trichostatin A (TSA), and depsipeptide or FK228 (FR901228, trade name Romidepsin). All of them inhibit HDACs by binding to the active site (reviewed in (Marks & Dokmanovic, 2005), (Martinet & Bertrand, 2011)), resulting in release of epigenetic repression. HDAC inhibitors also synergize with DNA damaging treatments such as radiotherapy or chemotherapy with nucleoside analogs (Munshi et al., 2005,Chinnaiyan et al., 2005), probably because HDACs are also involved in DNA replication and DNA repair (section 2.2) (Spange et al., 2009). The three most popular HDAC inhibitors in widespread clinical use today are VPA, SAHA, and depsipeptide, while Trichostatin A is not used clinically due to toxic side effects. VPA has the longest clinical history as it has been used for treatment of epilepsy since the 1960s. It inhibits proliferation of cultured cancer cells at millimolar concentrations and shows synergistic effects in combination with decitabine or hydralazine (Table 1), and it is currently in clinical trials for multiple kinds of cancer (section 5.4). SAHA is more potent, as it was shown to induce growth inhibition, differentiation or apoptosis in cultured cancer cells (Richon et al., 1996,Butler et al., 2000) and inhibit cancer cell growth synergistically with decitabine or zebularine at micromolar concentrations (Table 1); however, it has a short biological half-life of about 2 hours (Kelly et al., 2005). Depsipeptide, discovered in 1994 (Ueda et al., 1994), is a cyclic tetrapeptide that preferentially targets class I HDACs (Furumai et al., 2002). It shows synergistic effects in combination with decitabine, zebularine, Trichostatin A, and 5-aza, and inhibits cancer cell proliferation at sub-micromolar concentrations (Table 1). Currently, SAHA and depsipeptide have been approved by FDA for treatment of cancer, while VPA is still in clinical trials (reviewed in section 5.4).

5.3 DNA methylation and HDAC inhibitors in combination in cancer cells and mouse models

DNMT inhibitors and HDAC inhibitors in combination show synergistic growth inhibition in cancer cell lines and in animal models of cancer. Table 1 summarizes combined treatments with DNMT and HDAC inhibitors in cancer cell lines, organized by cancer type, in alphabetical order. Combination treatments caused synergistic growth inhibition and/or differentiation in the majority of cancer cell lineages tested, including leukemia and lymphoma, small-cell and non-small cell lung cancer, esophageal, liver, breast, and pancreatic cancers (Table 1 for references). This growth inhibition is generally followed by apoptosis as drug dose is increased.

DNMT inhibitors and HDAC inhibitors synergistically affect chromatin state and lead to a more pronounced re-expression of epigenetically silenced tumor suppressor genes and cell cycle regulators (Cameron et al., 1999) (see Figure 1). For example, decitabine plus VPA synergistically induce NY-ESO-1 antigen in glioma cells (Oi et al., 2009), making them a target for immunotherapy. Epigenetic agents are more effective when key tumor suppressor

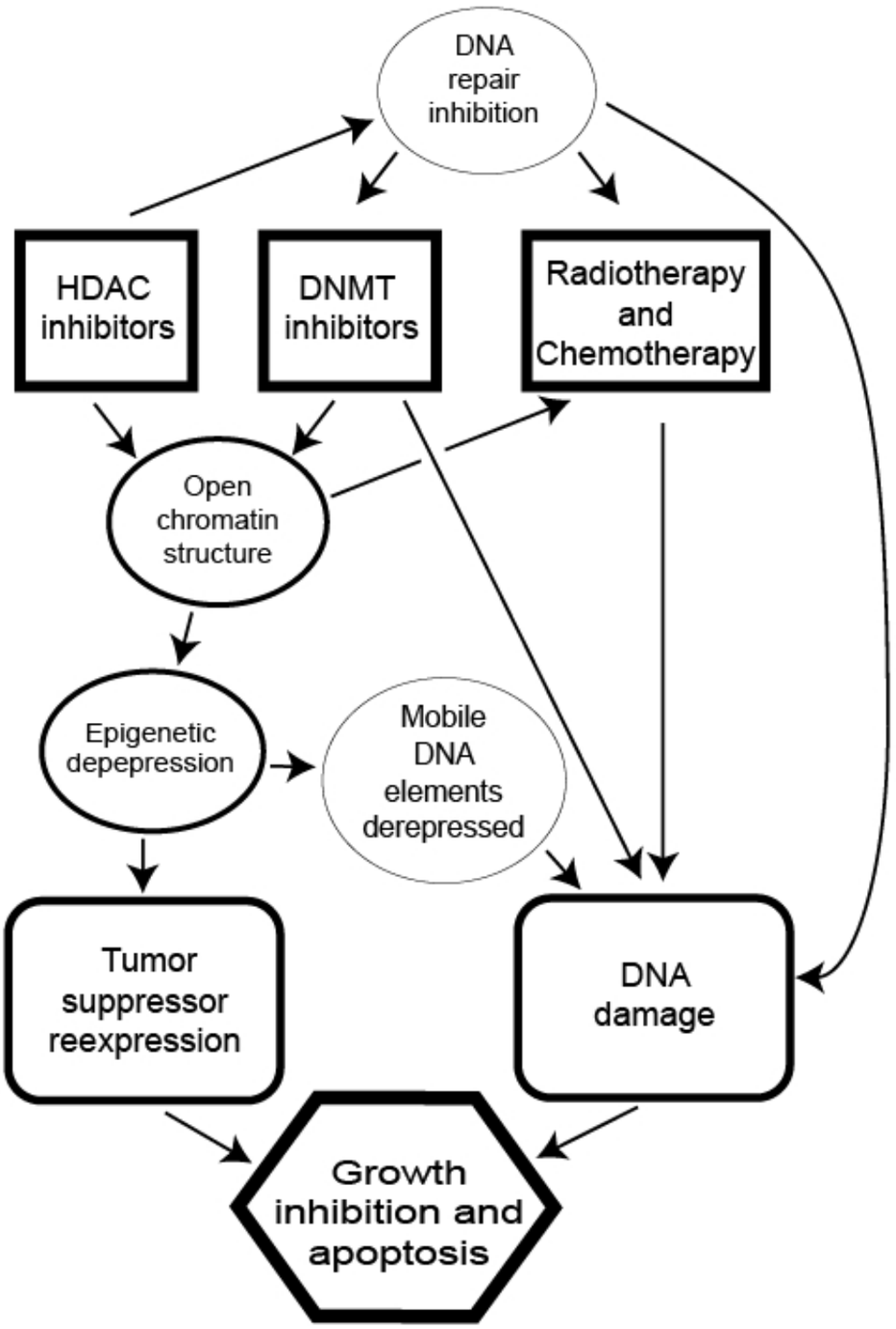


Fig. 1. HDAC and DNMT inhibitors synergistically affect chromatin state, leading to an open chromatin structure and derepression of tumor suppressors, resulting in growth inhibition and apoptosis. Open chromatin structure, as well as DNA repair inhibition induced by HDAC inhibitors, makes cells more susceptible to DNA damage induced by DNMT inhibitors, DNA damaging treatments such as radiotherapy, and epigenetic derepression of mobile DNA elements. DNA damage also leads to cell death.

genes, such as CDKN2A (p16) and p18, are epigenetically silenced (Table 1) (Gore et al., 2006), suggesting that the induction of silenced tumor suppressor genes may be critical for therapeutic effect. For example, the efficacy of DNMT inhibitors decitabine or zebularine combined with the HDAC inhibitor depsipeptide in lung and breast tumor cells with defined CDKN2A status was described recently (Chen et al., 2010). It was shown that non-small cell lung cancer cells with methylated CDKN2A are significantly more sensitive to methylation inhibitors than cell lines with deleted CDKN2A, and the combination of zebularine/depsipeptide results in a synergistic effect on cell growth inhibition that is also linked with the presence of epigenetically silenced CDKN2A (Chen et al., 2010). These data strongly support the importance of prospective pre-selection of patients in future clinical trials and suggest CDKN2A status as a key biomarker for DNMT/HDAC inhibition studies.

DNMT inhibitors and HDAC inhibitors do not necessarily act only on DNA methylation and histone acetylation, respectively, but have other functions as well (also see section 2). It was shown that HDAC inhibitors can affect DNA methylation (Sarkar et al., 2011), and DNMT inhibitors can affect histone methylation (Kondo et al., 2003). When these agents are used in combination, one inhibitor can affect epigenetic changes brought about by the other (Dobosy & Selker, 2001,Oi et al., 2009). DNMT inhibitors can also inhibit proliferation of cancer cells by causing DNA damage and chromosomal instability. Most DNMT inhibitors in widespread use are cytidine analogs (decitabine, 5-aza, zebularine, etc.) that can integrate into DNA and introduce perturbations in its normal structure leading to strand breaks (Karon & Benedict, 1972), or derepress endogenous transposons and retroviral sequences silenced by methylation (Groudine et al., 1981), which can lead to DNA damage and chromosomal instability as well (see Figure 1). These effects are likely augmented by HDAC inhibitors, which can introduce further changes into DNA structure, contribute to the activation of silenced transposons and retroviral sequences, or inhibit DNA damage repair (see Figure 1). For example, it was shown that depsipeptide can slow down removal of decitabine from the DNA (Chai et al., 2008) (possibly by inhibiting DNA repair mechanisms), suggesting an explanation for synergy between these compounds as decitabine staying on the DNA longer would lead to greater DNA demethylation and DNA damage. Also, DNA repair inhibition or chromatin structure changes may account for the fact that the interactions between DNMT inhibitors and HDAC inhibitors can depend on the order in which they are used. For example, depsipeptide treatment for 24 hours followed by 5-aza for 24 hours caused a synergistic induction of Gadd45, but this effect was not present when the compounds were used simultaneously or in the reverse order (Jiang et al., 2007).

HDAC inhibitors and DNMT inhibitors can also synergize with radiotherapy(Munshi et al., 2005,Chinnaiyan et al., 2005), CDK inhibitors (Almenara et al., 2002), TRAIL cytokine (Kaminskyy et al., 2011), and conventional chemotherapy agents such as cisplatin (Shang et al., 2008), paclitaxel, doxorubicin , and 5-fluorouracil (Mirza et al., 2010). Furthermore, they can induce a response in tumors which are resistant to chemotherapeutic agents (Plumb et al., 2000). In addition to the observation that HDAC and DNMT inhibitors can reactivate epigenetically silenced tumor suppressor genes and/or inhibit DNA repair, making conventional treatment regimens more effective, another possible mechanism behind this synergy is that the open chromatin structure induced by epigenetic agents makes DNA more accessible for chemotherapy drugs and radiation (Falk et al., 2008)(see Figure 1). These results show clinical potential for using combinations of epigenetic drugs and conventional agents, especially to overcome or prevent chemotherapy resistance (reviewed by (Gravina et al., 2010) and (Thurn et al., 2011)).

Several other conclusions can be drawn from the work summarized in Table 1. Cytidine analogs (decitabine, 5-aza, and zebularine) synergize well with HDAC inhibitors when acting on cultured cancer cells, making these combinations good candidates for future clinical trials. However, there are long-term cancer risks associated with using nucleoside analogs, as they induce DNA damage, increasing the probability of mutations. Among the HDAC inhibitors, depsipeptide is the most tested in culture over the recent years, because it preferentially inhibits class I HDACs (Furumai et al., 2002) that have been shown to be overexpressed and associated with poor prognosis in multiple kinds of cancer(Nakagawa et al., 2007,Weichert et al., 2008,Weichert et al., 2008).

The effects of epigenetic drugs on tumor growth in xenograft and in genetically engineered mouse models (GEMM) (Tables 2 and 3) are similar to the effects observed in cancer cell lines (Table 1).

Treatment of mice with DNMT1 and HDAC inhibitors causes induction of tumor suppressor genes and pro-apoptotic proteins leading to inhibition of tumor growth and apoptosis. Epigenetic agents were tested in xenografts (Table 2), and GEMMs, which more closely resemble human cancers (Becher & Holland, 2006)(Table 3). Consistent growth inhibition by epigenetic agents (Tables 2 and 3) further indicated that DMNTs and HDACs were potential targets for future clinical trials. The effects of epigenetic agents were similar using several distinct drugs and tumor models – e.g. p21 protein expression was induced with five different drugs (SAHA, MS-275, PXD101, LBH-589, decitabine) in xenograft models of breast, lung, and ovarian cancer (Table 2). CDKN1A/p21 was also induced by zebularine in a transgenic mouse model of breast cancer (Table 3). Other genes induced by epigenetic agents in mouse models included tumor suppressors such as p16 and MLH-1 and pro-apoptotic proteins Bax, caspase 3/7, and death receptor proteins 4 and 5 (Table 2). In contrast, expression of proto-oncogenes such as cyclin D1 and VEGF and anti-apoptotic proteins Bcl-2 and Bcl-XL was suppressed (Tables 2). These results are similar to what was observed in cancer cell lines, where epigenetic agents induced the expression of p16, p21, caspase 3 and MLH1, while downregulating Bcl-2 and other anti-apoptotic proteins (Table 1).

Most epigenetic agents that were tested in mouse models, e.g. decitabine, SAHA, and MS-275, are currently in clinical trials (see section 5.4). Several xenograft and GEMM studies focused on a novel DNMT inhibitor, zebularine, that is not yet approved for human use (Table 2 and 3). It has been shown that zebularine can be administered orally due to its longer half-life than other DNMT inhibitors and can inhibit tumor growth and induce expression of tumor suppressor genes (Cheng et al., 2003) and our unpublished observations (Chen et al, unpublished data). Subsequent xenograft studies showed that short-term treatment with zebularine (Dote et al., 2005) or even a single injection (Neureiter et al., 2007) can inhibit tumor growth. The GEMM studies focused on long-term therapy with an oral formulation of zebularine for intestinal adenomas (Yoo et al., 2008) and mammary tumors (Chen et al, unpublished data). In our unpublished study, high-dose zebularine treatment delayed tumor growth and reduced tumor burden in MMTV-PyMT mouse model. In the Yoo et al. study, continuous treatment of Min transgenic mice with low-dose zebularine prevented polyp formation in the majority of treated animals (while controls all developed intestinal polyps). This study makes low-dose oral zebularine an attractive target for future clinical trials.

Cancer	Drugs	Cell lines	Effects on growth	Genes affected	Ref
Brain cancer	Decitabine & VPA	U251, T98 glioma cells		Strong synergistic induction of NY-ESO-1 reexpression in both cell lines.	(Oi et al., 2009)
Breast	Decitabine & Depsipeptide	MDA-MB-231, MDA-MB-435, and MCF-7	Synergistic growth inhibition, enchanced apoptosis	Induced NY-ESO-1, Maspin, gelsolin.	{Weiser, 2001; Murakami, 2004} (Primeau et al., 2003)
	Hydralazine &VPA	MCF-7	Synergistic growth inhibition		(Chavez-Blanco et al., 2006)
	Zebularine & Depsipeptide	MDA-MB231, MDA-MB435	Synergistic growth inhibition in a cell line where CDKN2A was silenced by methylation (MDA-MB-435), but not in MDA-MB-231 cells with deleted CDKN2A.		(Chen et al., 2010)
Cervical cancer	Hydralazine & VPA	Hela	Reduced resistance to standard chemotherapy agents such as cisplatin, adrimycin or gemcitabine		(Chavez-Blanco et al., 2006)
Colon Cancer	Hydralazine & VPA	SW480	Synergistic growth inhibition.	Synergistic effect on gene reexpression (352 genes induced by the combination vs 153 and 178 by hydralazine and VPA alone, respectively)	(Chavez-Blanco et al., 2006)
	Decitabine & TSA	HCT116, HCT15, SW48		Synergistic reexpression of p16 and p14.	(Magdinier & Wolffe, 2001)
Esophageal	Decitabine & depsipeptide	BE-3, SKGT-5, TE-1, -2, -3, -12, and -13	Enhanced apoptosis after sequential treatment.	Induced NY-ESO-1(CTA) reexpression.	(Weiser et al., 2001)

Cancer	Drugs	Cell lines	Effects on growth	Genes affected	Ref
Leukemia	Decitabine & TSA/ depsiptide	HL-60, KG1a	Both combinations produced much greater growth inhibition than single agents. Colony formation assay showed synergy.		(Shaker et al., 2003)
	Decitabine & VPA	HL-60 and MOLT4 cells	Synergistic growth inhibition and apoptosis induction	Induced expression of p21CIP1 and p57KIP2.	(Yang et al., 2005)
	Decitabine & depsipeptide	Patient cells and Kasumi-1, NB-4 and K562 cells	Synergistic reduction of cell viability in all cell lines	Very strong synergistic effect inducing IL-3 expression in Kasumi-1 cells.	(Klisovic et al., 2003)
	Decitabine & MS-275	MV4-11. Leukemic blood and bone marrow patient cells	Synergistic effects inhibiting the proliferation of both cultured cell lines and patient-derived cells.	Downregulated Mcl-1 and upregulated caspase-3 and $p21^{waf1}$. Effects of the combination were reduced when caspase cascade was inhibited.	(Nishioka et al., 2011)
Liver cancer	5-aza& Depsipeptide	HepG2 carcinoma cells	Synergistic growth inhibition	Synergistic induction of Gadd45.	(Jiang et al., 2007)
Lymphoma	Decitabine & LBH589	OCI-Ly1, OCI-Ly7 and Su-DHL6 germinal center DLBCL cell lines. OCI-Ly10, RIVA and Su-DHL2 activated B-cell DLBCL lines. Primary tumor cells from patients.	All drugs had an effect on cell proliferation. DAC and LBH589 combination showed clear synergy in inhibiting proliferation and inducing apoptosis in DLBCL cell lines and CD19-positive primary tumor cells from patients.	Synergistic induction of caspase 3 expression and histone H3 acetylation. Other genes afffected: GATA1, GATA4, SMAD ,DNMT3A, ABCD3, C20orf75, CD19, CEACAM5, CHKA, DIRAS3, FUBP1, GALT, HSPC138, HSPC268, METAP1, PGM2L1, TCEB1, VHL, WT1 and ZFP95. Skp2 was down-regulated, p27 was upregulated.	(Kalac et al., 2011)

Cancer	Drugs	Cell lines	Effects on growth	Genes affected	Ref
Lung cancer: Non-small cell	Decitabine & Depsipeptide	NSLC Calu-6, H-358, H-596, H-1299, H-1355, H-1650, H-2087, and H-2228 cells; mesothelioma H-2373 cells	Enhanced apoptosis after combination treatment.	Induced NY-ESO-1 expression	(Weiser et al., 2001)
	Decitabine & TSA/ Depsipeptide	A549 and H719, cells	Combinations decreased proliferation much more than single agents.		(Chai et al., 2008)
Lung Cancer: Small-cell	Decitabine& Depsipeptide	H69, H82, H209, H211, H719, H792, H841, N417 cells	Synergistic growth inhibition	Induced expression of $p19^{INK4d}$, $p16^{INK4a}$ and $p18^{INK4c}$	(Zhu et al., 2001)
	Decitabine & LBH589 / MGCD0103	H82, H146, H196, H526, DMS114, SW1271, H1688, H1048, and H2195 cells	Both combinations caused synergistic growth inhibition and DNA damage in multiple cell lines, accompanied by an increased number of necrotic cells.		(Luszczek et al., 2010)
	Decitabine & VPA/CI-994	H69, H82, H1417, H2171, and U1906 cells	Both combinations sensitized cells to chemotherapy drug TRAIL.	The combinations synergistically induced caspase-8 and Mcl-2.	(Kaminskyy et al., 2011)
	Zebularine /Decitabine & Depsipeptide	H719 and H865 cells	Combinations synergistically inhibited growth in H719 cell line where CDKN2A is silenced by methylation	In H719 cell line, induced CDKN2A derepression and p16 protein expression.	(Chen et al., 2010)
Melanoma	Decitabine & depsipeptide	F045, 586mel, 1300mel, and 1363mel cells	Enhanced apoptosis after sequential treatment compared to either agent alone.	Combination induced NY-ESO-1(CTA) reexpression.	(Weiser et al., 2001)

Cancer	Drugs	Cell lines	Effects on growth	Genes affected
Oral	Decitabine / Depsi peptide	HO-1-u-1, HSC2, HSC3, HSC4, SAS, KB, Hep2, Ca9-22, H-O-N-1, KOSC2, KOSC3, SCC25 cells		DAC and Depsipeptide induce the expression o
Ovarian	Decitabine & PXD101	A2780/cp7 - cisplatin-resistant A2780 cells		DAC & PXD101 synerg MLH1 expression
	Decitabine & VPA	Hey, SKOv3 cells	Synergistic growth inhibition and induction of apoptosis	Synergistic induction o activity. Dose-depende ARHI and PEG3
Pancreatic Endocrine	Decitabine & TSA	CM (metastatic insulinoma), BON (metastatic carcinoid), QGP-1 (somatostatinoma) cells	Very strong synergistic effect in all three cell lines, inducing growth inhibition and then apoptosis as dose was increased.	In QGP-1 cell line TIMM downregulated and DL Cathepsin B, SET were BON cell line, VDAC1, GRB2, DEC, TIMM50 w downregulated and Ca upregulated. In CM ce LGALS1, S100A4, HSP downregulated.
Pancreatic Exocrine	Zebularine & SAHA	YAP C, DAN G and Panc-89 cells	Strong additive effect both in inducing apoptosis and inhibiting cell proliferation.	Pro-apoptotic bax / ant ratio increased after tre

Abbreviations: 5-FU – 5-fluorouracil, AML - acute myeloid leukemia, CTA – cancer testis antigen, DLBCL diffus
LBH589 – trade name Panobinostat, PXD101 – trade name Belinostat

Table 1. Effects of HDAC and DNMT inhibitors on proliferation and gene expression in cancer cell l

Combinations of DNMT and HDAC inhibitors were tested in three xenograft models and two GEMM's. Four models showed synergy between DMNT and HDAC inhibitors in the delay or reduction of tumor growth (Tables 2, 3). In addition, epigenetic agents in combination with chemotherapy drugs such as cisplatin, etoposide, daclizumab or bortezomib, enhanced the antitumor effect (Table 2). Animal studies paved the way for clinical trials using DNA methylation and HDAC inhibitors alone or in combination with chemotherapy (Section 5.4. and Tables 4, 5, 6, 7).

5.4 DNA methylation and HDAC inhibitors in clinical trials

The first epigenetic modulator in clinical use was a demethylating agent, 5-azacytidine. A phase I clinical trial was first reported in 1972, in which 30 patients with advanced solid tumors were treated with 5-azacytidine (Weiss 1972). Responses were seen in 7 of 11 patients with breast cancer, 2 of 5 patients with melanoma, and 2 of 6 patients with colon cancer. However, significant myelosuppression, nausea and diarrhea were observed. In a second study conducted in 1973, 37 children with acute leukemia were treated with 5-azacytidine (Karon 1973). Of the 14 patients with acute myelogenous leukemia (AML) enrolled in the trial, 5 achieved a complete remission (CR) lasting at least 2 months. Toxicities included myelosuppression, nausea, vomiting, diarrhea, and a transient, pruritic rash. Interest in the drug waned in the 1970s and 1980s. However, in 1993, the promising results of a single-arm phase I/II trial in patients with myelodysplastic syndrome (MDS, types refractory anemia with excess blasts (RAEB-t) and RAEB in transformation (RAEB) with 5-azacytidine were published (Silverman 1993). Out of 43 evaluable patients, the response rate was 32%, with 5 CRs (10%) and 11 partial remissions (PRs) (22%). An interesting finding was that the median time to initial response for several of these trials was 90 or more days, suggesting that longer assessments were necessary to see a significant effect on outcome. Eventually, a phase III trial was conducted, which led to its approval by the FDA for use in patients with MDS in 2004 (Table 3) (Silverman 2002). In this study, 191 patients with all French-American-British (FAB) subtypes of MDS were randomized to either best supportive care (BSC), or 5-azacytidine with BSC. The BSC-only patients could crossover to the 5-azacytidine arm if they had worsening disease after 4 months on the study. The response rate was 23% (7% CR, 16% PR), and median time to leukemic transformation or death was 21 months with 5-azacytidine, compared to 13 months for BSC ($p=0.007$). A landmark analysis to eliminate the confounding effect of early crossover to the 5-azacytidine arm showed a median survival of an additional 18 months for 5-azacytidine and 11 months for BSC ($p=0.03$). The most common adverse events were myelosuppression and infection. In a Quality of Life analysis, patients on the treatment arm, and those who crossed over, experienced a significant improvement in fatigue, dyspnea and physical functioning.

5-aza-2'-deoxycytidine (decitabine), another demethylating agent that has been extensively studied, was first used in clinical trials in patients with acute leukemia, in which 21 children and adults with acute lymphocytic leukemia (ALL) and AML were treated (Marmparler 1985). The response rate was 37%, with 6 CRs (22%) and 4 PRs (15%). There was a suggestion that the drug was more effective in AML than ALL, although the sample sizes in each group were too small. An analysis of DNA methylation by high-performance liquid

Mouse model	Drug	Effect	Molecular target	Cell line	Reference
Bladder cancer xenograft	Zebularine	Inhibited tumor growth.	Reactivated p16 expression	EJ6	(Cheng et al., 2003)
Breast cancer xenograft	Decitabine		Upregulated tumor suppressor RASSF1A, but also oncogenes uPA, HEPARANASE, CXCR4,SNCG, TGF-β and VEGF.	MCF-7	(Ateeq et al., 2008)
	SAHA	Inhibited tumor growth.	Induced p21 reexpression	MDA-231	(Pratap et al., 2010)
	SAHA plus TRAIL	Inhibited tumor growth, angiogenesis, and spread of metastases. Induced apoptosis.	Upregulated genes: DR4, DR5, Bak, Bax, Bim, Noxa, PUMA, p21CIP1, TIMP-1, and TIMP-2. Downregulated genes: cyclin D1, Bcl-2, Bcl-XL, VEGF, HIF-1α, IL-6, IL-8, MMP-2, and MMP-9	TRAIL-resistant MDA-MB-468	(Shankar et al., 2009)
	MS-275	Induced apoptosis, inhibited tumor cell proliferation, angiogenesis, spread of metastases. Reversed epithelial-to-mesenchymal transition.	Upregulated genes: DR4, DR5, Bax, Bak, and p21/CIP1. Downregulated genes: cyclin D1, Bcl-2, Bcl-XL, VEGF, HIF-1a, IL-6, IL-8, MMP-2, and MMP-9.	MDA-MB-468	(Srivastava et al., 2010)
Breast cancer Orthotopic	Panobinostat plus MD5-1	Inhibited tumor growth.		4T1.2 or EO771	(Martin et al., 2011)

Mouse model	Drug	Effect	Molecular target	Cell line	Reference
Glioblastoma xenograft	Zebularine	Inhibited tumor growth.	Upregulated genes: RASSF1A, HIC-1, and 14-3-3r	U251	(Dote et al., 2005)
	SAHA	Inhibited tumor growth and increased survival.		U87, T98G and U118	(Yin et al., 2007)
Leukemia xenograft	Decitabine & Zebularine	Increased survival.		L1210	(Lemaire et al., 2005)
	Depsipeptide &Daclizumab	Enhanced antitumor effect and survival		MET-1	(Chen et al., 2009)
	MS-275 & RAD101	Inhibited tumor growth.		HL60	(Nishioka et al., 2008)
Myeloma xenograft	SAHA plus melphalan / bortezomib	Enhanced antitumor effect.		RPMI8226, U266, and MM1S	(Campbell et al., 2010)
Myeloma orthotopic	JNJ-26481585	Inhibited tumor growth and angiogenesis.		5TMM	(Deleu et al., 2009)
	JNJ-26481585 & bortezomib	Reduced osteoclasts		5TMM	(Deleu et al., 2009)
Lung cancer orthotopic rat model	Decitabine & MS-275	Reduced tumor burden.	Upregulated Bad, Bik, Bak, Bok and p21. Demethylated genes: p16, SFRP. Downregulated DNMT1	Calu-6	(Belinsky et al., 2003)
Lung cancer xenograft and orthotopic	Panobinostat & Etoposide	Enhanced antitumor effect.	Upregulated p21, caspase 3/7 and cleaved poly[ADP-ribose] polymerase. Decreased Bcl-2 and Bcl-XL protein expression	Murine TC-1, AE17, M30, A549, H69, BK-T, H526, RG1	(Crisanti et al., 2009)
Lymphoma Xenograft	Decitabine & Panobinostat	Induced apoptosis.		Ly1 line	(Kalac et al., 2011)

Mouse model	Drug	Effect	Molecular target	C
Ovarian carcinoma xenograft	Decitabine & Belinostat	Reduced tumor growth.	Upregulated MLH1 and MAGE-A1. Demethylated MAGE-A1	A
	Decitabine & Cisplatin or Carboplatin or Epirubicin or Temozolomide	Enhanced antitumor effect.	Upregulated MLH1 gene	A
	Belinostat		Upregulated genes CDKN1A, CTGF, DHRS2, DNAJB1, H1F0, MAP1LC3B, ODC, SAT, TACC1. Downregulated genes ABL1, CTPS, EIF4G2, KPNB1, CAD, RAN, TP53, TYMS, TD-60.	A
Pancreatic cancer xenograft	Zebularine	Inhibited tumor growth.		I
	Decitabine	Inhibited tumor growth and induced apoptosis.	Increased expression of ARHI protein.	I
Sarcoma xenograft	MS—275	Inhibited tumor growth.		N r a r r
	SAHA	Reduced tumor growth and induced apoptosis.		S c r
	Decitabine	Inhibited tumor growth.	Upregulated genes: GADD45A, HSPA9B, PAWR, PDCD5, NFKBIA, TNFAIP3, IGFBP6	U

Table 2. Effects and molecular tragets of DNMT and HDAC inhibitors in xenograft or orthotopic m

Mouse tumor model	Transgene or Knock out gene	Drug	Effect	Molecul
Colorectal	APCmin	SB939	Inhibited adenoma formation	
Intestinal adenomas	Min	Zebularine	Prevented polyp formation in Min females	Demethylat repetitive e
Lung	DNMT1 +/- (heterozygous)	Decitabine plus sodium phenylbutyrate	Inhibited tumor development	
Medulloblastoma (MB) and rhabdomyosarcoma (RMS)	Ptchtm1Zim	Decitabine/ VPA	Combination prevented MB and RMS formation	
Mammary gland	MMTV-PyMT	Zebularine	Delayed onset of tumor growth; statistically significant reduction in total tumor burden.	

Table 3. Effects and molecular targets of DNMT and HDAC inhibitors in transgenic mouse tumor m

chromatography (HPLC) on 2 patient samples showed that decitabine therapy produced > 70% inhibition in DNA methylation. Decitabine was subsequently studied in patients with solid tumors (Abele 1987), where only 1 response in 82 evaluable patients was observed (1%), but significant myelosuppression was seen in many patients. A phase III trial demonstrated its efficacy compared to BSC in patients with intermediate- (INT-1 and INT-2) and high-risk MDS (Table 3), which led to its approval by the FDA in 2006. The response rate was significantly better than BSC (30% versus 7%), but there was no significant difference in median time to leukemic transformation or death (12.1 versus 7.8 months, p = 0.16). Cytopenias were again the dominant adverse effect.

In 2010, Gurion et al. performed a meta-analysis of demethylating agents for the treatment of MDS (Gurion 2010). 952 patients with MDS enrolled in 4 randomized controlled clinical trials comparing demethylating agents with either BSC or chemotherapy. Treatment with a demethylating agent significantly improved overall survival (Hazard Ratio [HR] 0.72, 95% CI 0.60-0.85) and the median time to leukemic transformation or death (HR 0.69, 95% CI 0.58-0.82). In a subgroup analysis of the type of drug, these benefits were seen for 5-azacytidine but not for decitabine. Both agents improved response rates. However, a higher rate of grade 3/4 toxicities was also noted (RR 1.21, 95% CI 1.10 to 1.33). The data, however, were not all obtained in randomized trials, and it is possible that the patients who received 5-azacytidine or decitabine had a better prognosis despite a similar International Prognostic Scoring System (IPSS) score.

Valproic acid was one of the first HDAC inhibitors investigated in clinical trials in cancer patients. However, as an anticonvulsant, it was initially explored for seizure prophylaxis (Glantz 1996) and for neuropathic pain (Hardy 2001) in cancer patients. In 2005, three articles were published, detailing the results of phase II clinical trials of VPA in combination with all-trans retinoic acid (ATRA). The three trials included one in older (age ≥ 65) poor-risk patients with AML or MDS (RAEB) (6 of 11 had hematological improvements, with platelet and erythroid response) (Pailatrino 2005), one in poor-risk patients with AML (1 of 19 evaluable patients had a minor response and 2 patients had a PR and clearance of peripheral blasts (Bug 2005)) and one in elderly patients (age ≥ 70) with AML (1 of 11 patients had CR, 2 patients had CRi (morphological CR with incomplete blood count recovery and 2 patients had hematological improvement) (Raffoux 2005). The first clinical trial in solid tumors was conducted in 12 patients with cervical cancer (Chavez-Blanco 2005). No outcomes were reported, but hyperacetylation of both H3 and H4 in 7 of 12 patients were confirmed. The main adverse effects were sedation and fatigue. While valproic acid is FDA approved for seizure control, treatment of manic episodes and migraine prophylaxis, it is not yet approved for cancer.

Vorinostat (SAHA) is one of best-studied HDAC inhibitors in clinical trials. The first clinical trial was a phase I, dose-finding trial with an intravenous formulation, administered in 2 different schedules, in a total of 37 patients with advanced cancer (hematologic and solid tumors) (Kelly 2003). The maximum tolerated dose was determined (300 mg/m^2/day and 900 mg/m^2/day for 5 days for 3 weeks for hematologic and solid malignancies, respectively). Grade 3/4 toxicities in patients with hematologic malignancies included neutropenia (grade 4), anemia, thrombocytopenia, dyspnea (grade 4), thrombosis, diarrhea, constipation and fatigue. Grade 3/4 toxicities in patients with solid tumors included cardiac

ischemia (grade 4), acute respiratory distress syndrome, thrombosis, constipation and abdominal pain. Four patients with solid tumors had an objective response. Post-therapy tumor biopsies confirmed the presence of acetylated histones. FDA approval for vorinostat was given in 2006. It was based on the results of two phase II trials (Duvic 2007, Olsen 2007) (Table 3). The pivotal trial was a phase IIB single-arm trial with 74 patients with persistent, progressive or recurrent cutaneous T-cell lymphoma (CTCL; mycosis fungoides or Sezary syndrome subtypes) who were treated with oral vorinostat (Olsen 2007). The response rate was approximately 30%. The most common adverse effects were diarrhea, fatigue, nausea and anorexia. Grade 3/4 toxicities included fatigue, pulmonary embolism, thrombocytopenia and nausea. A second trial in support of vorinostat was a three-arm, non-randomized, single-center trial in patients with refractory CTCL (Duvic 2007). Thirty-three patients were enrolled, and 8 achieved a PR. Again, the most common adverse effects were fatigue, thrombocytopenia, diarrhea and nausea, with grade 3/4 thrombocytopenia and dehydration.

Depsipeptide is another HDAC inhibitor that was recently approved by the FDA. The first clinical trial reported with this agent was a phase I trial in refractory or advanced solid tumors, in which 37 patients received escalating doses of depsipeptide intravenously on days 1 and 5 every 21 days (Sandor 2002). The dose-limiting toxicities included fatigue, nausea/vomiting, thrombocytopenia (grade 4) and cardiac arrhythmia (grade 4, atrial fibrillation). Based on preclinical data, there was a concern for cardiac toxicity, but no myocardial damage was evident, with only frequent, reversible ECG ST/T-wave changes noted. The maximum tolerated dose was determined to be 17.8 mg/m^2. One PR was observed in a patient with renal cell carcinoma, and 8 patients had stable disease. Two phase II trials led to FDA approval of depsipeptide in 2009, in patients with cutaneous T-cell lymphoma who had received at least one prior systemic therapy (Table 3). In the first trial, a multi-institutional, international, single-arm study, 96 patients were enrolled (Whittaker 2010). The response rate was 34%, and 6 patients (6%) achieved a CR. The median duration of response was 15 months. The most common adverse events were nausea, vomiting, fatigue, anorexia and infections, with serious adverse events including infection, sepsis and pyrexia. In the second trial, another multi-institutional single-arm study, a total of 71 patients were enrolled (Piekarz 2009). The response rate was 34%, with 4 CRs (6%). The median duration of response was 13.7 months. The main toxicities included nausea, vomiting, fatigue, and transient thrombocytopenia and neutropenia, anemia, and ECG T-wave changes, with serious adverse events including infection, supraventricular arrhythmia, ventricular arrhythmia, edema, pyrexia, nausea, leukopenia and thrombocytopenia. There are several other HDAC inhibitors currently in clinical trials as single agents, with similar promising results.

There are several compelling clinical reasons to use these epigenetic modalities in combination. As described above (Section 5.3), combining a DNA demethylating agent and an HDAC inhibitor appears to improve efficacy. In addition, the combination could decrease toxicity. In general, demethylating agents and HDAC inhibitors can cause significant toxicity, including grades 3 and 4 myelosuppression. The combination of the demethylating agent, decitabine, and the HDAC inhibitor, depsipeptide, was recently demonstrated to synergistically inhibit growth of lung and breast cancer cells, at 1000-fold lower doses of depsipeptide than what is used clinically (Chen 2010).

Clinical trials with demethylating agents and HDAC inhibitors in combination are still in the early stages of investigation, in phase I and II trials (Tables 4-6). At this time only eight trials have been published (Table 4). In a phase II trial with valproic acid in combination with hydralazine in 12 patients with refractory MDS, the CR/PR rate was 16%, with an overall RR of 50% (Candelaira 2011) (Table 4). The response rate includes a hematologic improvement (HI) rate of 34%, assessed per the International Working Group criteria (Cheson 2006). In comparison, a phase II trial with valproic acid in 43 MDS/AML patients showed a response in 18 patients (overall RR 24%), with no CR, 1 PR (2%) and 18 HI (22%) (Kuendgen 2005). It is important to note that the valproic acid dose in the Candelaira trial was fixed at 30 mg/kg/day, while valproic acid dose in the Kuendgen trial was titrated to valproic acid serum levels, which is more accurate. Therefore, the patients in the Candelaira trial might have been underdosed, and true outcomes in that trial may have been even better. However, while it appears that the addition of hydralazine to valproic acid might improve response, the study numbers for both trials were low, particularly the Candelaira trial (Candelaira 2011), and it would be premature to draw any conclusions about the superiority of this combination treatment.

Decitabine and valproic acid were combined in a phase I/II trial with 54 patients with MDS and AML (Garcia-Manero 2006) (Table 4). Six of the patients had MDS, of whom 3 responded (CR and PR) (50%). The number of CRs in this group was not reported. The sample size is too small to draw any conclusions about the efficacy of adding valproic acid to decitabine. Nevertheless, this combination warrants further investigation in an MDS-only population, as the response rate in a phase III trial with decitabine alone in 170 MDS patients was only 17% (Kantarjian 2006). The remaining 48 patients in the phase I/II trial had AML, among whom 9 patients experienced a response (19%). The number of CRs among AML patients was not reported. In a smaller phase I trial, 11 AML patients were also treated with decitabine and valproic acid (Blum 2007). Six patients (55%) responded, with 2 CRs (18%). As a comparison, in a phase II trial of decitabine monotherapy, limited to 55 previously-untreated older patients (age > 60), the overall response rate was 25%, with a 24% CR rate (Cashen 2010). The 19% RR for the AML patients in the phase I/II combination trial (Garcia-Manero 2006) is comparable to the 25% RR in the monotherapy trial (Cashen 2010), while the 55% RR observed in the small phase I trial (Blum 2007) is impressive. However, the interpretation of the small phase I trial in particular is limited by its small sample size (n=11).

In a study by Soriano et al. (2007), in which 5-azacytidine and valproic acid in combination with all-trans retinoic acid (ATRA) were administered to 53 AML and high-risk MDS patients, patients had impressive outcomes (Table 4). They had a RR of 42%, and a CR rate of 22%. However, another study of 5-azacytidine, valproic acid and ATRA by Raffoux et al. (Raffoux 2010) in 65 patients, also with AML and high-risk MDS, showed a markedly lower RR of 26%, with a similar CR rate of 21%. It is difficult to determine the reason for the discrepancies between these studies in response rates. They may be due to slightly different dosing schedules. In comparison to three clinical trials with valproic acid and ATRA in older and/or poor-risk patients with AML, with response rates of 11-55% (Pailatrino 2005, Bug 2005, Raffoux 2005), it does not appear that adding 5-azacytidine to valproic acid and ATRA adds to efficacy.

In solid tumors, combination therapy has not appeared to be effective. Stathis et al. did not observe any responses with decitabine and vorinostat (Stathis 2011) (Table 4). However, it was a phase I trial, and most of the 43 patients were not treated with the optimal doses (i.e.

recommended phase II doses) of the drugs. Nevertheless, the lack of response is similar to the lack of response observed with each individual agent in solid tumors. For example, Abele et al. (Abele 1987) observed only 1 response in 82 evaluable patients (1%) with advanced solid tumors treated with decitabine. Similarly, in a phase I trial in 73 patients with advanced solid tumors treated with oral vorinostat, only 1 CR (1%) and 3 PRs were noted (4%) (Kelly 2005). However, in another phase I trial with intravenous vorinostat, 17 advanced solid tumor patients were enrolled, of whom 4 had a response (23%, all PR) (Kelly 2003) (Table 4). It is unclear if this response is due to the use of intravenous vorinostat, but this method of delivery is no longer in development, and all current trials with vorinostat are using the oral form. Candelaria's 2007 trial with hydralazine, valproic acid and chemotherapy in 17 patients with solid tumors looks promising, with a 27% response rate (Table 4) (Candelaria 2007). It is especially impressive since valproic acid as monotherapy in solid tumors is not effective, as demonstrated in a phase I clinical trial in which none of 18 evaluable patients had a response (Atmaca 2007). However, it is difficult to assess the effect of chemotherapy itself. Since each patient received a different chemotherapy regimen, comparisons to historical controls with chemotherapy-only are difficult. In this trial, the authors noted that hydralazine and valproic administration permitted a lower chemotherapy dose intensity (Candelaria 2007), which might have led to lower rates of toxicity. This outcome warrants further clinical trials of chemotherapy administered sequentially after epigenetic agents.

One of the most impressive results with combination therapy appears to be the trial of hydralazine and valproic acid with chemotherapy (doxorubicin [Adriamycin]/ cyclophosphamide [AC]) as neoadjuvant therapy in 16 patients with locally-advanced breast cancer, where the response rate was 81%, with a clinical CR rate of 31% and pathologic CR rate of 6.6% (Arce-Salinas 2006) (Table 4). However, these outcomes are similar to those observed with AC chemotherapy alone in the neoadjuvant setting, with a response rate of 75%, clinical CR of 31%, and pathologic CR of 16%, albeit for patients with early breast cancer (Smith 2004). Currently, taxanes are often used with anthracyclines as neoadjuvant chemotherapy in the locally-advanced setting. The response rates range from 71 to 92%, with clinical CR rates from 17 to 31%, and pathologic CR rates 5-26% (Guarneri 2007), which are similar to the outcomes of AC with hydralazine and valproic acid (Arce-Salinas 2006), with potentially less hematologic toxicity.

To date, all of the studies with epigenetic agents in combination have been phase I or phase II trials, with sub-optimal historical controls with which we can compare response rates. Most have demonstrated transient hypomethylation or induction of histone acetylation (Table 4). Currently, there are many combination phase I and II trials in progress (Tables 6-7), using newer and potentially less toxic HDAC inhibitors, or studying the combination in non-hematologic malignancies (e.g. lung cancer). However, in order to truly assess the utility of combination epigenetic therapy, large, randomized, placebo-controlled phase III trials should be performed, directly comparing monotherapy with combination therapy, to assess overall survival, the most clinically meaningful endpoint. Furthermore, future clinical trial participation should be based on molecular biomarkers to predict which patients would respond best to the epigenetic agents. For example, it was demonstrated that a combination of a demethylating agent, zebularine, and HDAC inhibitor, depsipeptide, was only effective in tumor cells with silenced but not deleted CDKN2A (Chen 2010). A future clinical trial might focus on patients with silenced CDKN2A.

Cancer Type	Study Population	Agent	Phase	Schema	Number of Patients	Endpoints	
Lymphoma	Refractory/relapsed CTCL (stage IB to IV) who failed 2 systemic therapies	Vorinostat	IIB	Vorinostat 400 mg daily	74	RR 30% (CR 1%, PR 29%); Median Time To Progression: 4.9 mo; Median duration of response: ≥185 days	C p e t n
	CTCL (stage IIB to IV) - up to 2 prior cytotoxic therapies	Depsipeptide	II	Depsipeptide 14 mg/m2 on days 1, 8 and 15, every 28 days	71	RR 33% (CR 7%, PR 26%); Med. duration of response: 13.7 mo	C M C N f
	Refractory/relapsed CTCL (stage IB to IVA)	Depsipeptide	II	Depsipeptide 14 mg/m2 on days 1, 8 and 15, every 28 days	96	RR 34% (CR 6%, PR 28%); Med. duration of response: 15 mo	C w
MDS	MDS (all subtypes, all IPSS risk groups)	5-aza	III	5-aza (75 mg/m2/d s.c. x 7 days every 28 days) vs BSC	191	5-aza vs BSC: med OS 20 mo vs 14 mo (p = NS); Time to leukemic trans-formation/death: 21 mo vs 13 mo (p = 0.007)	5 n n
	MDS (all FAB subtypes, high-risk, INT-2, INT-1)	Decitabine	III	Decitabine (15 mg/m2 i.v. every 8 hrs for 3 days, every 6 wks) vs BSC	170	Decitabine vs BSC: med OS 14 mo vs 14.9 mo (p=NS); Time to leukemic trans-formation/death: 12.1 mo vs 7.8 mo, p=NS)	D n p h f

CTCL, cutaneous T-cell lymphoma; RR, response rate; CR, complete response; PR, partial response; mo, month; tests; GI, gastrointestinal; IPSS, International Prognostic Scoring System; BSC, best-supportive care; FAB, French intermediate-risk.

Table 4. Clinical trials leading to FDA approval of hypomethylating agents or HDAC inhibitors.

Cancer Type	Study Population	Agents	Phase	Schema	Number of Patients	Endpoints	Correlative Studies	Major Toxicities	Reference
Breast	Locally-advanced breast cancer	Hydralazine + VPA + chemo	-	Hydralazine (182 mg or 83 mg) + VPA (30 mg/kg) + chemo (AC every 21 days for 4 cycles beginning on day 7) THEN surgery	16	RR 81% (CR 31%, PR 50%); pCR 6.6% (1/15 operated pts)	Significant decrease in global methylation and HDAC activity	Grade 3/4: Myelo-suppression, tremor, somnolence, nausea, vomiting; Other toxicites: fatigue, headache, edema, diarrhea, anorexia	(Arce-Salinas et al., 2011)
Leukemia	Untreated or relapsed AML	Decitabine + VPA	I	Decitabine alone (dose-escalated; 14 pts), then Decitabine (at OBD) + VPA (dose-escalated) (11 pts)	25	RR 52% (21 pts) (CR 19%, PR 33%); Decitabine OBD 20 mg/m2/d on days 1-10; MTD VPA 20 mg/kg/d on days 5-21	Decitabine vs Decitabine+ VPA: no significant difference in histone acetylation	Neutropenic fever, fatigue, infection, pneumonia, encephalopathy, differentiation syndrome	(Karpenko et al., 2008)
MDS	Refractory MDS	Hydralazine + VPA	II	Hydralazine (182 mg [fast acetylators] or 83 mg [slow acetylators]) + VPA (30 mg/kg t.i.d.)	12	RR 16% (CR 8%, PR 8%); Progression to leukemia 16.6%	NR	No grade 3/4 toxicities; Grade 1/2 toxicities: Somnolence, nausea, headache, edema, tremor	(Juergens et al., 2008)

Cancer Type	Study Population	Agents	Phase	Schema	Number of Patients	Endpoints	Correlative Studies	Major Toxicities	Reference
MDS or Leukemia	Phase I - acute or chronic leukemia or MDS; Phase II - AML or high-risk MDS	Decitabine + VPA	I/II	Decitabine (15 mg/m2/d i.v. x 10 days) + VPA (escalating doses p.o. daily x 10 days)	54 (22 in Phase I, of whom 8 were evaluable for phase II)	VPA MTD 50 mg/kg; RR 22% (CR 19%, PR 3%); med. OS 6 mo (15.3 mo in responders); med. remission duration 7.2 mo	Transient DNA hypo-methylation and induction of histone acetylation (associated with p15 reactivation)	Non-hematologic: fatigue, somnolence, nausea, vomiting, anorexia/weight loss, diarrhea, confusion/ mental status changes, dizziness, mucositis	(Garcia-Manero et al., 2007)
	Refractory/ relapsed AML or high-risk MDS	5-aza + VPA + ATRA	I/II	5-Aza (75 mg/m2/d s.c. x 7 days every 28 days) + VPA (dose-escalated p.o. daily x 7 days) + ATRA (45 mg/m2/d p.o. x 5 days beginning on day 3)	53 (19 in phase I; 34 in phase II, including 10 pts in an expansion cohort treated at the MTD)	RR 42% (CR 22%, PR 18%); med. remission duration 26 wks	Transient decrease in global DNA methylation ($p<0.05$) and induction of histone acetylation	DLT: reversible neurotoxicity; Most common: somnolence, confusion, nausea/vomiting, fatigue, hepatic dysfunction, diarrhea	(Silverman et al., 2008)
	High-risk AML or MDS (untreated or refractory/ relapsed)	5-aza + VPA + ATRA	II	5-Aza + VPA x 7 days, then ATRA x 21 days for 6 cycles	65	RR 26% (CR 21%, PR 5%); med OS 12.4 mo; No restoration of ATRA-induced differentiation with therapy	Demethylation of FZD9, ALOX12, HPN and CALCA genes associated with clinical response;	Infection (septicemia, pneumonia), confusion, fatigue, constipation, hemorrhage, somnolence, nausea/vomiting, injection site reaction, mucosal dryness	(Raffoux et al., 2010)

Cancer Type	Study Population	Agents	Phase	Schema	Number of Patients	Endpoints	Correlative Studies
Solid tumors	Refractory solid tumors (ovarian, cervix, breast, lung, testis)	Hydralazine + VPA + chemo	II	Hydralazine (182 mg [fast acetylators] or 83 mg [slow acetylators]) + VPA (40 mg/kg p.o. daily) + chemo (beginning 1 wk after hydralazine/ VPA)	17 (15 evaluable for response)	27% (CR 0%, PR 27%), SD 53%; med OS 6.1 mo; med PFS 3.3 months	Reduction in global DNA methylation (in 6/8 evaluable pts), and HDAC activity (in 7/7 evaluable pts)
	Advanced solid tumors (25% colorectal, 9% NHL, 7% breast, 7% melanoma)	Decitabine + vorinostat	I	9 dose levels with sequential vs concurrent DAC/vorinostat	43	RP2D: Decitabine 10 mg/m2/d days 1-5 AND vorinostat 200 mg b.i.d. days 3-9; RR 0%; SD for 4+ cycles: 29%	NR

VPA, valproic acid; AC, Adriamycin/cyclophosphamide; RR, response rate; CR, complete response; PR, partial
complete response; HDAC, histone deacetylase; AML, acute myelogenous leukemia; OBD, optimal biologic dos
NR, not reported; MDS, myelodysplastic syndrome; med, median; 5-aza, 5-azacytidine; ATRA, All-trans retinoi
PFS, Progression-Free Survival; DAC, Decitabine; RP2D, recommended phase II dose

Table 5. Published trials of methylation and HDAC inhibitors in combination.

Cancer Type	Specific Population	Agents	Phase	Schema	Number of Patients	Endpoints	Correlative Studies	Major Toxicities	Reference
Breast	Operable and locally-advanced breast cancer	VPA + hydralazine + chemo	II	VPA + hydralazine vs placebo for one week, then FAC x 4 cycles then paclitaxel weekly x 12 wks, then surgery	33	VPA/hydralazine vs placebo: pCR in 3/17 pts (30%) vs 7/16 (70%) (p=NS); Inoperable pts: 3 vs 1 (p=NS)	NR	NR	(Arce-Salinas et al., 2011)
Lung	Metastatic NSCLC	Decitabine + VPA	I	Decitabine (5-15 mg/m2 on days 1-10 of 28-day cycle) and VPA (10-20 mg/kg/d on days 5-21)	8	MTD Decitabine 5 mg/m2 + VPA 10 mg/kg/d; No responses; SD 1/8 pt	Decreased MGMT methylation in 1 pt; increase in HbF levels in 5/7 pts	DLTs: Neurologic, neutropenia; Gr 3 toxicities: neutropenia, muscle weakness, pleural effusion	(Karpenko et al., 2008)
	Relapsed advanced NSCLC	5-aza + Entinostat	I	Escalating doses of 5-aza (days 1-6 and 8-10 of 28-day cycle) + Entinostat (7 mg on days 3 and 10)	10	PR 1/10 pt, SD 2/10 pt	PK and PD analysis pending	DLTs: Neutropenia, thrombocytopenia; Other toxicities: injection site reactions, nausea/ vomiting, constipation, fatigue	(Juergens et al., 2008)
MDS or AML	High-risk MDS (≥ 10% marrow blasts) or relapsed/ refractory AML	5-aza + Mocetinostat	I/II	Phase I: 5-aza 75 mg/m2 on days 1-7, Mocetinostat (escalating dose) 3X/ week starting on day 5 (28-day cycles)	24	MTD of Mocetinostat 110 mg; CR 3/14 pts, PR 4/14 pts	7/9 pts had reduction of whole cell HDAC activity	DLTs: Nausea, vomiting, anorexia,	(Garcia-Manero et al., 2007)

Cancer Type	Specific Population	Agents	Phase	Schema	Number of Patients	Endpoints	Correlative Studies
	MDS, AML	5-aza + vorinostat	I/II	Phase I: 5-aza (55 or 75 mg/m2/d on days 1-7) + Vorinostat (escalating doses)	Phase I: 20	CR 5/11 pts, PR 4/11 pts	NR
MDS, AML or PMF	Refractory/relapsed AML (53%), t-AML/MDS (18%), MDS/CMML (27%), PMF (2%)	5-aza + Belinostat	I	Part 1: 5-aza (75 mg/m2/d on days 1-5) + escalating doses of Belinostat; Part 2: 5-aza vs 5-aza/Bel (at MTD) for one cycle then all got 5-aza/Bel	56	MTD 1000 mg/m2 (max dose); CR 6/56 pts, PR 3/56	MDR1 transcripts increased 3.1-fold in 5-aza/Be vs 5-aza alone (day 0 vs 5), no change in p21 o HIST1H3H transcript levels
Solid tumors	Advanced solid tumors	5-aza + VPA	I	VPA 75-100 mcg/ml + 5-aza x 10 days (dose escalated)	47	MTD of 5-aza 75 mg/m2; SD 34%	Transient DNA hypomethylatio in all pts (median methylation 65% on day 0, 61% o day 10, 65% on day 0 of next cycle); histone acetylation in 53% of pts

VPA, valproic acid; FAC, 5-fluorouracil, doxorubicin, cyclophosphamide; pCR, pathologic complete response; p
NR, not reported; NSCLC, non-small cell lung cancer; MTD, maximum tolerated dose; SD, stable disease; MGM
methyltransferase; HgF, hemoglobin F; DLT, dose-limiting toxicity; Gr, grade; 5-aza, 5-azacytidine; PR, partial r
pharmacodynamics; MDS, myelodysplastic syndrome; AML, acute myelogenous leukemia; CR, complete respo
histone deacetylase; t-AML, therapy-related AML; CMML, chronic myelomonocytic leukemia; PMF, primary m
magnesium

Table 6. Trials in progress with preliminary results

Cancer Type	Specific Population	Agents	Phase	Schema	Planned # of Patients	Endpoints	Clinical Trials.gov Identifier
ALL or lymphoblastic lymphoma	ALL or relapsed/refractory lymphoblastic lymphoma	Decitabine &vorinostat + chemo	II	Decitabine (15 mg/m2 on days 1-4) and vorinostat (230 mg/m2 on days 1-4) THEN induction chemo	40	Remission rate at day 33 (by BM) or day 42 (by RECIST criteria), toxicity, methylation status (blood, BM) before and after treatment	NCT00882206
AML	Relapsed/refractory AML age ≥ 50 (non-APL)	5-aza & Vorinostat & Gemtu zumab	I/II	Fixed-dose 5-aza + dose-escalated Vorinostat + fixed-dose Gemtuzumab	52	MTD, CR rate, incomplete count recovery rate, DFS, whether characteristics associated with gemtuzumab efficacy predict clinical benefit	NCT00895934
Breast cancer	Advanced breast cancer (triple negative or hormone-resistant and HER2 negative)	5-aza & Entinostat	II	5-aza + Entinostat	60	RR, toxicities, PFS, OS, clinical benefit rate	NCT01349959
Colorectal cancer	Recurrent metastatic colorectal cancer	5-aza & Entinostat	II	5-aza + Entinostat	41	RR; PFS; toxicity; gene expression by qRT-PCR; changes in gene methylation, HDAC activity and acetylation of H3 and H4 histones, and their correlation with response and time to progression	NCT01105377
Lung cancer	Recurrent, advanced NSCLC	5-aza & Entinostat	I/II	5-aza (days 1-6 and 8-10 of each 28-day cycle) + Entinostat (days 3 and 10)	76	MTD, RR, toxicity, PK, PFS (1-year), OS (1-year), DNA methylation, histone acetylation, gene re-expression (blood, sputum, tissue biopsies)	NCT00387465

Cancer Type	Specific Population	Agents	Phase	Schema	Planned # of Patients	Endpoints	ClinicalTrials.gov Identifier
Lung cancer	Resected stage I NSCLC	5-aza & Entinostat	II	Randomized 5-aza + Entinostat versus BSC	258	PFS (3-year), toxicity, OS, DNA methylation, gene re-expression, predict clinical outcome in patients by GWAS on pre-treatment tumor, PFS of patients with methylated vs unmethylated N2 lymph nodes	NCT01207726
	Previously-treated advanced lung cancer	Hydralazine & VPA	I	Hydralazine (25 mg days 1-28) + VPA (titrated to serum level)	30	MTD, DLT, RR, clinical benefit, time to tumor response, TTP, OS	NCT00996060
Lymphoma	Relapsed/refractory DLBCL	5-aza & Vorinostat	I/II	Dose-escalated 5-aza + Vorinostat	32	MTD, RR	NCT01120834
MDS or AML	Patients age ≥ 60 with IPSS Int-2 or high-risk MDS, or AML	Decitabine & Panobinostat	I/II	Fixed dose Decitabine (20 mg/m2/day on days 1-5) and dose-escalated Panobinostat	66	MTD, DLT, CR rate, cytogenic CR rate with incomplete count recovery, RR, QOL, time to response, response duration, PFS, EFS, OS, toxicity	NCT00691938
MDS or AML	Newly-diagnosed MDS (IPSS Int-1, Int-2, high-risk) or AML (non-favorable cytogenetics)	5-aza & Vorinostat	II	5-aza (75 mg/m2 on days 1-5) + Vorinostat (200 mg p.o. TID on days 1-5)	30	60-day survival rate, RR, toxicity	NCT00948064

Cancer Type	Specific Population	Agents	Phase	Schema	Planned Number of Patients	Endpoints
MDS, AML or CMML	MDS (IPSS INT-2 or high-risk), CMML, AML with multi-lineage dysplasia and ≤ 30% marrow blasts	5-aza & Panobinostat	Ib (Dose-finding and Expansion phases)	Dose-finding phase: 5-aza (75 mg/m2 on days 1-7) + Panobinostat (escalating doses)	At least 9 for dose-finding phase, then 11 additional pts in expansion phase	MTD, toxici expression, status
	MDS (any IPSS), CMML (dysplastic type), AML with multilineage dysplasia (formerly RAEB-t)	5-aza & Entinostat	II	Randomized: 5-aza (days 1-10) ***or*** 5-aza (days 1-10) + Entinostat (on days 3 and 10)	196	RR (CR, PR, response), t methylation methylation PCR
Melanoma	Metastatic melanoma	Decitabine & Panobinostat & temozolomide	Ib/II	Fixed dose temozolomide & dose-escalated Decitabine & dose-escalated Panobinostat	70	Safety/toler (compared t historical co DLT, OS, ev response wi and compar conventiona

ALL, Acute Lymphblastic Leukemia; BM, bone marrow; RECIST, Response Evaluation Criteria In Solid Tumors; trial in progress; AML, acute myelogenous leukemia; APL, acute promyelocytic leukemia; 5-aza, 5-azacytidine; M complete response; DFS, disease-free survival; HER2, Human Epidermal Growth Factor Receptor 2; RR, respons survival; OS, overall survival; qRT-PCR, quantitative real-time polymerase chain reaction; HDAC, histone deace cancer; PK, pharmokinetics; GWAS, genome-wide association study; VPA, valproic acid; DLT, dose-limiting toxi DLBCL, diffuse large B-cell lymphoma; MDS, myelodysplastic syndrome; IPSS, International Prognostic Scoring QOL, quality of life; EFS, event-free survival; TID, three times a day; CMML, chronic myelomonocytic leukemia; excess blasts in transformation; PR, partial response; DTIC, dacarbazine; FDG-PET, fluorodeoxyglucose positron

Table 7. Trials in progress.

6. References

Al-Romaih, K.;Somers, G. R.;Bayani, J.;Hughes, S.;Prasad, M.;Cutz, J. C.;Xue, H.;Zielenska, M.;Wang, Y. & Squire, J. A. (2007) Modulation by decitabine of gene expression and growth of osteosarcoma U2OS cells in vitro and in xenografts: identification of apoptotic genes as targets for demethylation. *Cancer Cell Int* Vol.7, pp.14

Almenara, J.;Rosato, R. & Grant, S. (2002) Synergistic induction of mitochondrial damage and apoptosis in human leukemia cells by flavopiridol and the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). *Leukemia* Vol.16, No.7, pp.1331-1343

Angrisano, T.;Lembo, F.;Pero, R.;Natale, F.;Fusco, A.;Avvedimento, V. E.;Bruni, C. B. & Chiariotti, L. (2006) TACC3 mediates the association of MBD2 with histone acetyltransferases and relieves transcriptional repression of methylated promoters. *Nucleic Acids Res* Vol.34, No.1, pp.364-372

Arce-Salinas, C.;Dueñas-González, A.;Mohar, A.;Lara-Medina, F. U.;Perez-Sanchez, V. & Bargallo, E. (2011) Randomized, double blind, phase II trial: Epigenetic therapy (valproic acid and hydralazine) plus chemotherapy versus placebo plus neoadjuvant chemotherapy in operable and locally advanced breast cancer. *Journal of Clinical Oncology* Vol.29, No.18S, pp.e13550

Arzenani, M. K.;Zade, A. E.;Ming, Y.;Vijverberg, S. J.;Zhang, Z.;Khan, Z.;Sadique, S.;Kallenbach, L.;Hu, L.;Vukojevic, V. & Ekstrom, T. J. (2011) Genomic DNA hypomethylation by HDAC inhibition implicates DNMT1 nuclear dynamics. *Mol Cell Biol*

Ashktorab, H.;Belgrave, K.;Hosseinkhah, F.;Brim, H.;Nouraie, M.;Takkikto, M.;Hewitt, S.;Lee, E. L.;Dashwood, R. H. & Smoot, D. (2009) Global histone H4 acetylation and HDAC2 expression in colon adenoma and carcinoma. *Dig Dis Sci* Vol.54, No.10, pp.2109-2117

Ateeq, B.;Unterberger, A.;Szyf, M. & Rabbani, S. A. (2008) Pharmacological inhibition of DNA methylation induces proinvasive and prometastatic genes in vitro and in vivo. *Neoplasia* Vol.10, No.3, pp.266-278

Balasubramanian, S.;Ramos, J.;Luo, W.;Sirisawad, M.;Verner, E. & Buggy, J. J. (2008) A novel histone deacetylase 8 (HDAC8)-specific inhibitor PCI-34051 induces apoptosis in T-cell lymphomas. *Leukemia* Vol.22, No.5, pp.1026-1034

Balch, C.;Yan, P.;Craft, T.;Young, S.;Skalnik, D. G.;Huang, T. H. & Nephew, K. P. (2005) Antimitogenic and chemosensitizing effects of the methylation inhibitor zebularine in ovarian cancer. *Mol Cancer Ther* Vol.4, No.10, pp.1505-1514

Ballas, N.;Grunseich, C.;Lu, D. D.;Speh, J. C. & Mandel, G. (2005) REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* Vol.121, No.4, pp.645-657

Beaulieu, N.;Morin, S.;Chute, I. C.;Robert, M. F.;Nguyen, H. & MacLeod, A. R. (2002) An essential role for DNA methyltransferase DNMT3B in cancer cell survival. *J Biol Chem* Vol.277, No.31, pp.28176-28181

Becher, O. J. & Holland, E. C. (2006) Genetically engineered models have advantages over xenografts for preclinical studies. *Cancer Res* Vol.66, No.7, pp.3355-3358, discussion 3358-3359

Belinsky, S. A.;Nikula, K. J.;Palmisano, W. A.;Michels, R.;Saccomanno, G.;Gabrielson, E.;Baylin, S. B. & Herman, J. G. (1998) Aberrant methylation of p16(INK4a) is an

early event in lung cancer and a potential biomarker for early diagnosis. *Proc Natl Acad Sci U S A* Vol.95, No.20, pp.11891-11896

Belinsky, S. A.;Klinge, D. M.;Stidley, C. A.;Issa, J. P.;Herman, J. G.;March, T. H. & Baylin, S. B. (2003) Inhibition of DNA methylation and histone deacetylation prevents murine lung cancer. *Cancer Res* Vol.63, No.21, pp.7089-7093

Berger, S. L. (2007) The complex language of chromatin regulation during transcription. *Nature* Vol.447, No.7143, pp.407-412

Bouchard, J. & Momparler, R. L. (1983) Incorporation of 5-Aza-2'-deoxycytidine-5'-triphosphate into DNA. Interactions with mammalian DNA polymerase alpha and DNA methylase. *Mol Pharmacol* Vol.24, No.1, pp.109-114

Bouras, T.;Fu, M.;Sauve, A. A.;Wang, F.;Quong, A. A.;Perkins, N. D.;Hay, R. T.;Gu, W. & Pestell, R. G. (2005) SIRT1 deacetylation and repression of p300 involves lysine residues 1020/1024 within the cell cycle regulatory domain 1. *J Biol Chem* Vol.280, No.11, pp.10264-10276

Bourc'his, D.;Xu, G. L.;Lin, C. S.;Bollman, B. & Bestor, T. H. (2001) Dnmt3L and the establishment of maternal genomic imprints. *Science* Vol.294, No.5551, pp.2536-2539

Brueckner, B.;Kuck, D. & Lyko, F. (2007) DNA methyltransferase inhibitors for cancer therapy. *Cancer J* Vol.13, No.1, pp.17-22

Butler, L. M.;Agus, D. B.;Scher, H. I.;Higgins, B.;Rose, A.;Cordon-Cardo, C.;Thaler, H. T.;Rifkind, R. A.;Marks, P. A. & Richon, V. M. (2000) Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. *Cancer Res* Vol.60, No.18, pp.5165-5170

Cameron, E. E.;Bachman, K. E.;Myohanen, S.;Herman, J. G. & Baylin, S. B. (1999) Synergy of demethylation and histone deacetylase inhibition in the re-expression of genes silenced in cancer. *Nat Genet* Vol.21, No.1, pp.103-107

Campbell, R. A.;Sanchez, E.;Steinberg, J.;Shalitin, D.;Li, Z. W.;Chen, H. & Berenson, J. R. (2010) Vorinostat enhances the antimyeloma effects of melphalan and bortezomib. *Eur J Haematol* Vol.84, No.3, pp.201-211

Candelaria, M.;Gallardo-Rincón, D.;Arce, C.;Cetina, L.;Aguilar-Ponce, J.;Arrieta, Ó.;González-Fierro, A.;Chávez-Blanco, A.;de la Cruz-Hernández, E.;Camargo, M.;Trejo-Becerril, C.;Pérez-Cárdenas, E.;Pérez-Plasencia, C.;Taja-Chayeb, L.;Wegman-Ostrosky, T.;Revilla-Vazquez, A. & Dueñas-González, A. (2007) A phase II study of epigenetic therapy with hydralazine and magnesium valproate to overcome chemotherapy resistance in refractory solid tumors. *Annals of Oncology* Vol.18, No.9, pp.1529-1538

Candido, E. P.;Reeves, R. & Davie, J. R. (1978) Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* Vol.14, No.1, pp.105-113

Cardoso, M. C. & Leonhardt, H. (1999) DNA methyltransferase is actively retained in the cytoplasm during early development. *J Cell Biol* Vol.147, No.1, pp.25-32

Carlson, L. L.;Page, A. W. & Bestor, T. H. (1992) Properties and localization of DNA methyltransferase in preimplantation mouse embryos: implications for genomic imprinting. *Genes Dev* Vol.6, No.12B, pp.2536-2541

Cecconi, D.;Donadelli, M.;Dalla Pozza, E.;Rinalducci, S.;Zolla, L.;Scupoli, M. T.;Righetti, P. G.;Scarpa, A. & Palmieri, M. (2009) Synergistic effect of trichostatin A and 5-aza-2'-

deoxycytidine on growth inhibition of pancreatic endocrine tumour cell lines: a proteomic study. *Proteomics* Vol.9, No.7, pp.1952-1966

Chahrour, M.;Jung, S. Y.;Shaw, C.;Zhou, X.;Wong, S. T.;Qin, J. & Zoghbi, H. Y. (2008) MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* Vol.320, No.5880, pp.1224-1229

Chai, G.;Li, L.;Zhou, W.;Wu, L.;Zhao, Y.;Wang, D.;Lu, S.;Yu, Y.;Wang, H.;McNutt, M. A.;Hu, Y. G.;Chen, Y.;Yang, Y.;Wu, X.;Otterson, G. A. & Zhu, W. G. (2008) HDAC inhibitors act with 5-aza-2'-deoxycytidine to inhibit cell proliferation by suppressing removal of incorporated abases in lung cancer cells. *PLoS One* Vol.3, No.6, pp.e2445

Chauchereau, A.;Mathieu, M.;de Saintignon, J.;Ferreira, R.;Pritchard, L. L.;Mishal, Z.;Dejean, A. & Harel-Bellan, A. (2004) HDAC4 mediates transcriptional repression by the acute promyelocytic leukaemia-associated protein PLZF. *Oncogene* Vol.23, No.54, pp.8777-8784

Chavez-Blanco, A.;Perez-Plasencia, C.;Perez-Cardenas, E.;Carrasco-Legleu, C.;Rangel-Lopez, E.;Segura-Pacheco, B.;Taja-Chayeb, L.;Trejo-Becerril, C.;Gonzalez-Fierro, A.;Candelaria, M.;Cabrera, G. & Duenas-Gonzalez, A. (2006) Antineoplastic effects of the DNA methylation inhibitor hydralazine and the histone deacetylase inhibitor valproic acid in cancer cell lines. *Cancer Cell Int* Vol.6, pp.2

Chekhun, V. F.;Lukyanova, N. Y.;Kovalchuk, O.;Tryndyak, V. P. & Pogribny, I. P. (2007) Epigenetic profiling of multidrug-resistant human MCF-7 breast adenocarcinoma cells reveals novel hyper- and hypomethylated targets. *Mol Cancer Ther* Vol.6, No.3, pp.1089-1098

Chen, J.;Zhang, M.;Ju, W. & Waldmann, T. A. (2009) Effective treatment of a murine model of adult T-cell leukemia using depsipeptide and its combination with unmodified daclizumab directed toward CD25. *Blood* Vol.113, No.6, pp.1287-1293

Chen, M.;Voeller, D.;Marquez, V. E.;Kaye, F. J.;Steeg, P. S.;Giaccone, G. & Zajac-Kaye, M. (2010) Enhanced growth inhibition by combined DNA methylation/HDAC inhibitors in lung tumor cells with silenced CDKN2A. *Int J Oncol* Vol.37, No.4, pp.963-971

Chen, M. Y.;Liao, W. S.;Lu, Z.;Bornmann, W. G.;Hennessey, V.;Washington, M. N.;Rosner, G. L.;Yu, Y.;Ahmed, A. A. & Bast, R. C., Jr. (2011) Decitabine and suberoylanilide hydroxamic acid (SAHA) inhibit growth of ovarian cancer cell lines and xenografts while inducing expression of imprinted tumor suppressor genes, apoptosis, G2/M arrest, and autophagy. *Cancer*

Chen M.Y; Shabashvili D.E.;, Nawab A; Yang S. X.; Hollingshead M.; Hunter K. W.; Kaye F. J.; Hochwald S. N.; Marquez V; Steeg P & Zajac-Kaye M (2011) Induction of apoptosis and delay of tumor growth by DNA methyltransferase inhibitor in genetically engineered mouse model of breast cancer. Unpublished

Chen, T.;Ueda, Y.;Dodge, J. E.;Wang, Z. & Li, E. (2003) Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by Dnmt3a and Dnmt3b. *Mol Cell Biol* Vol.23, No.16, pp.5594-5605

Chen, T. & Li, E. (2004) Structure and function of eukaryotic DNA methyltransferases. *Curr Top Dev Biol* Vol.60, pp.55-89

Chen, T.;Hevi, S.;Gay, F.;Tsujimoto, N.;He, T.;Zhang, B.;Ueda, Y. & Li, E. (2007) Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. *Nat Genet* Vol.39, No.3, pp.391-396

Chen, W. Y.;Wang, D. H.;Yen, R. C.;Luo, J.;Gu, W. & Baylin, S. B. (2005) Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses. *Cell* Vol.123, No.3, pp.437-448

Chen, Z. X. & Riggs, A. D. (2011) DNA methylation and demethylation in mammals. *J Biol Chem* Vol.286, No.21, pp.18347-18353

Cheng, J. C.;Matsen, C. B.;Gonzales, F. A.;Ye, W.;Greer, S.;Marquez, V. E.;Jones, P. A. & Selker, E. U. (2003) Inhibition of DNA methylation and reactivation of silenced genes by zebularine. *J Natl Cancer Inst* Vol.95, No.5, pp.399-409

Cheng, J. C.;Weisenberger, D. J.;Gonzales, F. A.;Liang, G.;Xu, G. L.;Hu, Y. G.;Marquez, V. E. & Jones, P. A. (2004) Continuous zebularine treatment effectively sustains demethylation in human bladder cancer cells. *Mol Cell Biol* Vol.24, No.3, pp.1270-1278

Chinnaiyan, P.;Vallabhaneni, G.;Armstrong, E.;Huang, S. M. & Harari, P. M. (2005) Modulation of radiation response by histone deacetylase inhibition. *Int J Radiat Oncol Biol Phys* Vol.62, No.1, pp.223-229

Cho, Y.;Griswold, A.;Campbell, C. & Min, K. T. (2005) Individual histone deacetylases in Drosophila modulate transcription of distinct genes. *Genomics* Vol.86, No.5, pp.606-617

Choi, J. H.;Kwon, H. J.;Yoon, B. I.;Kim, J. H.;Han, S. U.;Joo, H. J. & Kim, D. Y. (2001) Expression profile of histone deacetylase 1 in gastric cancer tissues. *Jpn J Cancer Res* Vol.92, No.12, pp.1300-1304

Chotalia, M.;Smallwood, S. A.;Ruf, N.;Dawson, C.;Lucifero, D.;Frontera, M.;James, K.;Dean, W. & Kelsey, G. (2009) Transcription is required for establishment of germline methylation marks at imprinted genes. *Genes Dev* Vol.23, No.1, pp.105-117

Christman, J. K. (2002) 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* Vol.21, No.35, pp.5483-5495

Cihak, A. (1974) Biological effects of 5-azacytidine in eukaryotes. *Oncology* Vol.30, No.5, pp.405-422

Cohen, H. Y.;Miller, C.;Bitterman, K. J.;Wall, N. R.;Hekking, B.;Kessler, B.;Howitz, K. T.;Gorospe, M.;de Cabo, R. & Sinclair, D. A. (2004) Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* Vol.305, No.5682, pp.390-392

Crisanti, M. C.;Wallace, A. F.;Kapoor, V.;Vandermeers, F.;Dowling, M. L.;Pereira, L. P.;Coleman, K.;Campling, B. G.;Fridlender, Z. G.;Kao, G. D. & Albelda, S. M. (2009) The HDAC inhibitor panobinostat (LBH589) inhibits mesothelioma and lung cancer cells in vitro and in vivo with particular efficacy for small cell lung cancer. *Mol Cancer Ther* Vol.8, No.8, pp.2221-2231

Daniel, F. I.;Cherubini, K.;Yurgel, L. S.;de Figueiredo, M. A. & Salum, F. G. (2011) The role of epigenetic transcription repression and DNA methyltransferases in cancer. *Cancer* Vol.117, No.4, pp.677-687

David, G.;Alland, L.;Hong, S. H.;Wong, C. W.;DePinho, R. A. & Dejean, A. (1998) Histone deacetylase associated with mSin3A mediates repression by the acute promyelocytic leukemia-associated PLZF protein. *Oncogene* Vol.16, No.19, pp.2549-2556

David, G.;Neptune, M. A. & DePinho, R. A. (2002) SUMO-1 modification of histone deacetylase 1 (HDAC1) modulates its biological activities. *J Biol Chem* Vol.277, No.26, pp.23658-23663

De Smet, C.;Loriot, A. & Boon, T. (2004) Promoter-dependent mechanism leading to selective hypomethylation within the 5' region of gene MAGE-A1 in tumor cells. *Mol Cell Biol* Vol.24, No.11, pp.4781-4790

Deleu, S.;Lemaire, M.;Arts, J.;Menu, E.;Van Valckenborgh, E.;Vande Broek, I.;De Raeve, H.;Coulton, L.;Van Camp, B.;Croucher, P. & Vanderkerken, K. (2009) Bortezomib alone or in combination with the histone deacetylase inhibitor JNJ-26481585: effect on myeloma bone disease in the 5T2MM murine model of myeloma. *Cancer Res* Vol.69, No.13, pp.5307-5311

Deplus, R.;Brenner, C.;Burgers, W. A.;Putmans, P.;Kouzarides, T.;de Launoit, Y. & Fuks, F. (2002) Dnmt3L is a transcriptional repressor that recruits histone deacetylase. *Nucleic Acids Res* Vol.30, No.17, pp.3831-3838

Derks, S.;Postma, C.;Moerkerk, P. T.;van den Bosch, S. M.;Carvalho, B.;Hermsen, M. A.;Giaretti, W.;Herman, J. G.;Weijenberg, M. P.;de Bruine, A. P.;Meijer, G. A. & van Engeland, M. (2006) Promoter methylation precedes chromosomal alterations in colorectal cancer development. *Cell Oncol* Vol.28, No.5-6, pp.247-257

Dobosy, J. R. & Selker, E. U. (2001) Emerging connections between DNA methylation and histone acetylation. *Cell Mol Life Sci* Vol.58, No.5-6, pp.721-727

Dodge, J. E.;Okano, M.;Dick, F.;Tsujimoto, N.;Chen, T.;Wang, S.;Ueda, Y.;Dyson, N. & Li, E. (2005) Inactivation of Dnmt3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization. *J Biol Chem* Vol.280, No.18, pp.17986-17991

Dote, H.;Cerna, D.;Burgan, W. E.;Carter, D. J.;Cerra, M. A.;Hollingshead, M. G.;Camphausen, K. & Tofilon, P. J. (2005) Enhancement of in vitro and in vivo tumor cell radiosensitivity by the DNA methylation inhibitor zebularine. *Clin Cancer Res* Vol.11, No.12, pp.4571-4579

Dryden, S. C.;Nahhas, F. A.;Nowak, J. E.;Goustin, A. S. & Tainsky, M. A. (2003) Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol Cell Biol* Vol.23, No.9, pp.3173-3185

Du, Z.;Song, J.;Wang, Y.;Zhao, Y.;Guda, K.;Yang, S.;Kao, H. Y.;Xu, Y.;Willis, J.;Markowitz, S. D.;Sedwick, D.;Ewing, R. M. & Wang, Z. (2010) DNMT1 stability is regulated by proteins coordinating deubiquitination and acetylation-driven ubiquitination. *Sci Signal* Vol.3, No.146, pp.ra80

Duong, V.;Bret, C.;Altucci, L.;Mai, A.;Duraffourd, C.;Loubersac, J.;Harmand, P. O.;Bonnet, S.;Valente, S.;Maudelonde, T.;Cavailles, V. & Boulle, N. (2008) Specific activity of class II histone deacetylases in human breast cancer cells. *Mol Cancer Res* Vol.6, No.12, pp.1908-1919

Ecke, I.;Petry, F.;Rosenberger, A.;Tauber, S.;Monkemeyer, S.;Hess, I.;Dullin, C.;Kimmina, S.;Pirngruber, J.;Johnsen, S. A.;Uhmann, A.;Nitzki, F.;Wojnowski, L.;Schulz-Schaeffer, W.;Witt, O. & Hahn, H. (2009) Antitumor effects of a combined 5-aza-

2'deoxycytidine and valproic acid treatment on rhabdomyosarcoma and medulloblastoma in Ptch mutant mice. *Cancer Res* Vol.69, No.3, pp.887-895

Eden, A.;Gaudet, F.;Waghmare, A. & Jaenisch, R. (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* Vol.300, No.5618, pp.455

Espada, J.;Ballestar, E.;Fraga, M. F.;Villar-Garea, A.;Juarranz, A.;Stockert, J. C.;Robertson, K. D.;Fuks, F. & Esteller, M. (2004) Human DNA methyltransferase 1 is required for maintenance of the histone H3 modification pattern. *J Biol Chem* Vol.279, No.35, pp.37175-37184

Etoh, T.;Kanai, Y.;Ushijima, S.;Nakagawa, T.;Nakanishi, Y.;Sasako, M.;Kitano, S. & Hirohashi, S. (2004) Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers. *Am J Pathol* Vol.164, No.2, pp.689-699

Falk, M.;Lukasova, E. & Kozubek, S. (2008) Chromatin structure influences the sensitivity of DNA to gamma-radiation. *Biochim Biophys Acta* Vol.1783, No.12, pp.2398-2414

Farthing, C. R.;Ficz, G.;Ng, R. K.;Chan, C. F.;Andrews, S.;Dean, W.;Hemberger, M. & Reik, W. (2008) Global mapping of DNA methylation in mouse promoters reveals epigenetic reprogramming of pluripotency genes. *PLoS Genet* Vol.4, No.6, pp.e1000116

Fatemi, M. & Wade, P. A. (2006) MBD family proteins: reading the epigenetic code. *J Cell Sci* Vol.119, No.Pt 15, pp.3033-3037

Feng, Q. & Zhang, Y. (2001) The MeCP1 complex represses transcription through preferential binding, remodeling, and deacetylating methylated nucleosomes. *Genes Dev* Vol.15, No.7, pp.827-832

Ferrara, F. F.;Fazi, F.;Bianchini, A.;Padula, F.;Gelmetti, V.;Minucci, S.;Mancini, M.;Pelicci, P. G.;Lo Coco, F. & Nervi, C. (2001) Histone deacetylase-targeted treatment restores retinoic acid signaling and differentiation in acute myeloid leukemia. *Cancer Res* Vol.61, No.1, pp.2-7

Figueroa, M. E.;Lugthart, S.;Li, Y.;Erpelinck-Verschueren, C.;Deng, X.;Christos, P. J.;Schifano, E.;Booth, J.;van Putten, W.;Skrabanek, L.;Campagne, F.;Mazumdar, M.;Greally, J. M.;Valk, P. J.;Lowenberg, B.;Delwel, R. & Melnick, A. (2010) DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. *Cancer Cell* Vol.17, No.1, pp.13-27

Finkel, T.;Deng, C. X. & Mostoslavsky, R. (2009) Recent progress in the biology and physiology of sirtuins. *Nature* Vol.460, No.7255, pp.587-591

Fischle, W.;Dequiedt, F.;Hendzel, M. J.;Guenther, M. G.;Lazar, M. A.;Voelter, W. & Verdin, E. (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell* Vol.9, No.1, pp.45-57

Florl, A. R.;Lower, R.;Schmitz-Drager, B. J. & Schulz, W. A. (1999) DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. *Br J Cancer* Vol.80, No.9, pp.1312-1321

Foglietti, C.;Filocamo, G.;Cundari, E.;De Rinaldis, E.;Lahm, A.;Cortese, R. & Steinkuhler, C. (2006) Dissecting the biological functions of Drosophila histone deacetylases by RNA interference and transcriptional profiling. *J Biol Chem* Vol.281, No.26, pp.17968-17976

Fraga, M. F.;Ballestar, E.;Villar-Garea, A.;Boix-Chornet, M.;Espada, J.;Schotta, G.;Bonaldi, T.;Haydon, C.;Ropero, S.;Petrie, K.;Iyer, N. G.;Perez-Rosado, A.;Calvo, E.;Lopez, J. A.;Cano, A.;Calasanz, M. J.;Colomer, D.;Piris, M. A.;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* Vol.37, No.4, pp.391-400

Fryxell, K. J. & Zuckerkandl, E. (2000) Cytosine deamination plays a primary role in the evolution of mammalian isochores. *Mol Biol Evol* Vol.17, No.9, pp.1371-1383

Fu, M.;Liu, M.;Sauve, A. A.;Jiao, X.;Zhang, X.;Wu, X.;Powell, M. J.;Yang, T.;Gu, W.;Avantaggiati, M. L.;Pattabiraman, N.;Pestell, T. G.;Wang, F.;Quong, A. A.;Wang, C. & Pestell, R. G. (2006) Hormonal control of androgen receptor function through SIRT1. *Mol Cell Biol* Vol.26, No.21, pp.8122-8135

Furumai, R.;Matsuyama, A.;Kobashi, N.;Lee, K. H.;Nishiyama, M.;Nakajima, H.;Tanaka, A.;Komatsu, Y.;Nishino, N.;Yoshida, M. & Horinouchi, S. (2002) FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. *Cancer Res* Vol.62, No.17, pp.4916-4921

Gao, L.;Cueto, M. A.;Asselbergs, F. & Atadja, P. (2002) Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. *J Biol Chem* Vol.277, No.28, pp.25748-25755

Garcia-Manero, G.;Yang, A. S.;Klimek, V.;Luger, S.;Newsome, W. M.;Berman, N.;Patterson, T.;Maroun, C.;Li, Z.;Ward, R. & Martell, R. (2007) Phase I/II study of a novel oral isotype-selectie histone deacetylase (HDAC) inhibitor MGCD01013 in combination with azacitidine in patients (pts) with high-risk myelodysplastic syndrome (MDS) or acute myelogenous leukemia (AML). *Journal of Clinical Oncology* Vol.25, No.18S, pp.7062

Gaudet, F.;Hodgson, J. G.;Eden, A.;Jackson-Grusby, L.;Dausman, J.;Gray, J. W.;Leonhardt, H. & Jaenisch, R. (2003) Induction of tumors in mice by genomic hypomethylation. *Science* Vol.300, No.5618, pp.489-492

Geiman, T. M. & Robertson, K. D. (2002) Chromatin remodeling, histone modifications, and DNA methylation-how does it all fit together? *J Cell Biochem* Vol.87, No.2, pp.117-125

Girdwood, D.;Bumpass, D.;Vaughan, O. A.;Thain, A.;Anderson, L. A.;Snowden, A. W.;Garcia-Wilson, E.;Perkins, N. D. & Hay, R. T. (2003) P300 transcriptional repression is mediated by SUMO modification. *Mol Cell* Vol.11, No.4, pp.1043-1054

Glozak, M. A.;Sengupta, N.;Zhang, X. & Seto, E. (2005) Acetylation and deacetylation of non-histone proteins. *Gene* Vol.363, pp.15-23

Gopalakrishnan, S.;Van Emburgh, B. O. & Robertson, K. D. (2008) DNA methylation in development and human disease. *Mutat Res* Vol.647, No.1-2, pp.30-38

Gore, S. D.;Baylin, S.;Sugar, E.;Carraway, H.;Miller, C. B.;Carducci, M.;Grever, M.;Galm, O.;Dauses, T.;Karp, J. E.;Rudek, M. A.;Zhao, M.;Smith, B. D.;Manning, J.;Jiemjit, A.;Dover, G.;Mays, A.;Zwiebel, J.;Murgo, A.;Weng, L. J. & Herman, J. G. (2006) Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res* Vol.66, No.12, pp.6361-6369

Gravina, G. L.;Festuccia, C.;Marampon, F.;Popov, V. M.;Pestell, R. G.;Zani, B. M. & Tombolini, V. (2010) Biological rationale for the use of DNA methyltransferase

inhibitors as new strategy for modulation of tumor response to chemotherapy and radiation. *Mol Cancer* Vol.9, pp.305

Groudine, M.;Eisenman, R. & Weintraub, H. (1981) Chromatin structure of endogenous retroviral genes and activation by an inhibitor of DNA methylation. *Nature* Vol.292, No.5821, pp.311-317

Grozinger, C. M. & Schreiber, S. L. (2000) Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization. *Proc Natl Acad Sci U S A* Vol.97, No.14, pp.7835-7840

Grozinger, C. M. & Schreiber, S. L. (2002) Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chem Biol* Vol.9, No.1, pp.3-16

Guardiola, A. R. & Yao, T. P. (2002) Molecular cloning and characterization of a novel histone deacetylase HDAC10. *J Biol Chem* Vol.277, No.5, pp.3350-3356

Guenther, M. G.;Lane, W. S.;Fischle, W.;Verdin, E.;Lazar, M. A. & Shiekhattar, R. (2000) A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. *Genes Dev* Vol.14, No.9, pp.1048-1057

Guenther, M. G.;Barak, O. & Lazar, M. A. (2001) The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. *Mol Cell Biol* Vol.21, No.18, pp.6091-6101

Gui, C. Y.;Ngo, L.;Xu, W. S.;Richon, V. M. & Marks, P. A. (2004) Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. *Proc Natl Acad Sci U S A* Vol.101, No.5, pp.1241-1246

Guo, X.;Williams, J. G.;Schug, T. T. & Li, X. (2010) DYRK1A and DYRK3 promote cell survival through phosphorylation and activation of SIRT1. *J Biol Chem* Vol.285, No.17, pp.13223-13232

Haberland, M.;Johnson, A.;Mokalled, M. H.;Montgomery, R. L. & Olson, E. N. (2009) Genetic dissection of histone deacetylase requirement in tumor cells. *Proc Natl Acad Sci U S A* Vol.106, No.19, pp.7751-7755

Haberland, M.;Mokalled, M. H.;Montgomery, R. L. & Olson, E. N. (2009) Epigenetic control of skull morphogenesis by histone deacetylase 8. *Genes Dev* Vol.23, No.14, pp.1625-1630

Haberland, M.;Montgomery, R. L. & Olson, E. N. (2009) The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet* Vol.10, No.1, pp.32-42

Haigis, M. C. & Guarente, L. P. (2006) Mammalian sirtuins--emerging roles in physiology, aging, and calorie restriction. *Genes Dev* Vol.20, No.21, pp.2913-2921

Hajra, K. M. & Fearon, E. R. (2002) Cadherin and catenin alterations in human cancer. *Genes Chromosomes Cancer* Vol.34, No.3, pp.255-268

Halkidou, K.;Gaughan, L.;Cook, S.;Leung, H. Y.;Neal, D. E. & Robson, C. N. (2004) Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. *Prostate* Vol.59, No.2, pp.177-189

Hansen, K. D.;Timp, W.;Bravo, H. C.;Sabunciyan, S.;Langmead, B.;McDonald, O. G.;Wen, B.;Wu, H.;Liu, Y.;Diep, D.;Briem, E.;Zhang, K.;Irizarry, R. A. & Feinberg, A. P. (2011) Increased methylation variation in epigenetic domains across cancer types. *Nat Genet* Vol.43, No.8, pp.768-775

Hattori, N.;Nishino, K.;Ko, Y. G.;Hattori, N.;Ohgane, J.;Tanaka, S. & Shiota, K. (2004) Epigenetic control of mouse Oct-4 gene expression in embryonic stem cells and trophoblast stem cells. *J Biol Chem* Vol.279, No.17, pp.17063-17069

Hattori, N.;Imao, Y.;Nishino, K.;Hattori, N.;Ohgane, J.;Yagi, S.;Tanaka, S. & Shiota, K. (2007) Epigenetic regulation of Nanog gene in embryonic stem and trophoblast stem cells. *Genes Cells* Vol.12, No.3, pp.387-396

Hawes, S. E.;Stern, J. E.;Feng, Q.;Wiens, L. W.;Rasey, J. S.;Lu, H.;Kiviat, N. B. & Vesselle, H. (2010) DNA hypermethylation of tumors from non-small cell lung cancer (NSCLC) patients is associated with gender and histologic type. *Lung Cancer* Vol.69, No.2, pp.172-179

He, L. Z.;Tolentino, T.;Grayson, P.;Zhong, S.;Warrell, R. P., Jr.;Rifkind, R. A.;Marks, P. A.;Richon, V. M. & Pandolfi, P. P. (2001) Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. *J Clin Invest* Vol.108, No.9, pp.1321-1330

Hellebrekers, D. M.;Griffioen, A. W. & van Engeland, M. (2007) Dual targeting of epigenetic therapy in cancer. *Biochim Biophys Acta* Vol.1775, No.1, pp.76-91

Hendrich, B. & Bird, A. (1998) Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol* Vol.18, No.11, pp.6538-6547

Hendrich, B.;Guy, J.;Ramsahoye, B.;Wilson, V. A. & Bird, A. (2001) Closely related proteins MBD2 and MBD3 play distinctive but interacting roles in mouse development. *Genes Dev* Vol.15, No.6, pp.710-723

Hernandez-Vargas, H.;Lambert, M. P.;Le Calvez-Kelm, F.;Gouysse, G.;McKay-Chopin, S.;Tavtigian, S. V.;Scoazec, J. Y. & Herceg, Z. (2010) Hepatocellular carcinoma displays distinct DNA methylation signatures with potential as clinical predictors. *PLoS One* Vol.5, No.3, pp.e9749

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* Vol.27, No.3, pp.404-408

Hrzenjak, A.;Moinfar, F.;Kremser, M. L.;Strohmeier, B.;Petru, E.;Zatloukal, K. & Denk, H. (2010) Histone deacetylase inhibitor vorinostat suppresses the growth of uterine sarcomas in vitro and in vivo. *Mol Cancer* Vol.9, pp.49

Hu, E.;Chen, Z.;Fredrickson, T.;Zhu, Y.;Kirkpatrick, R.;Zhang, G. F.;Johanson, K.;Sung, C. M.;Liu, R. & Winkler, J. (2000) Cloning and characterization of a novel human class I histone deacetylase that functions as a transcription repressor. *J Biol Chem* Vol.275, No.20, pp.15254-15264

Huang, B. H.;Laban, M.;Leung, C. H.;Lee, L.;Lee, C. K.;Salto-Tellez, M.;Raju, G. C. & Hooi, S. C. (2005) Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/WAF1 expression, independent of histone deacetylase 1. *Cell Death Differ* Vol.12, No.4, pp.395-404

Illingworth, R.;Kerr, A.;Desousa, D.;Jorgensen, H.;Ellis, P.;Stalker, J.;Jackson, D.;Clee, C.;Plumb, R.;Rogers, J.;Humphray, S.;Cox, T.;Langford, C. & Bird, A. (2008) A novel CpG island set identifies tissue-specific methylation at developmental gene loci. *PLoS Biol* Vol.6, No.1, pp.e22

Irvine, R. A.;Lin, I. G. & Hsieh, C. L. (2002) DNA methylation has a local effect on transcription and histone acetylation. *Mol Cell Biol* Vol.22, No.19, pp.6689-6696

Jaboin, J.;Wild, J.;Hamidi, H.;Khanna, C.;Kim, C. J.;Robey, R.;Bates, S. E. & Thiele, C. J. (2002) MS-27-275, an inhibitor of histone deacetylase, has marked in vitro and in vivo antitumor activity against pediatric solid tumors. *Cancer Res* Vol.62, No.21, pp.6108-6115

Jackson-Grusby, L.;Beard, C.;Possemato, R.;Tudor, M.;Fambrough, D.;Csankovszki, G.;Dausman, J.;Lee, P.;Wilson, C.;Lander, E. & Jaenisch, R. (2001) Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nat Genet* Vol.27, No.1, pp.31-39

Januchowski, R.;Dabrowski, M.;Ofori, H. & Jagodzinski, P. P. (2007) Trichostatin A down-regulate DNA methyltransferase 1 in Jurkat T cells. *Cancer Lett* Vol.246, No.1-2, pp.313-317

Jiang, C.;Zhou, B.;Fan, K.;Heung, E.;Xue, L.;Liu, X.;Kirschbaum, M. & Yen, Y. (2007) A sequential treatment of depsipeptide followed by 5-azacytidine enhances Gadd45beta expression in hepatocellular carcinoma cells. *Anticancer Res* Vol.27, No.6B, pp.3783-3789

Jin, S. G.;Jiang, C. L.;Rauch, T.;Li, H. & Pfeifer, G. P. (2005) MBD3L2 interacts with MBD3 and components of the NuRD complex and can oppose MBD2-MeCP1-mediated methylation silencing. *J Biol Chem* Vol.280, No.13, pp.12700-12709

Jones, P. L.;Veenstra, G. J.;Wade, P. A.;Vermaak, D.;Kass, S. U.;Landsberger, N.;Strouboulis, J. & Wolffe, A. P. (1998) Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat Genet* Vol.19, No.2, pp.187-191

Juan, L. J.;Shia, W. J.;Chen, M. H.;Yang, W. M.;Seto, E.;Lin, Y. S. & Wu, C. W. (2000) Histone deacetylases specifically down-regulate p53-dependent gene activation. *J Biol Chem* Vol.275, No.27, pp.20436-20443

Juergens, R. A.;Vendetti, F.;Coleman, B.;Sebree, R. S.;Rudek, M. A.;Belinsky, S. A.;Brock, M. V.;Herman, J. G.;Baylin, S. B. & Rudin, C. M. (2008) Phase I trial of 5-azacitidine (5AC) and SNDX-275 in advanced lung cancer (NSCLC). *Journal of Clinical Oncology* Vol.26, No.15S, pp.19036

Kalac, M.;Scotto, L.;Marchi, E.;Amengual, J.;Seshan, V. E.;Bhagat, G.;Ulahannan, N.;Leshchenko, V. V.;Temkin, A. M.;Parekh, S.;Tycko, B. & O'Connor, O. A. (2011) HDAC inhibitors and decitabine are highly synergistic and associated with unique gene expression and epigenetic profiles in models of DLBCL. *Blood*

Kaludov, N. K. & Wolffe, A. P. (2000) MeCP2 driven transcriptional repression in vitro: selectivity for methylated DNA, action at a distance and contacts with the basal transcription machinery. *Nucleic Acids Res* Vol.28, No.9, pp.1921-1928

Kaminskyy, V.;Surova, O.;Vaculova, A. & Zhivotovsky, B. (2011) Combined inhibition of DNA methyltransferase and histone deacetylase restores caspase-8 expression and sensitizes SCLC cells to TRAIL. *Carcinogenesis*

Kaneda, M.;Okano, M.;Hata, K.;Sado, T.;Tsujimoto, N.;Li, E. & Sasaki, H. (2004) Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* Vol.429, No.6994, pp.900-903

Kantarjian, H.;Issa, J.-P. J.;Rosenfeld, C. S.;Bennett, J. M.;Albitar, M.;DiPersio, J.;Klimek, V.;Slack, J.;de Castro, C.;Ravandi, F.;Helmer, R.;Shen, L.;Nimer, S. D.;Leavitt, R.;Raza, A. & Saba, H. (2006) Decitabine improves patient outcomes in myelodysplastic syndromes. *Cancer* Vol.106, No.8, pp.1794-1803

Karon, M. & Benedict, W. F. (1972) Chromatid breakage: differential effect of inhibitors of DNA synthesis during G 2 phase. *Science* Vol.178, No.56, pp.62

Karpenko, M. J.;Liu, Z.;Aimiuwu, J.;Wang, L.;Wu, X.;Villalona-Calero, M. A.;Young, D.;Chan, K. K.;Grever, M. R. & Otterson, G. A. (2008) Phase I study of 5-aza-2'-deoxycytidine in combination with valproic acid in patients with NSCLC. *Journal of Clinical Oncology* Vol.26, No.15S, pp.3502

Kelly, W. K.;O'Connor, O. A.;Krug, L. M.;Chiao, J. H.;Heaney, M.;Curley, T.;MacGregore-Cortelli, B.;Tong, W.;Secrist, J. P.;Schwartz, L.;Richardson, S.;Chu, E.;Olgac, S.;Marks, P. A.;Scher, H. & Richon, V. M. (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol* Vol.23, No.17, pp.3923-3931

Kitamura, K.;Hoshi, S.;Koike, M.;Kiyoi, H.;Saito, H. & Naoe, T. (2000) Histone deacetylase inhibitor but not arsenic trioxide differentiates acute promyelocytic leukaemia cells with t(11;17) in combination with all-trans retinoic acid. *Br J Haematol* Vol.108, No.4, pp.696-702

Klisovic, M. I.;Maghraby, E. A.;Parthun, M. R.;Guimond, M.;Sklenar, A. R.;Whitman, S. P.;Chan, K. K.;Murphy, T.;Anon, J.;Archer, K. J.;Rush, L. J.;Plass, C.;Grever, M. R.;Byrd, J. C. & 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* Vol.17, No.2, pp.350-358

Kondo, Y.;Shen, L. & Issa, J. P. (2003) Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. *Mol Cell Biol* Vol.23, No.1, pp.206-215

Kondo, Y. & Issa, J. P. (2010) DNA methylation profiling in cancer. *Expert Rev Mol Med* Vol.12, pp.e23

Kudo, S. (1998) Methyl-CpG-binding protein MeCP2 represses Sp1-activated transcription of the human leukosialin gene when the promoter is methylated. *Mol Cell Biol* Vol.18, No.9, pp.5492-5499

Lagger, G.;O'Carroll, D.;Rembold, M.;Khier, H.;Tischler, J.;Weitzer, G.;Schuettengruber, B.;Hauser, C.;Brunmeir, R.;Jenuwein, T. & Seiser, C. (2002) Essential function of histone deacetylase 1 in proliferation control and CDK inhibitor repression. *Embo J* Vol.21, No.11, pp.2672-2681

Lee, H.;Rezai-Zadeh, N. & Seto, E. (2004) Negative regulation of histone deacetylase 8 activity by cyclic AMP-dependent protein kinase A. *Mol Cell Biol* Vol.24, No.2, pp.765-773

Lee, M. G.;Wynder, C.;Cooch, N. & Shiekhattar, R. (2005) An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. *Nature* Vol.437, No.7057, pp.432-435

Lei, H.;Oh, S. P.;Okano, M.;Juttermann, R.;Goss, K. A.;Jaenisch, R. & Li, E. (1996) De novo DNA cytosine methyltransferase activities in mouse embryonic stem cells. *Development* Vol.122, No.10, pp.3195-3205

Lemaire, M.;Momparler, L. F.;Bernstein, M. L.;Marquez, V. E. & Momparler, R. L. (2005) Enhancement of antineoplastic action of 5-aza-2'-deoxycytidine by zebularine on L1210 leukemia. *Anticancer Drugs* Vol.16, No.3, pp.301-308

Lembo, F.;Pero, R.;Angrisano, T.;Vitiello, C.;Iuliano, R.;Bruni, C. B. & Chiariotti, L. (2003) MBDin, a novel MBD2-interacting protein, relieves MBD2 repression potential and reactivates transcription from methylated promoters. *Mol Cell Biol* Vol.23, No.5, pp.1656-1665

Lewis, J. D.;Meehan, R. R.;Henzel, W. J.;Maurer-Fogy, I.;Jeppesen, P.;Klein, F. & Bird, A. (1992) Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* Vol.69, No.6, pp.905-914

Li, B.;Gogol, M.;Carey, M.;Lee, D.;Seidel, C. & Workman, J. L. (2007) Combined action of PHD and chromo domains directs the Rpd3S HDAC to transcribed chromatin. *Science* Vol.316, No.5827, pp.1050-1054

Li, E.;Bestor, T. H. & Jaenisch, R. (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* Vol.69, No.6, pp.915-926

Lin, R. J.;Sternsdorf, T.;Tini, M. & Evans, R. M. (2001) Transcriptional regulation in acute promyelocytic leukemia. *Oncogene* Vol.20, No.49, pp.7204-7215

Ling, Y.;Sankpal, U. T.;Robertson, A. K.;McNally, J. G.;Karpova, T. & Robertson, K. D. (2004) Modification of de novo DNA methyltransferase 3a (Dnmt3a) by SUMO-1 modulates its interaction with histone deacetylases (HDACs) and its capacity to repress transcription. *Nucleic Acids Res* Vol.32, No.2, pp.598-610

Linhart, H. G.;Lin, H.;Yamada, Y.;Moran, E.;Steine, E. J.;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* Vol.21, No.23, pp.3110-3122

Luo, J.;Su, F.;Chen, D.;Shiloh, A. & Gu, W. (2000) Deacetylation of p53 modulates its effect on cell growth and apoptosis. *Nature* Vol.408, No.6810, pp.377-381

Luszczek, W.;Cheriyath, V.;Mekhail, T. M. & Borden, E. C. (2010) Combinations of DNA methyltransferase and histone deacetylase inhibitors induce DNA damage in small cell lung cancer cells: correlation of resistance with IFN-stimulated gene expression. *Mol Cancer Ther* Vol.9, No.8, pp.2309-2321

Luxton, G. W. & Gundersen, G. G. (2007) HDAC6-pack: cortactin acetylation joins the brew. *Dev Cell* Vol.13, No.2, pp.161-162

Magdinier, F. & Wolffe, A. P. (2001) Selective association of the methyl-CpG binding protein MBD2 with the silent p14/p16 locus in human neoplasia. *Proc Natl Acad Sci U S A* Vol.98, No.9, pp.4990-4995

Marks, P. A. & Dokmanovic, M. (2005) Histone deacetylase inhibitors: discovery and development as anticancer agents. *Expert Opin Investig Drugs* Vol.14, No.12, pp.1497-1511

Martin, B. P.;Frew, A. J.;Bots, M.;Fox, S.;Long, F.;Takeda, K.;Yagita, H.;Atadja, P.;Smyth, M. J. & Johnstone, R. W. (2011) Antitumor activities and on-target toxicities mediated by a TRAIL receptor agonist following cotreatment with panobinostat. *Int J Cancer* Vol.128, No.11, pp.2735-2747

Martinet, N. & Bertrand, P. (2011) Interpreting clinical assays for histone deacetylase inhibitors. *Cancer Manag Res* Vol.3, pp.117-141

Mayer, W.;Fundele, R. & Haaf, T. (2000) Spatial separation of parental genomes during mouse interspecific (Mus musculus x M. spretus) spermiogenesis. *Chromosome Res* Vol.8, No.6, pp.555-558

Mayer, W.;Niveleau, A.;Walter, J.;Fundele, R. & Haaf, T. (2000) Demethylation of the zygotic paternal genome. *Nature* Vol.403, No.6769, pp.501-502

McKinsey, T. A.;Zhang, C. L.;Lu, J. & Olson, E. N. (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* Vol.408, No.6808, pp.106-111

Meehan, R. R.;Lewis, J. D.;McKay, S.;Kleiner, E. L. & Bird, A. P. (1989) Identification of a mammalian protein that binds specifically to DNA containing methylated CpGs. *Cell* Vol.58, No.3, pp.499-507

Meissner, A.;Mikkelsen, T. S.;Gu, H.;Wernig, M.;Hanna, J.;Sivachenko, A.;Zhang, X.;Bernstein, B. E.;Nusbaum, C.;Jaffe, D. B.;Gnirke, A.;Jaenisch, R. & Lander, E. S. (2008) Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* Vol.454, No.7205, pp.766-770

Milde, T.;Oehme, I.;Korshunov, A.;Kopp-Schneider, A.;Remke, M.;Northcott, P.;Deubzer, H. E.;Lodrini, M.;Taylor, M. D.;von Deimling, A.;Pfister, S. & Witt, O. (2010) HDAC5 and HDAC9 in medulloblastoma: novel markers for risk stratification and role in tumor cell growth. *Clin Cancer Res* Vol.16, No.12, pp.3240-3252

Miller, A. A.;Kurschel, E.;Osieka, R. & Schmidt, C. G. (1987) Clinical pharmacology of sodium butyrate in patients with acute leukemia. *Eur J Cancer Clin Oncol* Vol.23, No.9, pp.1283-1287

Minucci, S.;Nervi, C.;Lo Coco, F. & Pelicci, P. G. (2001) Histone deacetylases: a common molecular target for differentiation treatment of acute myeloid leukemias? *Oncogene* Vol.20, No.24, pp.3110-3115

Mirza, S.;Sharma, G.;Pandya, P. & Ralhan, R. (2010) Demethylating agent 5-aza-2-deoxycytidine enhances susceptibility of breast cancer cells to anticancer agents. *Mol Cell Biochem* Vol.342, No.1-2, pp.101-109

Miska, E. A.;Karlsson, C.;Langley, E.;Nielsen, S. J.;Pines, J. & Kouzarides, T. (1999) HDAC4 deacetylase associates with and represses the MEF2 transcription factor. *Embo J* Vol.18, No.18, pp.5099-5107

Mohn, F.;Weber, M.;Rebhan, M.;Roloff, T. C.;Richter, J.;Stadler, M. B.;Bibel, M. & Schubeler, D. (2008) Lineage-specific polycomb targets and de novo DNA methylation define restriction and potential of neuronal progenitors. *Mol Cell* Vol.30, No.6, pp.755-766

Monks, A.;Hose, C. D.;Pezzoli, P.;Kondapaka, S.;Vansant, G.;Petersen, K. D.;Sehested, M.;Monforte, J. & Shoemaker, R. H. (2009) Gene expression-signature of belinostat in cell lines is specific for histone deacetylase inhibitor treatment, with a corresponding signature in xenografts. *Anticancer Drugs* Vol.20, No.8, pp.682-692

Moreno, D. A.;Scrideli, C. A.;Cortez, M. A.;de Paula Queiroz, R.;Valera, E. T.;da Silva Silveira, V.;Yunes, J. A.;Brandalise, S. R. & Tone, L. G. (2010) Differential expression of HDAC3, HDAC7 and HDAC9 is associated with prognosis and survival in childhood acute lymphoblastic leukaemia. *Br J Haematol* Vol.150, No.6, pp.665-673

Mottet, D.;Pirotte, S.;Lamour, V.;Hagedorn, M.;Javerzat, S.;Bikfalvi, A.;Bellahcene, A.;Verdin, E. & Castronovo, V. (2009) HDAC4 represses p21(WAF1/Cip1) expression in human cancer cells through a Sp1-dependent, p53-independent mechanism. *Oncogene* Vol.28, No.2, pp.243-256

Munshi, A.;Kurland, J. F.;Nishikawa, T.;Tanaka, T.;Hobbs, M. L.;Tucker, S. L.;Ismail, S.;Stevens, C. & Meyn, R. E. (2005) Histone deacetylase inhibitors radiosensitize human melanoma cells by suppressing DNA repair activity. *Clin Cancer Res* Vol.11, No.13, pp.4912-4922

Murakami, J.;Asaumi, J.;Maki, Y.;Tsujigiwa, H.;Kuroda, M.;Nagai, N.;Yanagi, Y.;Inoue, T.;Kawasaki, S.;Tanaka, N.;Matsubara, N. & Kishi, K. (2004) Effects of demethylating agent 5-aza-2(')-deoxycytidine and histone deacetylase inhibitor FR901228 on maspin gene expression in oral cancer cell lines. *Oral Oncol* Vol.40, No.6, pp.597-603

Mutskov, V. J.;Farrell, C. M.;Wade, P. A.;Wolffe, A. P. & Felsenfeld, G. (2002) The barrier function of an insulator couples high histone acetylation levels with specific protection of promoter DNA from methylation. *Genes Dev* Vol.16, No.12, pp.1540-1554

Nakagawa, M.;Oda, Y.;Eguchi, T.;Aishima, S.;Yao, T.;Hosoi, F.;Basaki, Y.;Ono, M.;Kuwano, M.;Tanaka, M. & Tsuneyoshi, M. (2007) Expression profile of class I histone deacetylases in human cancer tissues. *Oncol Rep* Vol.18, No.4, pp.769-774

Nakagawa, T.;Kanai, Y.;Saito, Y.;Kitamura, T.;Kakizoe, T. & Hirohashi, S. (2003) Increased DNA methyltransferase 1 protein expression in human transitional cell carcinoma of the bladder. *J Urol* Vol.170, No.6 Pt 1, pp.2463-2466

Nakagawa, T.;Kanai, Y.;Ushijima, S.;Kitamura, T.;Kakizoe, T. & Hirohashi, S. (2005) DNA hypermethylation on multiple CpG islands associated with increased DNA methyltransferase DNMT1 protein expression during multistage urothelial carcinogenesis. *J Urol* Vol.173, No.5, pp.1767-1771

Nan, X.;Campoy, F. J. & Bird, A. (1997) MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell* Vol.88, No.4, pp.471-481

Nan, X.;Ng, H. H.;Johnson, C. A.;Laherty, C. D.;Turner, B. M.;Eisenman, R. N. & Bird, A. (1998) Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* Vol.393, No.6683, pp.386-389

Neureiter, D.;Zopf, S.;Leu, T.;Dietze, O.;Hauser-Kronberger, C.;Hahn, E. G.;Herold, C. & Ocker, M. (2007) Apoptosis, proliferation and differentiation patterns are influenced by Zebularine and SAHA in pancreatic cancer models. *Scand J Gastroenterol* Vol.42, No.1, pp.103-116

Ng, H. H.;Zhang, Y.;Hendrich, B.;Johnson, C. A.;Turner, B. M.;Erdjument-Bromage, H.;Tempst, P.;Reinberg, D. & Bird, A. (1999) MBD2 is a transcriptional repressor belonging to the MeCP1 histone deacetylase complex. *Nat Genet* Vol.23, No.1, pp.58-61

Ng, H. H. & Bird, A. (2000) Histone deacetylases: silencers for hire. *Trends Biochem Sci* Vol.25, No.3, pp.121-126

Ng, H. H.;Jeppesen, P. & Bird, A. (2000) Active repression of methylated genes by the chromosomal protein MBD1. *Mol Cell Biol* Vol.20, No.4, pp.1394-1406

Nishioka, C.;Ikezoe, T.;Yang, J.;Koeffler, H. P. & Yokoyama, A. (2008) Blockade of mTOR signaling potentiates the ability of histone deacetylase inhibitor to induce growth arrest and differentiation of acute myelogenous leukemia cells. *Leukemia* Vol.22, No.12, pp.2159-2168

Nishioka, C.;Ikezoe, T.;Yang, J.;Udaka, K. & Yokoyama, A. (2011) Simultaneous inhibition of DNA methyltransferase and histone deacetylase induces p53-independent

apoptosis via down-regulation of Mcl-1 in acute myelogenous leukemia cells. *Leuk Res* Vol.35, No.7, pp.932-939

North, B. J.;Marshall, B. L.;Borra, M. T.;Denu, J. M. & Verdin, E. (2003) The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol Cell* Vol.11, No.2, pp.437-444

Novotny-Diermayr, V.;Sangthongpitag, K.;Hu, C. Y.;Wu, X.;Sausgruber, N.;Yeo, P.;Greicius, G.;Pettersson, S.;Liang, A. L.;Loh, Y. K.;Bonday, Z.;Goh, K. C.;Hentze, H.;Hart, S.;Wang, H.;Ethirajulu, K. & Wood, J. M. (2010) SB939, a novel potent and orally active histone deacetylase inhibitor with high tumor exposure and efficacy in mouse models of colorectal cancer. *Mol Cancer Ther* Vol.9, No.3, pp.642-652

Odenike, O.;Godley, L. A.;Madzo, J.;Karrison, T.;Green, M.;Artz, A. S.;Mattison, R. J.;Yee, K. W. L.;Bennett, M.;Fulton, N.;Koval, G.;Malnassy, G.;Larson, R. A.;Ratain, M. J. & Stock, W. (2011) A phase I and pharmacodynamic (PD) study of the histone deacetylase (HDAC) inhibitor belinostat (BEL) plus azacitidine (AZC) in advanced myeloid malignancies. *Journal of Clinical Oncology* Vol.29, No.18S, pp.6521

Oh, Y. M.;Kwon, Y. E.;Kim, J. M.;Bae, S. J.;Lee, B. K.;Yoo, S. J.;Chung, C. H.;Deshaies, R. J. & Seol, J. H. (2009) Chfr is linked to tumour metastasis through the downregulation of HDAC1. *Nat Cell Biol* Vol.11, No.3, pp.295-302

Oi, S.;Natsume, A.;Ito, M.;Kondo, Y.;Shimato, S.;Maeda, Y.;Saito, K. & Wakabayashi, T. (2009) Synergistic induction of NY-ESO-1 antigen expression by a novel histone deacetylase inhibitor, valproic acid, with 5-aza-2'-deoxycytidine in glioma cells. *J Neurooncol* Vol.92, No.1, pp.15-22

Okano, M.;Bell, D. W.;Haber, D. A. & Li, E. (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* Vol.99, No.3, pp.247-257

Olsen, E. A.;Kim, Y. H.;Kuzel, T. M.;Pacheco, T. R.;Foss, F. M.;Parker, S.;Frankel, S. R.;Chen, C.;Ricker, J. L.;Arduino, J. M. & Duvic, M. (2007) Phase IIB Multicenter Trial of Vorinostat in Patients With Persistent, Progressive, or Treatment Refractory Cutaneous T-Cell Lymphoma. *Journal of Clinical Oncology* Vol.25, No.21, pp.3109-3115

Ooi, S. K.;Qiu, C.;Bernstein, E.;Li, K.;Jia, D.;Yang, Z.;Erdjument-Bromage, H.;Tempst, P.;Lin, S. P.;Allis, C. D.;Cheng, X. & Bestor, T. H. (2007) DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature* Vol.448, No.7154, pp.714-717

Oshimo, Y.;Nakayama, H.;Ito, R.;Kitadai, Y.;Yoshida, K.;Chayama, K. & Yasui, W. (2003) Promoter methylation of cyclin D2 gene in gastric carcinoma. *Int J Oncol* Vol.23, No.6, pp.1663-1670

Oswald, J.;Engemann, S.;Lane, N.;Mayer, W.;Olek, A.;Fundele, R.;Dean, W.;Reik, W. & Walter, J. (2000) Active demethylation of the paternal genome in the mouse zygote. *Curr Biol* Vol.10, No.8, pp.475-478

Ou, J. N.;Torrisani, J.;Unterberger, A.;Provencal, N.;Shikimi, K.;Karimi, M.;Ekstrom, T. J. & Szyf, M. (2007) Histone deacetylase inhibitor Trichostatin A induces global and gene-specific DNA demethylation in human cancer cell lines. *Biochem Pharmacol* Vol.73, No.9, pp.1297-1307

Ouaissi, M.;Sielezneff, I.;Silvestre, R.;Sastre, B.;Bernard, J. P.;Lafontaine, J. S.;Payan, M. J.;Dahan, L.;Pirro, N.;Seitz, J. F.;Mas, E.;Lombardo, D. & Ouaissi, A. (2008) High

histone deacetylase 7 (HDAC7) expression is significantly associated with adenocarcinomas of the pancreas. *Ann Surg Oncol* Vol.15, No.8, pp.2318-2328

Palmisano, W. A.;Divine, K. K.;Saccomanno, G.;Gilliland, F. D.;Baylin, S. B.;Herman, J. G. & Belinsky, S. A. (2000) Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res* Vol.60, No.21, pp.5954-5958

Paredes, J.;Albergaria, A.;Oliveira, J. T.;Jeronimo, C.;Milanezi, F. & Schmitt, F. C. (2005) P-cadherin overexpression is an indicator of clinical outcome in invasive breast carcinomas and is associated with CDH3 promoter hypomethylation. *Clin Cancer Res* Vol.11, No.16, pp.5869-5877

Park, S. Y.;Jun, J. A.;Jeong, K. J.;Heo, H. J.;Sohn, J. S.;Lee, H. Y.;Park, C. G. & Kang, J. (2011) Histone deacetylases 1, 6 and 8 are critical for invasion in breast cancer. *Oncol Rep* Vol.25, No.6, pp.1677-1681

Peinado, H.;Ballestar, E.;Esteller, M. & Cano, A. (2004) Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol Cell Biol* Vol.24, No.1, pp.306-319

Pereira, C. V.;Lebiedzinsk, M.;Wieckowski, M. R. & Oliveira, P. J. (2011) Regulation and protection of mitochondrial physiology by sirtuins. *Mitochondrion*

Perissi, V.;Jepsen, K.;Glass, C. K. & Rosenfeld, M. G. (2010) Deconstructing repression: evolving models of co-repressor action. *Nat Rev Genet* Vol.11, No.2, pp.109-123

Piekarz, R. L.;Frye, R.;Turner, M.;Wright, J. J.;Allen, S. L.;Kirschbaum, M. H.;Zain, J.;Prince, H. M.;Leonard, J. P.;Geskin, L. J.;Reeder, C.;Joske, D.;Figg, W. D.;Gardner, E. R.;Steinberg, S. M.;Jaffe, E. S.;Stetler-Stevenson, M.;Lade, S.;Fojo, A. T. & Bates, S. E. (2009) Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma. *J Clin Oncol* Vol.27, No.32, pp.5410-5417

Plumb, J. A.;Strathdee, G.;Sludden, J.;Kaye, S. B. & Brown, R. (2000) Reversal of drug resistance in human tumor xenografts by 2'-deoxy-5-azacytidine-induced demethylation of the hMLH1 gene promoter. *Cancer Res* Vol.60, No.21, pp.6039-6044

Pradhan, S.;Bacolla, A.;Wells, R. D. & Roberts, R. J. (1999) Recombinant human DNA (cytosine-5) methyltransferase. I. Expression, purification, and comparison of de novo and maintenance methylation. *J Biol Chem* Vol.274, No.46, pp.33002-33010

Pratap, J.;Akech, J.;Wixted, J. J.;Szabo, G.;Hussain, S.;McGee-Lawrence, M. E.;Li, X.;Bedard, K.;Dhillon, R. J.;van Wijnen, A. J.;Stein, J. L.;Stein, G. S.;Westendorf, J. J. & Lian, J. B. (2010) The histone deacetylase inhibitor, vorinostat, reduces tumor growth at the metastatic bone site and associated osteolysis, but promotes normal bone loss. *Mol Cancer Ther* Vol.9, No.12, pp.3210-3220

Primeau, M.;Gagnon, J. & Momparler, R. L. (2003) Synergistic antineoplastic action of DNA methylation inhibitor 5-AZA-2'-deoxycytidine and histone deacetylase inhibitor depsipeptide on human breast carcinoma cells. *Int J Cancer* Vol.103, No.2, pp.177-184

Pruitt, K.;Zinn, R. L.;Ohm, J. E.;McGarvey, K. M.;Kang, S. H.;Watkins, D. N.;Herman, J. G. & Baylin, S. B. (2006) Inhibition of SIRT1 reactivates silenced cancer genes without loss of promoter DNA hypermethylation. *PLoS Genet* Vol.2, No.3, pp.e40

Qiu, Y.;Zhao, Y.;Becker, M.;John, S.;Parekh, B. S.;Huang, S.;Hendarwanto, A.;Martinez, E. D.;Chen, Y.;Lu, H.;Adkins, N. L.;Stavreva, D. A.;Wiench, M.;Georgel, P. T.;Schiltz,

R. L. & Hager, G. L. (2006) HDAC1 acetylation is linked to progressive modulation of steroid receptor-induced gene transcription. *Mol Cell* Vol.22, No.5, pp.669-679

Raffoux, E.;Cras, A.;Recher, C.;Boëlle, P.-Y.;de Labarthe, A.;Turlure, P.;Marolleau, J.-P.;Reman, O.;Gardin, C.;Victor, M.;Maury, S.;Rousselot, P.;Malfuson, J.-V.;Maarek, O.;Daniel, M.-T.;Fenaux, P.;Degos, L.;Chomienne, C.;Chevret, S. & Dombret, H. (2010) Phase 2 clinical trial of 5-azacitidine, valproic acid, and all-trans retinoic acid in patients with high-risk acute myeloid leukemia or myelodysplastic syndrome. *Oncotarget* Vol.1, No.1, pp.34-42

Rai, K.;Sarkar, S.;Broadbent, T. J.;Voas, M.;Grossmann, K. F.;Nadauld, L. D.;Dehghanizadeh, S.;Hagos, F. T.;Li, Y.;Toth, R. K.;Chidester, S.;Bahr, T. M.;Johnson, W. E.;Sklow, B.;Burt, R.;Cairns, B. R. & Jones, D. A. (2010) DNA demethylase activity maintains intestinal cells in an undifferentiated state following loss of APC. *Cell* Vol.142, No.6, pp.930-942

Reik, W. & Walter, J. (2001) Genomic imprinting: parental influence on the genome. *Nat Rev Genet* Vol.2, No.1, pp.21-32

Richon, V. M.;Webb, Y.;Merger, R.;Sheppard, T.;Jursic, B.;Ngo, L.;Civoli, F.;Breslow, R.;Rifkind, R. A. & Marks, P. A. (1996) Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. *Proc Natl Acad Sci U S A* Vol.93, No.12, pp.5705-5708

Rideout, W. M., 3rd;Coetzee, G. A.;Olumi, A. F. & Jones, P. A. (1990) 5-Methylcytosine as an endogenous mutagen in the human LDL receptor and p53 genes. *Science* Vol.249, No.4974, pp.1288-1290

Robert, M. F.;Morin, S.;Beaulieu, N.;Gauthier, F.;Chute, I. C.;Barsalou, A. & MacLeod, A. R. (2003) DNMT1 is required to maintain CpG methylation and aberrant gene silencing in human cancer cells. *Nat Genet* Vol.33, No.1, pp.61-65

Robertson, K. D. (2001) DNA methylation, methyltransferases, and cancer. *Oncogene* Vol.20, No.24, pp.3139-3155

Rodriguez-Gonzalez, A.;Lin, T.;Ikeda, A. K.;Simms-Waldrip, T.;Fu, C. & Sakamoto, K. M. (2008) Role of the aggresome pathway in cancer: targeting histone deacetylase 6-dependent protein degradation. *Cancer Res* Vol.68, No.8, pp.2557-2560

Ropero, S. & Esteller, M. (2007) The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* Vol.1, No.1, pp.19-25

Rougier, N.;Bourc'his, D.;Gomes, D. M.;Niveleau, A.;Plachot, M.;Paldi, A. & Viegas-Pequignot, E. (1998) Chromosome methylation patterns during mammalian preimplantation development. *Genes Dev* Vol.12, No.14, pp.2108-2113

Rountree, M. R.;Bachman, K. E. & Baylin, S. B. (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet* Vol.25, No.3, pp.269-277

Saito, Y.;Kanai, Y.;Nakagawa, T.;Sakamoto, M.;Saito, H.;Ishii, H. & Hirohashi, S. (2003) Increased protein expression of DNA methyltransferase (DNMT) 1 is significantly correlated with the malignant potential and poor prognosis of human hepatocellular carcinomas. *Int J Cancer* Vol.105, No.4, pp.527-532

Saji, S.;Kawakami, M.;Hayashi, S.;Yoshida, N.;Hirose, M.;Horiguchi, S.;Itoh, A.;Funata, N.;Schreiber, S. L.;Yoshida, M. & Toi, M. (2005) Significance of HDAC6 regulation via estrogen signaling for cell motility and prognosis in estrogen receptor-positive breast cancer. *Oncogene* Vol.24, No.28, pp.4531-4539

Sakuma, M.;Akahira, J.;Ito, K.;Niikura, H.;Moriya, T.;Okamura, K.;Sasano, H. & Yaegashi, N. (2007) Promoter methylation status of the Cyclin D2 gene is associated with poor prognosis in human epithelial ovarian cancer. *Cancer Sci* Vol.98, No.3, pp.380-386

Sarkar, S.;Abujamra, A. L.;Loew, J. E.;Forman, L. W.;Perrine, S. P. & Faller, D. V. (2011) Histone Deacetylase Inhibitors Reverse CpG Methylation by Regulating DNMT1 through ERK Signaling. *Anticancer Res* Vol.31, No.9, pp.2723-2732

Seigneurin-Berny, D.;Verdel, A.;Curtet, S.;Lemercier, C.;Garin, J.;Rousseaux, S. & Khochbin, S. (2001) Identification of components of the murine histone deacetylase 6 complex: link between acetylation and ubiquitination signaling pathways. *Mol Cell Biol* Vol.21, No.23, pp.8035-8044

Sen, G. L.;Reuter, J. A.;Webster, D. E.;Zhu, L. & Khavari, P. A. (2010) DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* Vol.463, No.7280, pp.563-567

Sengupta, N. & Seto, E. (2004) Regulation of histone deacetylase activities. *J Cell Biochem* Vol.93, No.1, pp.57-67

Shaker, S.;Bernstein, M.;Momparler, L. F. & Momparler, R. L. (2003) Preclinical evaluation of antineoplastic activity of inhibitors of DNA methylation (5-aza-2'-deoxycytidine) and histone deacetylation (trichostatin A, depsipeptide) in combination against myeloid leukemic cells. *Leuk Res* Vol.27, No.5, pp.437-444

Shang, D.;Liu, Y.;Matsui, Y.;Ito, N.;Nishiyama, H.;Kamoto, T. & Ogawa, O. (2008) Demethylating agent 5-aza-2'-deoxycytidine enhances susceptibility of bladder transitional cell carcinoma to Cisplatin. *Urology* Vol.71, No.6, pp.1220-1225

Shankar, S.;Davis, R.;Singh, K. P.;Kurzrock, R.;Ross, D. D. & Srivastava, R. K. (2009) Suberoylanilide hydroxamic acid (Zolinza/vorinostat) sensitizes TRAIL-resistant breast cancer cells orthotopically implanted in BALB/c nude mice. *Mol Cancer Ther* Vol.8, No.6, pp.1596-1605

Sharp, A. J.;Stathaki, E.;Migliavacca, E.;Brahmachary, M.;Montgomery, S. B.;Dupre, Y. & Antonarakis, S. E. (2011) DNA methylation profiles of human active and inactive X chromosomes. *Genome Res*

Shen, L.;Kondo, Y.;Guo, Y.;Zhang, J.;Zhang, L.;Ahmed, S.;Shu, J.;Chen, X.;Waterland, R. A. & Issa, J. P. (2007) Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet* Vol.3, No.10, pp.2023-2036

Shi, Y.;Sawada, J.;Sui, G.;Affar el, B.;Whetstine, J. R.;Lan, F.;Ogawa, H.;Luke, M. P.;Nakatani, Y. & Shi, Y. (2003) Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature* Vol.422, No.6933, pp.735-738

Silverman, L. R.;Demakos, E. P.;Peterson, B. L.;Kornblith, A. B.;Holland, J. C.;Odchimar-Reissig, R.;Stone, R. M.;Nelson, D.;Powell, B. L.;DeCastro, C. M.;Ellerton, J.;Larson, R. A.;Schiffer, C. A. & Holland, J. F. (2002) Randomized Controlled Trial of Azacitidine in Patients With the Myelodysplastic Syndrome: A Study of the Cancer and Leukemia Group B. *Journal of Clinical Oncology* Vol.20, No.10, pp.2429-2440

Silverman, L. R.;Verma, A.;Odchimar-Reissig, R.;Cozza, A.;Najfeld, V.;Licht, J. D. & Zwiebel, J. A. (2008) A phase I/II study of vorinostat, an oral histone deacetylase inhibitor, in combination with azacitidine in patients with the myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Initial results of the phase I trial: A

New York Cnacer Consortium. *Journal of Clinical Oncology* Vol.2008, No.15S, pp.7000

Smith, K. M.;Dobosy, J. R.;Reifsnyder, J. E.;Rountree, M. R.;Anderson, D. C.;Green, G. R. & Selker, E. U. (2010) H2B- and H3-specific histone deacetylases are required for DNA methylation in Neurospora crassa. *Genetics* Vol.186, No.4, pp.1207-1216

Song, J.;Noh, J. H.;Lee, J. H.;Eun, J. W.;Ahn, Y. M.;Kim, S. Y.;Lee, S. H.;Park, W. S.;Yoo, N. J.;Lee, J. Y. & Nam, S. W. (2005) Increased expression of histone deacetylase 2 is found in human gastric cancer. *Apmis* Vol.113, No.4, pp.264-268

Soriano, A. O.;Braiteh, F.;Garcia-Manero, G.;Camacho, L. H.;Hong, D.;Moulder, S.;Ng, C. & Kurzrock, R. (2007) Combination of 5-azacytidine (5-AZA) and valproic acid (VPA) in advanced solid cancers: A phase I study. *Journal of Clinical Oncology* Vol.25, No.18S, pp.3547

Sorm, F.;Piskala, A.;Cihak, A. & Vesely, J. (1964) 5-Azacytidine, a new, highly effective cancerostatic. *Experientia* Vol.20, No.4, pp.202-203

Spange, S.;Wagner, T.;Heinzel, T. & Kramer, O. H. (2009) Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *Int J Biochem Cell Biol* Vol.41, No.1, pp.185-198

Spurling, C. C.;Suhl, J. A.;Boucher, N.;Nelson, C. E.;Rosenberg, D. W. & Giardina, C. (2008) The short chain fatty acid butyrate induces promoter demethylation and reactivation of RARbeta2 in colon cancer cells. *Nutr Cancer* Vol.60, No.5, pp.692-702

Srivastava, R. K.;Kurzrock, R. & Shankar, S. (2010) MS-275 sensitizes TRAIL-resistant breast cancer cells, inhibits angiogenesis and metastasis, and reverses epithelial-mesenchymal transition in vivo. *Mol Cancer Ther* Vol.9, No.12, pp.3254-3266

Stathis, A.;Hotte, S. J.;Chen, E. X.;Hirte, H. W.;Oza, A. M.;Moretto, P.;Webster, S.;Laughlin, A.;Stayner, L.-A.;McGill, S.;Wang, L.;Zhang, W.-j.;Espinoza-Delgado, I.;Holleran, J. L.;Egorin, M. J. & Siu, L. L. (2011) Phase I Study of Decitabine in Combination with Vorinostat in Patients with Advanced Solid Tumors and Non-Hodgkin's Lymphomas. *Clinical Cancer Research* Vol.17, No.6, pp.1582-1590

Steele, N.;Finn, P.;Brown, R. & Plumb, J. A. (2009) Combined inhibition of DNA methylation and histone acetylation enhances gene re-expression and drug sensitivity in vivo. *Br J Cancer* Vol.100, No.5, pp.758-763

Sterner, D. E. & Berger, S. L. (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* Vol.64, No.2, pp.435-459

Strahl, B. D. & Allis, C. D. (2000) The language of covalent histone modifications. *Nature* Vol.403, No.6765, pp.41-45

Stresemann, C. & Lyko, F. (2008) Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *Int J Cancer* Vol.123, No.1, pp.8-13

Suetake, I.;Shinozaki, F.;Miyagawa, J.;Takeshima, H. & Tajima, S. (2004) DNMT3L stimulates the DNA methylation activity of Dnmt3a and Dnmt3b through a direct interaction. *J Biol Chem* Vol.279, No.26, pp.27816-27823

Sulewska, A.;Niklinska, W.;Kozlowski, M.;Minarowski, L.;Naumnik, W.;Niklinski, J.;Dabrowska, K. & Chyczewski, L. (2007) DNA methylation in states of cell physiology and pathology. *Folia Histochem Cytobiol* Vol.45, No.3, pp.149-158

Thurn, K. T.;Thomas, S.;Moore, A. & Munster, P. N. (2011) Rational therapeutic combinations with histone deacetylase inhibitors for the treatment of cancer. *Future Oncol* Vol.7, No.2, pp.263-283

Tucker, K. L.;Talbot, D.;Lee, M. A.;Leonhardt, H. & Jaenisch, R. (1996) Complementation of methylation deficiency in embryonic stem cells by a DNA methyltransferase minigene. *Proc Natl Acad Sci U S A* Vol.93, No.23, pp.12920-12925

Ueda, H.;Nakajima, H.;Hori, Y.;Fujita, T.;Nishimura, M.;Goto, T. & Okuhara, M. (1994) FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. I. Taxonomy, fermentation, isolation, physico-chemical and biological properties, and antitumor activity. *J Antibiot (Tokyo)* Vol.47, No.3, pp.301-310

Uhl, M.;Csernok, A.;Aydin, S.;Kreienberg, R.;Wiesmuller, L. & Gatz, S. A. (2010) Role of SIRT1 in homologous recombination. *DNA Repair (Amst)* Vol.9, No.4, pp.383-393

Vaquero, A.;Scher, M.;Lee, D.;Erdjument-Bromage, H.;Tempst, P. & Reinberg, D. (2004) Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin. *Mol Cell* Vol.16, No.1, pp.93-105

Vaziri, H.;Dessain, S. K.;Ng Eaton, E.;Imai, S. I.;Frye, R. A.;Pandita, T. K.;Guarente, L. & Weinberg, R. A. (2001) hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell* Vol.107, No.2, pp.149-159

Vesely, J. & Cihak, A. (1977) Incorporation of a potent antileukemic agent, 5-aza-2'-deoxycytidine, into DNA of cells from leukemic mice. *Cancer Res* Vol.37, No.10, pp.3684-3689

Viegas-Pequignot, E. & Dutrillaux, B. (1976) Segmentation of human chromosomes induced by 5-ACR (5-azacytidine). *Hum Genet* Vol.34, No.3, pp.247-254

Wade, P. A.;Jones, P. L.;Vermaak, D. & Wolffe, A. P. (1998) A multiple subunit Mi-2 histone deacetylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. *Curr Biol* Vol.8, No.14, pp.843-846

Waltregny, D.;De Leval, L.;Glenisson, W.;Ly Tran, S.;North, B. J.;Bellahcene, A.;Weidle, U.;Verdin, E. & Castronovo, V. (2004) Expression of histone deacetylase 8, a class I histone deacetylase, is restricted to cells showing smooth muscle differentiation in normal human tissues. *Am J Pathol* Vol.165, No.2, pp.553-564

Wang, A. H. & Yang, X. J. (2001) Histone deacetylase 4 possesses intrinsic nuclear import and export signals. *Mol Cell Biol* Vol.21, No.17, pp.5992-6005

Wang, C.;Chen, L.;Hou, X.;Li, Z.;Kabra, N.;Ma, Y.;Nemoto, S.;Finkel, T.;Gu, W.;Cress, W. D. & Chen, J. (2006) Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage. *Nat Cell Biol* Vol.8, No.9, pp.1025-1031

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* Vol.138, No.4, pp.660-672

Wang, Z.;Zang, C.;Cui, K.;Schones, D. E.;Barski, A.;Peng, W. & Zhao, K. (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. *Cell* Vol.138, No.5, pp.1019-1031

Weichert, W.;Roske, A.;Gekeler, V.;Beckers, T.;Ebert, M. P.;Pross, M.;Dietel, M.;Denkert, C. & Rocken, C. (2008) Association of patterns of class I histone deacetylase expression

with patient prognosis in gastric cancer: a retrospective analysis. *Lancet Oncol* Vol.9, No.2, pp.139-148

Weichert, W.;Roske, A.;Niesporek, S.;Noske, A.;Buckendahl, A. C.;Dietel, M.;Gekeler, V.;Boehm, M.;Beckers, T. & Denkert, C. (2008) Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo. *Clin Cancer Res* Vol.14, No.6, pp.1669-1677

Weiser, T. S.;Guo, Z. S.;Ohnmacht, G. A.;Parkhurst, M. L.;Tong-On, P.;Marincola, F. M.;Fischette, M. R.;Yu, X.;Chen, G. A.;Hong, J. A.;Stewart, J. H.;Nguyen, D. M.;Rosenberg, S. A. & Schrump, D. S. (2001) Sequential 5-Aza-2 deoxycytidine-depsipeptide FR901228 treatment induces apoptosis preferentially in cancer cells and facilitates their recognition by cytolytic T lymphocytes specific for NY-ESO-1. *J Immunother* Vol.24, No.2, pp.151-161

Wen, Y. D.;Perissi, V.;Staszewski, L. M.;Yang, W. M.;Krones, A.;Glass, C. K.;Rosenfeld, M. G. & Seto, E. (2000) The histone deacetylase-3 complex contains nuclear receptor corepressors. *Proc Natl Acad Sci U S A* Vol.97, No.13, pp.7202-7207

Wermann, H.;Stoop, H.;Gillis, A. J.;Honecker, F.;van Gurp, R. J.;Ammerpohl, O.;Richter, J.;Oosterhuis, J. W.;Bokemeyer, C. & Looijenga, L. H. (2010) Global DNA methylation in fetal human germ cells and germ cell tumours: association with differentiation and cisplatin resistance. *J Pathol* Vol.221, No.4, pp.433-442

Whittaker, S. J.;Demierre, M.-F.;Kim, E. J.;Rook, A. H.;Lerner, A.;Duvic, M.;Scarisbrick, J.;Reddy, S.;Robak, T.;Becker, J. C.;Samtsov, A.;McCulloch, W. & Kim, Y. H. (2010) Final Results From a Multicenter, International, Pivotal Study of Romidepsin in Refractory Cutaneous T-Cell Lymphoma. *Journal of Clinical Oncology* Vol.28, No.29, pp.4485-4491

Wilson, A. J.;Byun, D. S.;Popova, N.;Murray, L. B.;L'Italien, K.;Sowa, Y.;Arango, D.;Velcich, A.;Augenlicht, L. H. & Mariadason, J. M. (2006) Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. *J Biol Chem* Vol.281, No.19, pp.13548-13558

Woodage, T.;Basrai, M. A.;Baxevanis, A. D.;Hieter, P. & Collins, F. S. (1997) Characterization of the CHD family of proteins. *Proc Natl Acad Sci U S A* Vol.94, No.21, pp.11472-11477

Worthley, D. L.;Whitehall, V. L.;Buttenshaw, R. L.;Irahara, N.;Greco, S. A.;Ramsnes, I.;Mallitt, K. A.;Le Leu, R. K.;Winter, J.;Hu, Y.;Ogino, S.;Young, G. P. & Leggett, B. A. (2010) DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. *Oncogene* Vol.29, No.11, pp.1653-1662

Wu, H.;Coskun, V.;Tao, J.;Xie, W.;Ge, W.;Yoshikawa, K.;Li, E.;Zhang, Y. & Sun, Y. E. (2010) Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes. *Science* Vol.329, No.5990, pp.444-448

Yang, H.;Hoshino, K.;Sanchez-Gonzalez, B.;Kantarjian, H. & Garcia-Manero, G. (2005) Antileukemia activity of the combination of 5-aza-2'-deoxycytidine with valproic acid. *Leuk Res* Vol.29, No.7, pp.739-748

Yang, H.;Lu, X.;Qian, J.;Xu, F.;Hu, Y.;Yu, Y.;Bast, R. C. & Li, J. (2010) Imprinted tumor suppressor gene ARHI induces apoptosis correlated with changes in DNA methylation in pancreatic cancer cells. *Mol Med Report* Vol.3, No.4, pp.581-587

Yang, X. J. & Gregoire, S. (2005) Class II histone deacetylases: from sequence to function, regulation, and clinical implication. *Mol Cell Biol* Vol.25, No.8, pp.2873-2884

Yang, X. J. & Seto, E. (2007) HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* Vol.26, No.37, pp.5310-5318

Yang, X. J. & Seto, E. (2008) The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat Rev Mol Cell Biol* Vol.9, No.3, pp.206-218

Yasui, W.;Oue, N.;Ono, S.;Mitani, Y.;Ito, R. & Nakayama, H. (2003) Histone acetylation and gastrointestinal carcinogenesis. *Ann N Y Acad Sci* Vol.983, pp.220-231

Yeung, F.;Hoberg, J. E.;Ramsey, C. S.;Keller, M. D.;Jones, D. R.;Frye, R. A. & Mayo, M. W. (2004) Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *Embo J* Vol.23, No.12, pp.2369-2380

Yin, D.;Ong, J. M.;Hu, J.;Desmond, J. C.;Kawamata, N.;Konda, B. M.;Black, K. L. & Koeffler, H. P. (2007) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor: effects on gene expression and growth of glioma cells in vitro and in vivo. *Clin Cancer Res* Vol.13, No.3, pp.1045-1052

Yoder, J. A.;Walsh, C. P. & Bestor, T. H. (1997) Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* Vol.13, No.8, pp.335-340

Yoo, C. B.;Cheng, J. C. & Jones, P. A. (2004) Zebularine: a new drug for epigenetic therapy. *Biochem Soc Trans* Vol.32, No.Pt 6, pp.910-912

Yoo, C. B.;Chuang, J. C.;Byun, H. M.;Egger, G.;Yang, A. S.;Dubeau, L.;Long, T.;Laird, P. W.;Marquez, V. E. & Jones, P. A. (2008) Long-term epigenetic therapy with oral zebularine has minimal side effects and prevents intestinal tumors in mice. *Cancer Prev Res (Phila)* Vol.1, No.4, pp.233-240

Yoshida, N.;Omoto, Y.;Inoue, A.;Eguchi, H.;Kobayashi, Y.;Kurosumi, M.;Saji, S.;Suemasu, K.;Okazaki, T.;Nakachi, K.;Fujita, T. & Hayashi, S. (2004) Prediction of prognosis of estrogen receptor-positive breast cancer with combination of selected estrogen-regulated genes. *Cancer Sci* Vol.95, No.6, pp.496-502

You, J. S.;Kelly, T. K.;De Carvalho, D. D.;Taberlay, P. C.;Liang, G. & Jones, P. A. (2011) OCT4 establishes and maintains nucleosome-depleted regions that provide additional layers of epigenetic regulation of its target genes. *Proc Natl Acad Sci U S A* Vol.108, No.35, pp.14497-14502

Yuki, Y.;Imoto, I.;Imaizumi, M.;Hibi, S.;Kaneko, Y.;Amagasa, T. & Inazawa, J. (2004) Identification of a novel fusion gene in a pre-B acute lymphoblastic leukemia with t(1;19)(q23;p13). *Cancer Sci* Vol.95, No.6, pp.503-507

Zhang, X.;Yuan, Z.;Zhang, Y.;Yong, S.;Salas-Burgos, A.;Koomen, J.;Olashaw, N.;Parsons, J. T.;Yang, X. J.;Dent, S. R.;Yao, T. P.;Lane, W. S. & Seto, E. (2007) HDAC6 modulates cell motility by altering the acetylation level of cortactin. *Mol Cell* Vol.27, No.2, pp.197-213

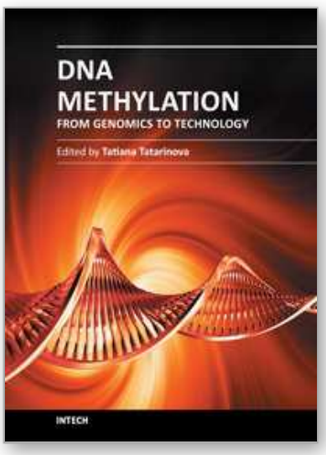
Zhang, Y.;LeRoy, G.;Seelig, H. P.;Lane, W. S. & Reinberg, D. (1998) The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. *Cell* Vol.95, No.2, pp.279-289

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* Vol.10, No.20, pp.6962-6968

Zhang, Z.;Yamashita, H.;Toyama, T.;Sugiura, H.;Ando, Y.;Mita, K.;Hamaguchi, M.;Hara, Y.;Kobayashi, S. & Iwase, H. (2005) Quantitation of HDAC1 mRNA expression in invasive carcinoma of the breast*. *Breast Cancer Res Treat* Vol.94, No.1, pp.11-16

Zhou, L.;Cheng, X.;Connolly, B. A.;Dickman, M. J.;Hurd, P. J. & Hornby, D. P. (2002) Zebularine: a novel DNA methylation inhibitor that forms a covalent complex with DNA methyltransferases. *J Mol Biol* Vol.321, No.4, pp.591-599

Zhu, W. G.;Dai, Z.;Ding, H.;Srinivasan, K.;Hall, J.;Duan, W.;Villalona-Calero, M. A.;Plass, C. & Otterson, G. A. (2001) Increased expression of unmethylated CDKN2D by 5-aza-2'-deoxycytidine in human lung cancer cells. *Oncogene* Vol.20, No.53, pp.7787-7796



DNA Methylation - From Genomics to Technology

Edited by Dr. Tatiana Tatarinova

ISBN 978-953-51-0320-2
Hard cover, 400 pages
Publisher InTech
Published online 16, March, 2012
Published in print edition March, 2012

Epigenetics is one of the most exciting and rapidly developing areas of modern genetics with applications in many disciplines from medicine to agriculture. The most common form of epigenetic modification is DNA methylation, which plays a key role in fundamental developmental processes such as embryogenesis and also in the response of organisms to a wide range of environmental stimuli. Indeed, epigenetics is increasing regarded as one of the major mechanisms used by animals and plants to modulate their genome and its expression to adapt to a wide range of environmental factors. This book brings together a group of experts at the cutting edge of research into DNA methylation and highlights recent advances in methodology and knowledge of underlying mechanisms of this most important of genetic processes. The reader will gain an understanding of the impact, significance and recent advances within the field of epigenetics with a focus on DNA methylation.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen