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Minireview

Epigenetics in cancer: Targeting chromatin modifications

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Abstract

Posttranslational modifications to histones affect chromatin structure and function resulting in altered gene expression and changes in cell behavior. Aberrant gene expression and altered epigenomic patterns are major features of cancer. Epigenetic changes including histone acetylation, histone methylation, and DNA methylation are now thought to play important roles in the onset and progression of cancer in numerous tumor types. Indeed dysregulated epigenetic modifications, especially in early neoplastic development, may be just as significant as genetic mutations in driving cancer development and growth. The reversal of aberrant epigenetic changes has therefore emerged as a potential strategy for the treatment of cancer. A number of compounds targeting enzymes that regulate histone acetylation, histone methylation, and DNA methylation have been developed as epigenetic therapies, with some demonstrating efficacy in hematological malignancies and solid tumors. This review highlights the roles of epigenetic modifications to histones and DNA in tumorigenesis and emerging epigenetic therapies being developed for the treatment of cancer. [Mol Cancer Ther 2009;8(6):1409–20]

Introduction

Cancer can evolve from a combination of epigenetic and genetic abnormalities resulting in dysregulated gene expression and function (1). The most common epigenetic modifications observed are increased methylation of CpG islands within gene promoter regions and deacetylation and or methylation of histone proteins (1–3). This review will focus on the role altered epigenetic regulation plays in mediating tumor onset and progression and the development of compounds that target enzymes that regulate the epigenome as anticancer agents.

Chromatin is a highly ordered structure consisting of repeats of nucleosomes connected by linker DNA. Chromatin consists of DNA, histones, and nonhistone proteins condensed into nucleoprotein complexes and it functions as the physiological template of all eukaryotic genetic information (4). Chromatin is divided into two distinct conformation states: (1) heterochromatin, which is densely compacted and transcriptionally inert and (2) euchromatin, which is decondensed and transcriptionally active (5, 6). Histones are small basic proteins containing a globular domain and a flexible charged NH_2 terminus known has the histone tail, which protrudes from the nucleosome. Regulation of gene expression occurs through posttranslational modifications of the histone tails provided by covalent modifications including acetylation, methylation, phosphorylation, ubiquitination, sumoylation, proline isomerization, and ADP-ribosylation (5, 7). Posttranslational modifications to histone tails govern the structural status of chromatin and the resulting transcriptional status of genes within a particular locus (5). In addition, hypermethylation of CpG dinucleotides within promoter regions also plays an important role in controlling gene expression (8). It is the complex interplay of posttranslational modification of specific residues on histone tails coupled with the DNA methylation status at a particular locus that determines if a particular gene(s) is transcriptionally active or repressed. Chromatin remodeling and DNA methylation is a highly regulated process controlled by enzymes that often exist in large macromolecular complexes. Posttranslational modifications such as histone acetylation and methylation and DNA methylation do not necessarily occur as mutually exclusive processes controlled by the sequential recruitment of different enzymes to a specific genomic region, but are more likely to be a dynamic process directed by large protein complexes containing different epigenetic enzymes (9). DNA methylation, histone modification, and subsequent modulation of nucleosome positioning are interlinked processes that act in a coordinated manner to determine the transcriptional status of a particular gene (10).

Epigenetic Deregulation in Cancer Development

Epigenetic mechanisms controlling transcription of genes involved in cell differentiation, proliferation, and survival

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are often targets for deregulation in malignant development. In addition to affecting transcription of protein-encoding mRNAs, noncoding microRNAs (miRs) that can regulate the expression of a myriad of cellular proteins by affecting mRNA stability and/or translation are similarly modulated by epigenetic means (10, 11). Altered patterns of epigenetic modifications are common in many human diseases including cancer (12) and there is evidence that epigenetic dysregulation can be a preliminary transforming event (13). For example epigenetic changes such as global DNA hypomethylation and promoter-specific hypermethylation are commonly observed in benign neoplasias as well as early-stage tumors (12). This suggests that epigenetic alterations are early events in the loss of cellular homeostasis, and may in some instances precede genetic mutations and genomic instability.

The deregulation of epigenetic modifiers has been characterized in many malignancies (Table 1) and the disruption of a number of histone modifying proteins, by mutations, deletions, or overexpression, is supportive of the critical role of epigenetic effectors in oncogenesis (14). Indeed individual genes, entire sets of genes, and miRs may be epigenetically deregulated to promote the "hallmarks of cancer" such as self-sufficiency in growth signals, insensitivity to growth inhibitory signals, evasion of apoptosis, increased proliferation potential, sustained angiogenesis, and capability of metastasis and invasion (8, 10). As detailed below, genetic abnormalities often result in the deregulated localization of enzymes that actively control DNA methylation or post-translational modifications of histone tails to specific loci. In these instances, loss of epigenetic regulation occurs downstream of oncogenic disruptions to the genome. In instances involving mutation of a single allele, epigenetic silencing of the other allele can provide a second "hit," resulting in loss of heterozygosity (LOH) and inactivation of tumor suppressor genes (i.e., *CDKN2A, PTEN, RB, APC*). Moreover, silencing of genes such as *CDKN2A* can cooperate with oncogenic mutations (i.e., mutations in *KRAS*) to promote tumor development and growth (Fig. 1A) (ref. 15). Interestingly, it is becoming apparent that the expression of epigenetic-regulatory enzymes such as DNA methyltransferases (DNMT), histone deacetylases (HAT), and histone methyltransferases (HMT) can be controlled by miRs (10). In particular, the miR-29 family can directly regulate the expression of DNMTs such that downregulation of this family of miRs in small cell lung cancer results in increased expression of DNMT3A and 3B causing a global genomic hypermethylation and specific methylation-induced silencing of tumor suppressor genes such as *FHIT* and *WWOX* (16). Importantly, re-expression of miR-29s in lung cancer cell lines restored normal patterns of DNA methylation and expression levels of *FHIT* and *WWOX*, and inhibited tumorigenicity both *in vitro* and *in vivo* (16).

Deregulated epigenetic mechanisms may initiate genetic instability, resulting in the acquisition of genetic mutations in tumor-suppressor genes and activating genetic mutations in oncogenes. Moreover, epigenetic disruptions in tumors are generally of a clonal nature, indicating occurrence in early generations of cells. There is a strong causative link between the silencing of genes involved in DNA repair (i.e., *hMLH1, BRCA1)* and cell transformation (17). In addition to negative effects on DNA repair, pathways such as the Wnt/β-catenin pathway that regulate cell proliferation and epithelial-to-mesenchymal transition (EMT) can be epigenetically controlled (18). Epigenetic silencing of genes including *APC*, Wnt inhibitory factor-1 (*WIF-1*), and frizzled-related proteins (*SFRP*) occurs in numerous cancer types, which often results in increased activity of β-catenin transcriptional activity (18, 19). There is clearly growing awareness of the importance of epigenetic deregulation in early cancer predisposition and development as evidenced by the growing list of genes with tumor suppressor activity that are often epigenetically silenced but rarely genetically mutated in the pre-invasive stages of many cancers (1).

DNA Methylation and Cancer

The maintenance of appropriate DNA methylation within CpG dinucleotide islands plays a significant role in regulation of a wide variety of molecular processes including stability of chromosomal structure and control of gene expression (20). In general, DNA methylation in gene promoter regions results in gene silencing likely because of steric inhibition of transcription complexes binding to regulatory DNA (20). DNA methylation can also result in the recruitment of proteins that bind methylated CpG sequences (methyl-CpG-binding domain [MBD] proteins) complexed with histone deacetylases (HDACs) and HMTs prompting coordinated epigenetic modifications of the surrounding chromatin (12).

Abnormal patterns in DNA methylation were the first examples of epigenetic deregulation to be characterized in human cancers, either as a result of DNMT overexpression or aberrant recruitment (Table 1). Tumor cell-specific promoter hypermethylation in genes that play important roles in regulating cell cycle, apoptosis, DNA repair, differentiation, and cell adhesion is often a hallmark of disease (8). In addition, hypomethylation of repetitive sequences may result in chromosomal and genetic instability, leading to further oncogenic events. Transcriptional silencing via DNA hypermethylation can often be associated with poor clinical outcome in several malignancies (21–23). For example silencing of *CDKN2A* and *CDKN1A* has been associated with poor clinical outcome in acute leukemias (21). The development of high-throughput approaches such as methylated DNA immunoprecipitation (Methyl-DIP) and differential methylation hybridization (DMH) (ref. 12) to survey the epigenome of normal and cancer cells for alterations in methylated regions of the genome should allow for the identification of other cancer-related silenced genes that will expand our knowledge of the epigenomic changes that occur during cellular transformation.

Histone Modifications

Although a number of histone modifications undoubtedly play important roles in epigenetic deregulation, acetylation

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Table 1. Proteins affecting epigenetic modifications are deregulated in cancer

Epigenetic protein category	Epigenetic proteins	Deregulation in cancer	Tumor	Reference
DNMTs	DNMT1	Mutation/overexpression	Colon/multiple	12, 14, 107
	DNMT3A	Overexpression	Multiple	
	DNMT3B	Overexpression	Multiple	
Methyl CpG Binding Proteins	MeCP2	Overexpression, mutation	Multiple	12, 108
	MBD1	Overexpression, mutation	Multiple	
	MBD2	Overexpression, mutation	Multiple	
	MBD3	Overexpression, mutation	Multiple	
	MBD4	Mutation	Colon, stomach, endometrium	
HATS	P300	Mutations, translocations, deletions	Multiple	12, 14, 39, 40
	CBP	Mutations, translocations, deletions	Multiple	
	pCAF	Mutations	Colon	
	MOZ	Translocations	Hematologic,	
	MORF	Translocations	Hematologic Uterine Leiomyomata	
	Tip60	Underexpression	Multiple	
HDACs	HDAC1	Overexpression/underexpression	Multiple/colon	12, 14, 49, 76, 109, 110
	HDAC2	Overexpression/mutation	Multiple/colon, gastric, endometrial	
	HDAC3	Overexpression	Colon	
	HDAC4	Overexpression/underexpression/ mutation	Prostate, breast/colon/breast	
	HDAC5	Underexpression	Colon, AML	
	HDAC6	Overexpression	Breast, AML	
	HDAC7	Overexpression	Colon	
	HDAC8	Overexpression	Colon	
	SIRT1	Overexpression/underexpression	Multiple/colon	
	SIRT2	Underexpression, deletion	Glioma	
	SIRT3	Overexpression	Breast	
	SIRT4	Underexpression	AML	
	SIRT7	Overexpression	Breast, thyroid carcinoma	
HMTs and PcG Proteins	MLL1	Translocation, amplification	Hematologic	12, 14, 111, 112
	MLL2	Amplification	Glioma, pancreas	
	MLL3	Mutation/deletion	Hematologic/colon	
	MLL4	Amplification	Solid tumors	
	NSD1	Translocation	AML	
	NSD2	Translocation	Multiple myeloma	
	NSD3	Translocation/amplification	AML/breast	
	EZH2	Amplification, overexpression	Multiple	
	BLIMP1	Mutations	B cell lymphomas	
	RIZ1	Underexpression, mutations	Multiple	
	EVI1	Chromosomal rearrangements	Myeloid leukemia	
	PFM9	Deletion	CML	
	MEL1	Translocation	Hematologic	
	SUV39H1	Mutation/overexpression	Ovarian/colon	
	ZMYND1	Overexpression	Multiple	
	HCP1	Underexpression	Breast	
	LBP1	Overexpression	Gastric	
	BMI1	Overexpression	Multiple	
	SUZ12	Overexpression	Multiple	
	CBX7	Underexpression	Thyroid carcinoma	
Histone Demethylase	GASC1	Amplification	Squamous cell carcinoma	14
	PUT1	Overexpression	Breast	

and methylation are the two histone modifications that have been clinically associated with pathological epigenetic disruptions in cancer cells (24, 25). In particular, the loss of acetylation and methylation of specific residues in core histones H3 and H4 have been identified as a marker of tumor cells (24, 25).

Histone Acetylation and Cancer

The acetylation status of histones H3 and H4 seem to largely dictate the fate of chromatin assembly, transcription, and gene expression (26). Histone acetylation is governed by the opposing activities of HATs and HDACs. Three main families of HATs transfer acetyl groups to lysine residues of

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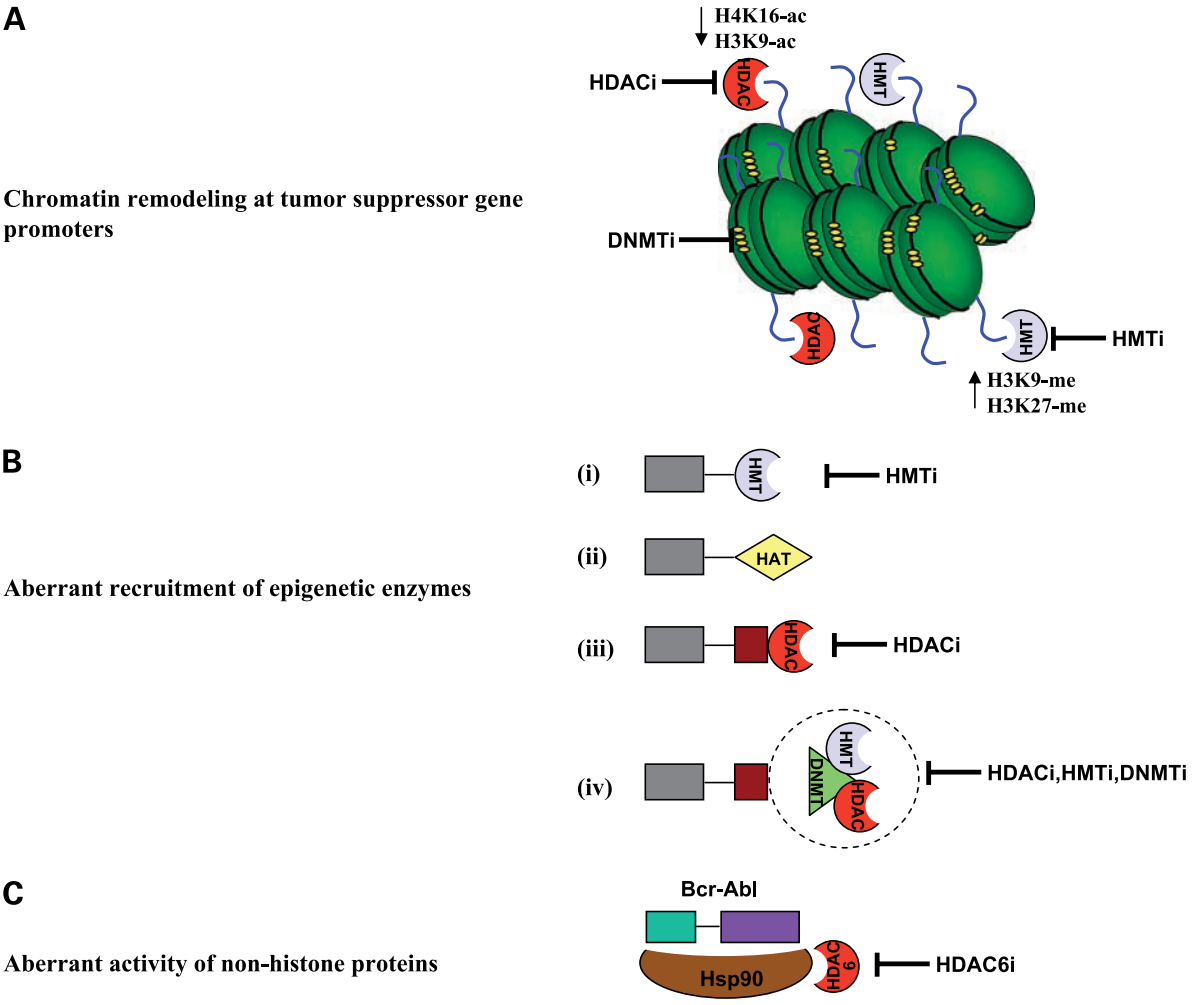

Figure 1. The role of epigenetic enzymes in tumorigenesis. **A,** silencing of tumor suppressor gene expression through DNA hypermethylation (yellow circles) by DNMTs, histone hypoacetylation at H3K9 and H4K16 following aberrant expression or activity of HDAC, and/or histone hypermethylation at H3K9 and H3K27 by HMT can induce cellular transformation. **B,** aberrant localization and or recruitment of (**i**) HMTs and (**ii**) histone acetylation transferases (HAT) following fusion of these enzymes to a variety of partner proteins as a result of chromosomal translocations can be oncogenic. Altered localization of corepressor complexes containing HDACs either (**iii**) alone or (**iv**) in large macromolecular complexes with DNMTs and or HMTs through interaction with oncogenic fusion proteins such as PML-RARα and AML1-ETO can also promote tumorigenesis. **C,** oncoproteins such as Bcr-Abl are stabilized through the chaperone activity of Hsp90 that is dependent on hypoacetylation of Hsp90 by HDAC6. Specific inhibition of enzymes that mediate these epigenetic defects can be exploited to induce antitumor activities.

the nucleosome core histones: the MOZ/YBF2/SAS2/TIP60 (MYST) family, the GCN5 *N*-acetyltransferase (GNAT) family, and the CBP/p300 family (7, 27). HATs are recruited as co-activators of transcription by transcription factors, usually in the context of large chromatin remodeling complexes. In addition, HATs such as PCAF, p300, and CBP acetylate multiple nonhistone proteins, many of which play prominent roles in oncogenesis (7, 28–30).

Altered HAT activity has been reported in both hematological and solid cancers, by inactivation of HAT activity through gene mutation or through deregulation of HAT activity by viral oncoproteins. For example, the adenovirus E1A and SV40 T antigen proteins target p300/CBP (31), and this interaction is important for cellular transformation (32, 33). Binding of these viral oncoproteins to p300 and CBP results in global hypoacetylation of histone H3 lysine 18 (34) and concomitant relocalization of these HATs to the promoter regions of a limited number of genes that promote cell growth and division, hyperacetylation of H3K18, and gene-specific transcriptional activation (35). These studies indicate that cellular transformation by E1A and SV40 T antigen is mediated through concerted epigenetic reprogramming following interaction with HATs such as p300 and CBP. Missense mutations of p300 have also been identified in a variety of solid tumors including colorectal, gastric, breast, and pancreatic cancers (30, 36). Tip60 is a HAT that may play an important role in regulating tumorigenesis through its involvement in modulating signaling events involving ATM following DNA damage and regulating the transcriptional activation activities of p53 and Myc

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(37). Indeed Tip60-mediated acetylation of p53 influences the specificity of p53 for certain gene promoters, directing a switch from cell cycle arrest to apoptosis (38, 39). Decreased expression of Tip60 results in hypo-acetylation of p53 and a defect in apoptotic signaling, putatively increasing the potential for malignant transformation. Consistent with the proposed role of Tip60 as a tumor suppressor protein, mono-allelic loss of the human Tip60 gene is frequently observed in lymphomas, mammary carcinomas, and head and neck tumors (40). Moreover, genetic studies using the Eμ-myc model of B cell lymphoma indicate that Tip60 suppresses myc-mediated lymphomagenesis in a haploinsufficient and p53-independent manner (40).

Chromosomal translocations involving HATs and their consequent fusion proteins have been implicated in the onset and progression of acute leukemia (Fig. 1B) (ref. 30). Examples of such translocations have been identified in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) cases in which the translocation t(11;16)(q23;p13) results in a fusion protein (MLL-CBP) consisting of the CBP and the mixed lineage leukemia protein (MLL). The effects of this fusion protein in the formation of AML are still unclear, but possible underlying mechanisms may involve deregulation of MLL target genes by CBP-mediated chromatin remodeling and increased chromatin accessibility (41). MLL can also be aberrantly fused to p300 in AML via the t(11;22)(q23;p13) translocation (42). In addition to p300 and CBP fusion proteins involving the HATs TIF2, MOZ and MORF that arise as a result of chromosomal translocations have also been identified in hematological malignancies (43–45). In AML the chromosomal translocation t(8;16)(p11;p13) causes the fusion of the MOZ gene and CBP. The resulting fusion protein has been recently shown to increase expression of genes regulated by NF-κB (46). MORF has been associated with childhood AML and myelodysplastic syndrome through the translocation 1(10;16)(q22;p13), in which the MORF gene is fused with the CBP gene (47). TIF2 is another HAT found to be a fusion partner that combines with MOZ through the chromosome inversion inv(8)(p11;q13) that has been identified in both AML and mixed lineage leukemia (45, 48). Taken together, it seems that the involvement of HATs in tumorigenesis usually stems from chromosomal abnormalities causing altered HAT activity, aberrant histone acetylation, and consequent alteration in gene expression.

Histone Deacetylation and Cancer

The principle role of HDACs is to oppose the activity of HATs and regulate transcription through the removal of acetyl groups from lysine resides of histone tails and through the targeted deacetylation of nonhistone substrates (49). There are four classes of HDACs: class I consists of HDAC 1, 2, 3, and 8, (localized to the nucleus); class II consists of HDAC 4, 5, 6, 7, 9, and 10, (found in both the nucleus and cytoplasm); class III consists of sirtuins (SIRT 1-7); and class IV consists of HDAC 11, which displays features of both class I and II HDACs (50). Class I, II, and IV HDACs share homology in both sequence and structure and all require a zinc ion for catalytic activity. In contrast, the sirtuin family of deacetylases shares no similarities in their sequence or structure with class I, II, or IV HDACs and requires nicotinamide adenine dinucleotide (NAD^+) for catalytic activity (49). Class I HDACs are nuclear proteins thought primarily to regulate histone acetylation and thus chromatin structure (49). It is clear that not all class I HDACs function in the same way, as they are often components of distinct corepressor complexes and functional knockdown or deletion of different class I HDACs can result in diverse cellular effects (51). For example, knockdown of HDAC2 and HDAC1 but not HDAC3 suppressed the proliferation of certain colon carcinoma cells *in vitro* (52) and sensitized chronic lymphocytic leukemia cells to TRAIL-induced apoptosis (53). Interestingly and in contrast to the study by Weichert and colleagues (52), knockdown of HDAC3 was more effective in inhibiting the growth of a different set of colon carcinoma cells than was knockdown of HDACs 1 or 2 (54). Moreover, knockdown of HDAC3 and HDAC2 induced DNA damage and concomitant apoptosis (55). Class II and IV HDACs are found predominantly in the cytoplasm and may preferentially deacetylate nonhistone proteins (49). Although we and others have identified a link between HDAC inhibitor (HDACi)-induced chromatin remodeling, gene expression changes, and apoptosis (56, 57), others have proposed that altered acetylation of nonhistone proteins (in particular Hsp90 that is deacetylated by HDAC6 and when hyperacetylated releases client oncoproteins such as c-kit, Her2/erbB2, and Bcr-Abl resulting in their degradation), is the key effector mechanism of broad-spectrum HDACi (Fig. 1C) (ref. 49). Interestingly, knockdown of specific class II HDACs also revealed functional differences between this family of proteins. Knockdown of HDAC4 resulted in inhibition of cell proliferation and induction of apoptosis (58), whereas knockdown of HDAC7 in endothelial cells did not affect cell growth or survival but inhibited cell migration and capacity to form capillary tube-like structures (59). A further role for class II HDACs in regulating angiogenesis was shown by the knockdown of HDAC6 and HDAC10 that resulted in the depletion of VEGFR1 and VEGFR2 (60). Clearly the activities of these enzymes is cell-type and/or context-dependent and therefore making broad predictions about the effect of functionally inactivating a single HDAC or class of HDAC is challenging at this stage. However, the functional studies done thus far indicate that class I HDACs predominantly regulate cell proliferation and apoptosis whereas class II HDACs are more specifically involved in regulating cell migration and angiogenesis (51).

Deregulation of HDAC activity by chromosomal translocations has been strongly implicated in aberrant gene silencing and the promotion of tumorigenesis, especially in leukemias (Fig. 1B). A well-understood link between tumorigenesis and aberrant HDAC activity occurs in acute promyelocytic leukemia (APL). The retinoic acid receptor (RAR) is important for myeloid differentiation and acts as a transcriptional regulator by binding its heterodimerization partner RXR, which in turn bind to retinoic acid response elements (RAREs) within the promoters of target genes (49). In the absence of retinoids, transcriptional complexes

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including RAR, RXR, and HDACs act to repress transcription, whereas the addition of ligand releases this transcriptional hold and allows the binding of HATs including TIF2 and CBP, to RAR/RXR resulting in activated transcription of RAR target genes (61–64). In APL, the chromosomal translocations t(15;17) and t(11;17) result in production of fusion proteins RARα-PML (promyelocytic leukemia protein) and RARα-PLZF (promyelocytic zinc finger), respectively. These aberrant proteins retain the ability to bind RAREs and HDACs with high affinity and are nonresponsive to retinoids, resulting in the deregulated transcriptional silencing of RAR-targeted genes and prevention of cell differentiation (61–64). Other examples of translocations in leukemogenesis that produce fusion proteins capable of altering protein acetylation include AML1-ETO that results from t(8;21) and CBFβ-MYH11 caused by the chromatin inversion 16(p13;q22) in cases of AML. Fusion proteins from these translocations result in the recruitment of HDACs to target gene promoters and consequent gene silencing (7, 21). Certain non-Hodgkin's lymphomas display irregular expression of the oncoprotein Bcl-6 that recruits HDACs resulting in the repression of target gene involved in cell cycle arrest and apoptosis (7, 21, 50, 65).

In addition to aberrant recruitment of HDACs to specific loci through their interaction with proto-oncogenes with DNA binding activity, altered expression of individual HDACs in tumor samples has also been reported. For example, HDAC1 is overexpressed in prostate, gastric, colon, and breast carcinomas (54, 66–68). HDAC2 is overexpressed in colorectal (69), cervical (70), and gastric cancer (71), whereas overexpression of HDAC6 is observed in breast cancer specimens (72). In contrast, truncating mutations in HDAC2 have been detected in a subset of microsatellite unstable colorectal cell lines and primary tumor samples resulting in loss of HDAC2 expression and enzymatic activity, and decreased sensitivity to HDACi-induced apoptosis (73). These studies, which propose that HDAC2 may function as a tumor suppressor, seem at odds with most other reports indicating that enhanced HDAC activity is tumor-promoting. At present, the relationship between aberrant expression of various HDACs and cancer remain largely correlative however there is some experimental evidence to suggest that increased HDAC expression can play a role in tumorigenesis and provides a molecular rationale for targeting HDAC activity in these tumors. Loss of the adenomatosis polyposis coli (APC) tumor suppressor gene resulted in enhanced expression of HDAC2 via activated β-catenin/c-myc, and specific siRNA-mediated knockdown of HDAC2 in APC-deficient colon carcinoma cells overexpressing HDAC2 resulted in robust induction of apoptosis (69). Moreover, ectopic expression of HDAC2 antagonized APC-induced apoptosis in colon carcinoma cells. Finally, studies using APC^{min} mice showed that HDAC2 was selectively upregulated in normal colonic mucosa cells and was further induced in tumors from these mice, and importantly, treatment of mice with the HDACi valproic acid (VPA) significantly reduced the number and size of adenomas. Knockout of HDAC2 in APC^{min} mice resulted in decreased intestinal tumor development (74). These findings were supported by others showing that siRNA-mediated knockdown of HDAC2 induced apoptosis in cervical carcinoma cells (70), and knockdown of HDAC1 or HDAC3 using siRNA suppressed growth and survival of cervical carcinoma cells (75).

Whereas much of the focus about HDACs and cancer has centered on class I, II, and IV HDACs, class III HDACs (sirtuins) may also play an important role in regulating tumor onset and/or progression. Sirtuins can deacetylate protein substrates including histones, and can mediate ADP-ribosylation (76–78). These enzymes were originally studied for their role in regulating calorie restriction and lifespan of lower organisms (76). However sirtuins may regulate cellular senescence, DNA repair, chromosomal stability, cell cycle progression, and the transcriptional activity of important proteins such as p53, p73, pRb, NF-κB, and the Foxo family of proteins providing circumstantial evidence that the deregulated expression or function of these enzymes may be oncogenic (76–79). Consistent with this notion, overexpression of SIRT1, SIRT2, SIRT3, and SIRT7 has been documented in a range of tumors (76, 77). A direct role of SIRT1 in regulating tumor development, growth, and survival was recently shown by studies showing that pharmacological inhibition of SIRT1 activity, expression of dominant-negative SIRT1, or knockdown of SIRT1 using siRNA resulted in reactivation of tumor suppressor genes including E-cadherin, SFRP1, SFRP2, and MLH1 and decreased clonogenic cell growth (80). Interestingly, the promoter regions of these reactivated genes remained heavily methylated indicating that in this instance, histone deacetylation mediated by SIRT1 is the dominant gene silencing mechanism.

In contrast to the findings above that indicate that class III HDACs likely function as oncoproteins, *SIRT2* is deleted in human gliomas and re-expression of SIRT2 in glioma-derived cell lines reduces their clonogenic capacity indicating the intriguing possibility that under certain circumstances sirtuins may function as tumor suppressors (76, 77). Moreover, SIRT1 can deacetylate androgen receptor (AR), and overexpression of SIRT1 in AR-positive prostate cancer cells inhibits their proliferation and SIRT1 can promote senescence during aging and can protect against tumorigenesis (77). Finally, chronic inflammation and concomitant activation of NF-κB is a major cancer risk factor and deacetylation of NF-κB by SIRT1 can inhibit its activity thereby putatively suppressing the inflammatory response and thus tumorigenesis (77). At present it remains unclear exactly what role sirtuins may play in oncogenesis and if their dominant role may be as tumor suppressors or oncogenes. However, these data support the notion that altered HDAC function may play an active role in tumor onset and progression and highlight HDACs as attractive targets for therapeutic intervention.

Histone Methylation and Cancer

Histones can be mono-, di-, or tri-methylated at lysine and arginine residues by HMTs, and the recent identification of histone lysine demethylases such as KDM1/LSD1 and the Jumonji-domain (JMJD)-containing protein family

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shows that histone methylation is an enzymatically dynamic process (81). In general, methylation at H3K4, H3K36, and H3K79 is associated with transcriptional activation, whereas H3K9, H3K27, and H3K20 methylation is associated with transcriptional repression (29). Recent advanced high resolution, whole genome analysis of histone methylation marks using chromatin immunoprecipitation (ChIP) assays coupled to next generation sequencing indicates that monomethylations of H3K27, H3K9, H4K20, H3K79, and H2BK5 are all linked to gene activation, whereas trimethylations of H3K27, H3K9, and H3K79 are linked to repression. The binding of heterochromatin protein (HP1) to methylated H3K9 promotes the formation of a transcriptional silent heterochromatin state via association with DNMTs and HDACs (7, 82).

The tumor cell-selective loss of trimethylation of H4K20 (24) and demonstration that knockout of the HMT SUV39H sensitizes mice to tumorigenesis (83) provides experimental evidence that altered histone methylation may play an important role in tumor onset and/or progression. As with HATs and HDACs, aberrant expression or activity of HMTs and demethylases in tumor cells has been reported (Table 1). This can be in the form of chromosomal translocations involving HMTs (i.e., MLL1, NSD1, NSD3) (Fig. 1B), gene overexpression or amplification (i.e., EZH2, MLL2, NSD3, BMI1, GASC1), gene silencing (i.e., RIZ1), and gene deletion (i.e., MLL3) (refs. 1, 8, 12, 14, 84). It is not yet clear if global changes in histone methylation across the genome or more specific effects at distinct loci are the basis for the proposed link between altered expression/function of proteins that directly or indirectly regulate histone methylation and tumorigenesis. However, the development of new technologies to rapidly and comprehensively survey the epigenomic landscape of tumor and normal cells should enhance the acquisition of this information.

Targeting Cancer Via Epigenetic Therapy

In contrast to genetic mutations, most epigenetic modifications may be reversible and preventable. The resetting of aberrant epigenetic states in neoplastic cells is an expanding therapeutic approach to treat or prevent cancer. Pharmacological targeting of DNA methylation and histone acetylation and methylation is now possible (Table 2) and as detailed below is a promising therapeutic approach.

Inhibitors of DNA Methylation

A number of DNA methylation inhibitors are currently under investigation, including the pyrimidine nucleoside analogs decitabine (Dacogen, SuperGen, Inc., Dublin, CA) and azacitidine (Vidaza, Celgene, Summit, NJ), and the nonnucleoside inhibitor hydralazine (Table 2). Azacitidine and decitabine are both U.S. Food and Drug Administration (FDA) approved for the treatment of a number of myelodysplastic syndrome subtypes, including refractory anemia and chronic myelogenous leukemia (CML) (refs. 1, 85, 86). Hydralazine was originally approved for use as an antihypertensive, and recently reported to be an inhibitor of DNA methylation able to reactivate the expression of tumor-suppressor genes in cancer (87). Pharmacologic inhibition of DNA methylation causes the trapping of DNMTs and their targeted degradation resulting in re-expression of genes that have been aberrantly silenced by hypermethylation, concomitant with inhibition of clonal expansion and tumor cell growth, induction of cell differentiation, and cancer cell death (85). How DNMT inhibitors (DNMTi) specifically affect tumor cells is not well understood however as these agents function by being incorporated into newly replicated DNA, only rapidly dividing cells such as tumor cells will be targeted. Moreover, as discussed above, the epigenetic silencing of tumor suppressor genes can be an essential oncogenic event and may therefore result in "addiction" of the transformed cell to the silencing of specific genes. Accordingly, tumor cells may be exquisitely sensitive to the reversion of this gene silencing phenotype by DNMTi. It is currently not known if the reactivation of epigenetically silenced genes is the only molecular event that underpins the biological and therapeutic effects of DNMTi, and if so if specific reactivation of only one or a number of epigenetically silenced genes is necessary. Extensive clinical studies indicate that DNMTi induce manageable short-term side effects at doses that show therapeutic efficacy (85, 88). However, the long-term effects of chronic epigenetic deregulation and inhibition of DNMT activity remains to be fully evaluated.

Histone Deacetylase Inhibitors

An extensive number of HDACi have been purified from natural sources or synthetically developed, and many of these HDACi have advanced to clinical development (49). The most extensively studied HDACi target class I and or II, and or IV HDACs and a separate set of inhibitors target class III HDACs (sirtuins) and for the purpose of this review will be referred to as SIRTi (Table 2). HDACi execute their antitumor effects by mediating a variety of biological responses such as induction of tumor cell death, inhibition of cell cycle progression, suppression of angiogenesis, and enhancing immune responses (49). Most HDACi currently in clinical development target multiple HDACs and inhibition of specific HDACs may affect different molecular processes. The earliest model to explain the biological effects of HDACi involved changes in gene expression as a direct result of histone hyperacetylation at specific gene loci. Indeed, gene expression analyses indicated that greater than 5% of genes were altered in expression following treatment with HDACi and many of these genes have been shown to be directly responsible for the functional (biological) effects of HDACi (49). However, we now realize that histones are not the only targets of HDACs and a range of nonhistone proteins are targeted by HDACs and show regulated function according to their acetylation status (89). These proteins include transcription factors such as p53, NF-κB, and E2F1 that play important roles in tumorigenesis and antitumor responses (89, 90), and in this way HDACi indirectly regulate gene expression. Moreover, proteins that regulate DNA

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Table 2. Epigenetic-based therapeutics in preclinical and clinical development

Epigenetic target	Compound	Developmental stage	References
HDACs (class I, IIa, IIb, IV)	Vorinostat (SAHA)	FDA approved for CTCL	49, 104, 113
	Panobinostat (LBH589)	Phase I/II	
	Belinostat (PXD101)	Phase I/II	
	ITF2357	Phase I	
	PCI-24781	Phase I	
HDACs (class I, IIa)	Phenylbutyrate	Phase I/II	49, 104, 113
	VPA	Phase I/II	
HDACS 1, 2	Romidepsin (depsipeptide)	Phase I/II	49, 104, 113
	SK-7041	Experimental	
	SK-7068	Experimental	
HDACs 1, 3	MS-275	Phase I/II	49, 104, 113
	CI-994	Phase I	
	MGCD0103	Phase I/II	
HDACs 2, 3	Apicidin	Experimental	49, 104, 113
HDAC 6	Tubacin	Experimental	49, 104, 113
HDAC8	SB-379872A	Experimental	49, 104, 113
	PCI-34051	Experimental	
DNMT	Vidaza (5-azacytidine)	FDA approved for myelodysplatic	86, 114–116
	Decitabine (5-aza-2′-deoxycytidine)	syndromes	
	Zebularine	Experimental	
	RG108	Experimental	
	Procaine	Experimental	
	Hydralazine	Phase I	
	(-)-epigallocatechin-3-gallate noncovalent enzyme (EGCG)	Experimental	
SIRT1-7	Nicotinamide	Experimental	93
SIRT1, 2	Tenovin-1-3, 5-6	Experimental	92
	Sirtinol	Experimental	93
	Splitomycin	Experimental	117
	Cambinol	Experimental	
SIRT1	SRT1720	Experimental	78, 118
	EX-527	Experimental	
	NF657		
SIRT2	AGK2	Experimental	119
SIRT5	Suramin	Experimental	93
HMT (G9a)	BIX-01294	Experimental	96
HMT (SU(VAR)3-9, G9a)	Chaetocin	Experimental	94
Polycomb group proteins	DZNep	Experimental	97
LSD1	Polyamine analogs	Experimental	98

Abbreviation: CTCL, cutaneous T-cell lymphoma.

repair (i.e., Ku70), protein stabilization (i.e., Hsp90), and the cellular cytoskeleton (i.e., tubulin) and have no direct role in regulating gene expression, can be directly acetylated. Accordingly, HDACi may affect the function of these proteins resulting in decreased tumor growth and/or survival (49). Of particular note is Hsp90, which is deacetylated by HDAC6, and following HDACi treatment, acetylated Hsp90 releases "client oncoproteins" such as Bcr-Abl, c-Kit, and Her2/ErbB2, which are subsequently targeted for degradation. Accordingly, tumor cells that are "addicted" to these oncoproteins may be particularly sensitive to HDACi that can inhibit HDAC6. Moreover, HDAC6 plays a more general role in regulating protein turnover through its involvement in the aggresome pathway (Fig. 1C) (ref. 91). Accordingly, inhibition of HDAC6 function could disrupt the activity of the aggresome, resulting in accumulation of polyubiquitinated proteins, which has been shown to lead to cell stress and ultimately caspase-dependent apoptosis (91).

As well as their intrinsic effects on tumor cells, HDACi may additionally affect neoplastic growth and survival by regulating host immune responses and tumor vasculature (49). The pleiotropic cellular effects of HDACi can act cooperatively to mediate potent antitumor activities, however the molecular processes underlying these effects of HDACi remain to be fully elucidated. Consequently, HDACi may have a much broader effect on cellular physiology than originally understood and defining the molecular events underpinning the various antitumor activities of HDACi will require a greater understanding of the effects of HDACi on diverse cellular proteins and pathways.

Regulators of sirtuin activity have been developed that either enhance or suppress the activity of the enzymes

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(Table 2). Natural product (i.e., resveratrol) and synthetically (i.e., SRT1720) derived activators of SIRT1 have been developed that might have therapeutic benefit for age-related diseases such as type 2 diabetes, cardiovascular disease, and neurodegeneration (78). Moreover, given the putative role for sirtuins as tumor suppressors under certain circumstances, sirtuin activators may be useful as chemopreventative agents (77), SIRTi have also been developed (Table 2) that may be applicable as anticancer agents in situations in which sirtuins have oncogeneic activities. Indeed, a recent screen for small molecule activators of p53 that have antitumor activity identified a series of compounds (tenovin-1-6), which were shown to specifically inhibit SIRT1 and SIRT2 (92). Other inhibitors of SIRT1 and SIRT2 such as splitomycin, sirtinol, and cambinol have antitumor activities *in vitro* or *in vivo* (93). The ability of SIRTi to kill tumor cells *in vitro* and inhibit xenotumor growth *in vivo* provides promising evidence that these agents may be utilized as anticancer agents.

Inhibition of Histone Methylation

The implied role of HMTs in tumorigenesis has lead to the development of small molecule regulators of these enzymes (Table 2). Chaetocin was one of the first HMT inhibitors (HMTi) developed and it seems that this compound has some selectivity for the SUV39 class of HMTs (94). Chaetocin killed human tumor cell lines and primary myeloma cells *in vitro* whereas normal human B cells were insensitive to the compound (95). Chaetocin also had potent antitumor activity *in vivo* providing some indication that further development of the compound as an anticancer therapeutic was warranted. However, it has not been shown that the inhibitory effect of chaetocin on HMTs confers the antitumor effect of the compound, and further mechanistic studies are required to address this important point. Using the SU(VAR)3-9, enhancer of zeste, trithorax-homology (SET) domain of the HMT G9a as the target enzyme, the small molecule inhibitor BIX-01294 was identified via a high-throughput chemical screen (96). This compound inhibited methylation at H3K9 at several G9a-target genes and it will be of great interest to determine if reactivation of genes by BIX-01294 affects tumor cell growth or survival. A very exciting recent study reported the identification of 3-Deazaneplanocin (DZNep) as a compound capable of depleting levels of the polycomb-recessive complex 2 (PRC2) components EZH2, SUZ12, and EED. Treatment of tumor cell lines with DZNep inhibited methylation at H3K27 but not H3K9 and reactivated a series of genes that are transcriptionally repressed by PRC2 and induced potent tumor cell-selective apoptosis (97). The effects of DZNep on H3K27 methylation and cell survival was phenocopied by siRNA-mediated knockdown of EZH2, EED, or SUZ12. Importantly, knockdown of FBXO32, a gene that is upregulated by DZNep across all tumor cell lines tested, significantly suppressed DZNep-induced apoptosis (97). These functional assays provide a very important mechanistic link between pharmacological inhibition of PRC2 activity, enhanced histone methylation, and induction of tumor cell apoptosis. An alternative way to reactivate epigenetically silenced genes is to inhibit the activity of histone demethylases. Recently, polyamine-based inhibitors of LSD1 have been developed that induce mono- and di-methylation at H3K4 and concomitant reactivation of previously silenced genes in treated tumor cell lines (98). The biological effects of these agents have not yet been evaluated but they represent an important step forward in the development of new agents to target the epigenome.

Epigenetic Therapies in Combination

Cooperation between different epigenetic modifications in driving oncogenic gene expression supports the rationale of combining epigenetic therapies. Both HDAC inhibition and DNA demethylating agents have shown clinical efficacy as single agents; yet combination of the two therapies has been shown to have strong synergistic effects on the reactivation of silenced genes and antiproliferative and cytotoxic effects on cancer cells (21, 99). Although demethylating agents have shown clinical efficacy in a subset of hematologic tumors, there is evidence that this treatment does not fully revert aberrant epigenetic states and may not protect against the recurrence of aberrant gene silencing directed by chromatin modifications (100). The dense methylation of genes precludes the activation of gene expression by HDAC inhibitors as single agents; however, combination with DNA demethylating agents has shown synergistic effects in inducing the expression of heavily methylated genes and inhibiting cancer cell proliferation and survival (22). Such combinations are currently being investigated in a number of clinical trials (85, 101, 102).

Treatments targeting epigenetic processes can potentiate the effects of other antineoplastic treatments, including traditional chemotherapy and radiation. Examples of such combinations include epigenetic therapies with agents targeting microtubule stability (docetaxel, paclitaxel), proteosomal degradation (bortezomib), and molecular protein chaperones (geldanomycin) (ref. 49). The rationale for these combinations is based on the observation that epigenetic therapies, especially HDAC inhibition, lower the apoptotic threshold of tumor cells, making them more sensitive to other agents.

Based on the rationale that epigenetic deregulation is a driver of tumorigenesis, the combination of epigenetic therapies with targeted anticancer treatments is also under clinical investigation. In preclinical models, combinations of HDAC inhibitors with targeted therapies such as signal transduction inhibitors have consistently shown additive or synergistic effects on induction of apoptosis in cancer cell lines and *in vivo* tumor models (49, 103–105). This includes combination of epigenetic with targeted therapies such as imatinib in CML, gefitinib in lung cancer, and trastuzumab in breast cancer, among others (49, 106). The molecular events underpinning the synergistic effects of HDACi and various targeted therapeutics remain to be

delineated, however the promising preclinical results indicate that such combination approaches may be clinically valuable.

Concluding Remarks

An appreciation of the significant role of epigenetic defects in cancer onset and progression has increased remarkably in recent years. It is now understood that deregulated epigenetic mechanisms can cause, as well as compound, the effects of oncogenic mutations to promote tumor development and growth. The management of aberrant epigenetic states as a way to target early tumor development as well as tumor progression is therefore a logical therapeutic approach. The efficacy of epigenetic therapies in the treatment of myelodysplastic syndromes and prevention of leukemic transformation reinforces the importance of epigenetic deregulation prior to cancer onset. As such, epigenetic therapy is a promising approach for the prevention and treatment of malignancies. One of the most exciting aspects of epigenetic therapy is the ability to potentiate responses to existing therapies, which effectively multiplies the arsenal against cancer progression. An understanding of the link between epigenetic deregulation and cancer is applicable to prognosis as well as treatment. Further definition and refinement of profiles of histone and DNA modification patterns should be invaluable for the purposes of detection, diagnosis, and prognosis of cancer as well as the prediction of therapeutic responses.

Disclosure of Potential Conflicts of Interest

P. Atadja: employee, Novartis. R. Johnstone: grant support, Novartis, Merck, Pfizer. No other potential conflicts of interest were disclosed.

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