Epigenetic Changes in Pediatric Solid Tumors: Promising New Targets

Elizabeth R. Lawlor1,2 and Carol J. Thiele3

Abstract

Cancer is being reinterpreted in the light of recent discoveries related to the histone code and the dynamic nature of epigenetic regulation and control of gene programs during development, as well as insights gained from whole cancer genome sequencing. Somatic mutations in or deregulated expression of genes that encode chromatin-modifying enzymes are being identified with high frequency. Nowhere is this more relevant than in pediatric embryonal solid tumors. A picture is emerging that shows that classic genetic alterations associated with these tumors ultimately converge on the epigenome to dysregulate developmental programs. In this review, we relate how alterations in components of the transcriptional machinery and chromatin modifier genes contribute to the initiation and progression of pediatric solid tumors. We also discuss how dramatic progress in our understanding of the fundamental mechanisms that contribute to epigenetic deregulation in cancer is providing novel avenues for targeted cancer therapy. Clin Cancer Res; 18(10); 2768–79. ©2012 AACR.

Introduction

Cancer is a developmental disease, and the hijacking of biologic processes that are central to normal embryonic development is an essential feature of human malignancy. Nowhere is this more apparent than in pediatric tumors, where disruptions to normal development are believed to underlie the genesis of many (if not all) childhood tumors (1). Normal mammalian development is a precisely orchestrated process that results in the creation of hundreds of differentiated cell types from a single pluripotent stem cell. This process of progressive lineage specification and cellular differentiation is dependent on epigenetic regulation, which directs heritable changes in gene expression independently of DNA sequence changes (2–4). In eukaryotic cells, DNA is wrapped around core histone proteins and packaged into compact chromatin structures termed nucleosomes (Fig. 1). Epigenetic regulation of gene expression is predominantly controlled by covalent modifications of histones, changes to nucleosome conformation and position (nucleosome remodeling), and DNA methylation (Figs. 1 and 2). In this article, we review normal epigenetic regulation and discuss how disruptions to the epigenetic machinery contribute to the initiation and progression of pediatric solid tumors. In addition, we discuss how an advanced understanding of epigenetic regulatory mechanisms is providing novel avenues for targeted cancer therapy.

Epigenetic Regulation in Normal Development

Histone modifications

Histone modifications such as acetylation, methylation, and ubiquitination lead to changes in chromatin structure that determine the accessibility of DNA to transcription factors (Fig. 1; refs. 5, 6). Histone acetylation and methylation are dynamically regulated by the competing actions of histone acetyltransferases and histone deacetylases (HDAC), and histone methyltransferases (HMT) and histone demethylases, respectively (6). Repressive histone modifications are largely driven by the action of Polycomb group (PcG) proteins (7, 8). PcG proteins function as multiprotein complexes (PRC1 and PRC2) that act cooperatively to silence transcription, mainly by trimethylating lysine residue 27 on histone 3 (H3K27me3). In stem cells, PcG proteins maintain self-renewal and pluripotency by repressing differentiation genes (2, 8). PcG proteins also interact with DNA methyltransferases (DNMT) to induce permanent transcriptional silencing by enabling DNA methylation (Fig. 2A and C; refs. 9, 10). In many cancers, PcG genes and proteins, especially BMI-1 and EZH2, are aberrantly overexpressed, resulting in persistent activation of stem cell programs and repression of cellular differentiation (Fig. 3; refs. 7, 11).

Transcriptionally active chromatin is acetylated and methylated at H3K4 (Figs. 1 and 3; ref. 5). H3K4 methylation is supported by Trithorax group proteins with HMT activity that are encoded by mixed lineage leukemia (MLL) genes (12). Alterations in H3K4 methylation lead to differentiation defects and rearrangements of MLL1, and mutations in MLL2 and MLL3 are common in high-risk leukemias of childhood (13) and pediatric medulloblastomas (14), respectively. Of importance, in embryonic stem cells, repressed genes that are involved in early lineage commitment maintain both repressive (H3K27me3) and activating (H3K4me3) histone modifications (15). These bivalent
genes are considered to be poised for rapid activation in response to appropriate differentiation signals (15), and they are frequently aberrantly irreversibly silenced in cancer (Fig. 3; ref. 16).

**Nucleosome remodeling**

Changes in nucleosome position along the DNA strand and conformational changes to DNA-histone interactions influence the accessibility of transcription factors to DNA. Such nucleosome remodeling is critical for normal differentiation and is controlled by ATP-dependent, multiprotein chromatin-remodeling complexes, in particular SWI/SNF (Fig. 2B; refs. 5, 17). In addition, SWI/SNF contributes to the epigenetic regulation of gene expression by competing with and antagonizing PcG protein function (18). The core subunit of SWI/SNF, SNF5 (also known as SMARCBI, INI1, and BAF47) is an established tumor suppressor that is mutated in aggressive malignant rhabdoid tumors in children (19). Mutations of other members of the SWI/SNF complex are also prominent in several aggressive adult malignancies (reviewed in ref. 17).

**DNA methylation**

DNA methylation at gene promoters is associated with gene silencing, and during differentiation, cellular reprogramming, or tumorigenesis, the pattern of global DNA
Figure 2. Protein modifications and complexes that regulate higher-order chromatin conformation. A, PcG-protein complexes. The PRC2 protein EZH2 is the key effector of PRC2 action, catalyzing trimethylation of H3K27 [H3K27me3 (7, 8)]. HDACs also bind the PRC2 complex, decreasing acetylation of H3K27 and favoring its methylation, and inhibiting gene transcription. In contrast, inhibitors of histone deacetylases (e.g., vorinostat and romidepsin) would be expected to counteract this activity, resulting in increased acetylation at these loci, favoring gene expression. For example, at steady state, EZH2 mediates increased H3K27Ac and increased gene transcription at this locus (57). PRC2 is targeted to DNA by JARID2, which binds GC-containing DNA regions. PRC1 in turn mono-ubiquitinates H2A, a task that is achieved by the PRC1 protein BMI-1 in cooperation with the E3 ubiquitin ligase RING1B (7, 8). In contrast, acetylation of lysine residues on histones 3 and 4 (H3K, H4K) and methylation of H3K residue 4 (H3K4me3) promote an open euchromatin state and active transcription. B, nucleosome remodeling. The ATP-dependent chromatin remodeling complexes are a family of proteins (SWI/SNF, ISWI, NURD/MI-2/CHD, and INO80) characterized by common DExx and HELICc domains. These chromatin-remodeling complexes use energy-dependent mechanisms to move the DNA around the histone octamer and also alter histone octamer composition. Most significantly, the SWI/SNF complex has been shown to play essential roles in regulating nucleosome remodeling, contributing to both the activation (left, denoted in green) and repression (right, denoted in red) of gene expression programs in a context-dependent manner (17). During lineage-specific differentiation, the SWI/SNF complex cooperates with transcription factors to promote the activation of differentiation genes and silencing of proliferation genes. C, DNA methylation. Methylation of CpG islands located in proximity to transcription initiation is associated with heritable silencing (denoted in red) of gene expression (gene OFF) and is responsible for physiologic processes that depend upon epigenetic mechanisms. DNA methylation may be mediated by transcription factor– or PcG-mediated recruitment of DNMTs to specific gene loci. D, DNA demethylation. More recent studies have identified DNA demethylases (TET1,2) that hydroxylate 5-mC in CpG dinucleotides to 5-hC. Evidence suggests that TET enzymes are capable of iterative oxidation on substrates to 5-fC or 5-caC (23, 24). This leads to substrates upon which base excision repair mechanisms mediated by TDG excise a modified C and replace it with an unmodified C, allowing for rapid reactivation (gene ON) of previously silenced genes (24). 5-caC, 5-carboxymethylcytosine; 5-fC, 5-formylmethylcytosine; 5-hC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; TDG, thymine-DNA glycosylase.
methyltransferase undergoes significant changes (Fig. 2C; refs. 20–22). In normal differentiated tissues, DNA methylation is quite stable and is characterized by methylated intergenic regions and unmethylated gene promoters (22). By contrast, in cancer, large regions of methylation are lost from intergenic regions and gene promoters are aberrantly hypermethylated (22). In addition, tissue-specific differentially methylated regions that are highly stable in normal tissues are highly unstable in malignant tissues, indicating epigenetic instability (22). Although DNA methylation was long considered to be irreversible, the recent discovery of TET family proteins has elucidated a physiologic mechanism of DNA demethylation (23). Through their action as 5′-methylcytosine dioxygenases, TET enzymes convert 5′-methylcytosine residues on methylated DNA to 5′-hydroxymethylcytosine (Fig. 2D; ref. 24). Of importance, mutations in TET-encoding genes and genes that alter TET function are common in human myeloid malignancies (25, 26) and gliomas (27), respectively, and are associated with abnormal DNA methylation phenotypes. Hypermethylated genes in cancer are highly enriched for bivalent genes as well as other genes that are subject to PcG-mediated silencing in
stem cells (16, 28). Together, these findings suggest a developmental model of cancer initiation in which tumor-initiating cells become locked in an undifferentiated state as a result of aberrant DNA methylation at PcG-marked loci (Fig. 3B).

Epigenetic Deregulation in Pediatric Solid Tumors

Whether a tumor arises from an embryonic stem cell or multipotent progenitor, or from the dedifferentiation of a more specialized postnatal cell, a unifying model is that genetic alterations associated with tumorigenesis lead to initiation of malignant transformation as a result of epigenetic deregulation. Recent and emerging data generate compelling support for the critical contribution of this model to the origin and progression of pediatric solid tumors, in particular to Ewing sarcoma, neuroblastoma, and brain tumors.

Ewing sarcoma

Ewing sarcoma family tumors (EFT) are characterized by an undifferentiated histology, a highly metastatic phenotype, and the presence of chromosomal translocations that result in the creation of fusion oncogenes (29). In 85% of cases, the resulting chimeric protein is a fusion between EWS and FLI1 (30). EWS-FLI1 functions as an aberrant transcription factor and is believed to be an initiating tumorigenic event. The very primitive neuroectodermal histology of EFTs and their diverse presentation in bones and soft tissues suggest an early stem or progenitor cell of origin. Indeed, recent experimental evidence supports this hypothesis, and bone progenitors, mesenchymal stem cells (MSC), and neural crest stem cells (NCSC) have all been implicated as potential cells of origin (31–35).

Initial investigations into the function of EWS-FLI1 focused largely on identifying genes that were induced by the fusion. In the course of those studies, several promising candidates were identified, and genes such as NR0B1, NR0B1, DAX1 (37), and EZH2 (38) are now well established as direct transcriptional targets of EWS-FLI1. However, EWS-FLI1 represses as many genes as it induces (34, 39, 40), and the mechanisms by which EWS-FLI1 represses transcription and the contribution of gene repression to the EFT phenotype have become the focus of intense investigation.

A significant portion of EWS-FLI1–repressed targets are downregulated by the activity of its target gene, NR0B1, which functions to repress transcription by recruiting TLE transcriptional corepressors and HDACs to target gene promoters (39). Similarly, EWS-FLI1–mediated induction of EZH2 blocks differentiation of MSC and leads to the repression of genes involved in neuroectodermal and endothelial differentiation (38, 41). In NCSC, EZH2 and BMI-1 are both induced by EWS-FLI1; however, unlike EZH2, the mechanism of BMI-1 induction is likely to be indirect (34). This upregulation of BMI-1 leads to repression of p16 and maintenance of stemness, supporting the hypothesis that epigenetic silencing of p16 may be a key step in EFT initiation (Fig. 3B; ref. 34). Sustained expression of EWS-FLI1 in NCSCs results in progressive downregulation of transcription factors that are normally required for neuroectodermal and skeletal differentiation (ref. 34; C. von Levetzow and E.R. Lawlor, unpublished data). In established EFT, tumorigenicity is dependent on continued overexpression of EZH2 and BMI-1 (32, 38, 42, 43). To exploit this property, Thiel and colleagues (44) recently proposed cell therapy with cytotoxic T cells directed against EZH2 as a novel approach to EFT therapy, showing that the abnormal cancer epigenome could be a source of novel antigenic targets for immunotherapy (45).

Finally, regulation of PcG protein expression and function is mediated by a complex network of microRNAs [miRNA (46)] and by long noncoding RNAs [lncRNAs (7)]. EWS-FLI1 modulates the expression of both miRNAs (47) and lncRNAs (48), so it will be interesting to discover whether EWS-FLI1–mediated deregulation of ncRNA transcripts contributes to PcG protein dysfunction. In addition, the contribution of altered DNA promoter methylation to EFT pathogenesis is beginning to come into focus. A global analysis of DNA promoter methylation has revealed that the CpG islands of PcG target genes are aberrantly hypermethylated in EFT, and the promoters of transcription factors that are involved in neural differentiation become progressively methylated over time in EWS-FLI1–expressing NCSCs (C. von Levetzow and E.R. Lawlor, unpublished data). Thus, EWS-FLI1 drives EFT pathogenesis by invoking global deregulation of the epigenome through diverse mechanisms (Fig. 3B).

Progression of established EFT depends on continued expression of EWS-FLI1 (36). Inhibition of tumor growth following EWS-FLI1 inhibition is associated with reexpression of numerous repressed target genes, suggesting that the oncogenic phenotype is dependent, at least in part, on maintaining epigenetic repression (36). In support of this notion, targeted knockdown of BMI-1 in EFT leads to reduced tumorigenicity and induction of differentiation genes (Fig. 4A; ref. 42). In addition, exposure of EFT cells to HDAC inhibitors (Table 1) restores the expression of numerous EWS-FLI1-repressed genes and also inhibits growth and tumorigenesis (39, 49). Combining HDAC inhibition with decitabine, an inhibitor of DNA methylation, potentiates the growth-inhibitory effects of either agent alone, supporting a role for combination approaches to epigenetic therapy (50). Direct pharmacologic targeting of transcription factors continues to be an immense therapeutic challenge. In EFT, targeting the epigenome may be an effective way to circumvent the oncogenic activity of EWS-FLI1 without having to target the oncogene itself directly.

Neuroblastoma

Neuroblastomas are neural-crest–derived tumors that express genes characteristic of sympathoadrenal cell lineages. Mutations in PHOX2B, a major regulator of sympathoadrenal development, have been identified in 6% of familial neuroblastomas and also, more rarely, in
In the 1980s, histologic classification of neuroblastomas into subsets based on the extent of differentiation was prognostically relevant. Functional studies contributed to the concept that neuroblastoma cells correspond to different stages of sympathoadrenal development and retain the potential to differentiate into a number of neural crest cell lineages, and thus represent a multipotent sympathoadrenal progenitor cell with distinct tumorigenic potential. Consistent with these concepts, aggressive neuroblastoma tumors are characterized by increased expression of cell-cycle genes, whereas ganglioneuroma and ganglioneuroblastoma express genes associated with neural development. EZH2 is elevated in poor-prognosis, undifferentiated neuroblastoma and is associated with enrichment of H3K27me3 at the promoters of tumor suppressor genes such as CASZ1, RUNX3, NGFR (p75), and NTRK1 [TrkA]. The cause of such elevated EZH2 levels is still unknown, but a number of genetic alterations at the EZH2 locus may contribute: 7q35-36 is amplified in 10% to 15% of tumors, and >50% of neuroblastoma tumors have gains of the entire Chr7 or 7q. BMI-1 is a direct transcriptional target of MYCN; it is also overexpressed by neuroblastomas and is essential for their tumorigenicity. In embryonic stem cells, LSD1 preferentially demethylates H3K4me3, thus favoring PcG-associated H3K27me3 silencing at bivalent loci and maintenance of stemness. This physiologic function of LSD1 and EZH2 overexpression most likely contributes to the poor prognosis that is associated with LSD1-overexpressing neuroblastoma compared with more-differentiated forms of the tumor.

The relationship between PcG protein dysregulation and DNA methylation in neuroblastoma tumors is tenuous but intriguing. The CHD5 tumor suppressor gene, which is part of a family of chromatin remodeling proteins, is in a chromosomal region that is subject to loss of heterozygosity in a number of tumors, including neuroblastoma. CHD5 contains a bivalent chromatin mark in stem cells, and in neuroblastoma cells with loss of heterozygosity, the remaining allele is silenced via DNA methylation. An examination of genome-wide expression microarray data from primary neuroblastoma tumors reveals an inverse correlation between EZH2 and CHD5 expression and a direct correlation between EZH2 and DNMT1. Thus, overexpression of PcG proteins in neuroblastoma may promote recruitment of DNMTs and induce
permanent gene silencing of key developmental loci. It is not clear whether silencing of a single gene or an entire pathway is needed for neuroblastoma initiation or progression, but a number of genes, including a commonly hypermethylated gene in cancer (HIC1), putative tumor suppressor genes (RASSF1A and PRKCDBP), differentiation genes (HOXA9), and apoptosis genes (CASP8, APAF1, and TMS1), have been consistently identified (62, 64–67). In addition, emerging data from unbiased, global approaches have confirmed established loci and identified novel DNA methylated regions (telomeric silencing of 1p, 3p, 11q, and 19p) and a methylator phenotype that is associated with a worse clinical outcome (65, 67, 68).

In addition to MYCN’s association with activating histone chromatin modifications (69), there is also an intriguing association of MYCN amplification with DNA methylation. This was first noted with CASP8 (70) and has now been associated with additional loci (66, 67). In neuroblastoma cell lines, genome-wide MYCN DNA binding is significantly associated with the binding of MeCP2, a CpG methyl-binding protein (71). At gene promoters, transcription is relatively high when MYCN is bound alone, intermediate when both MYCN and MeCP2 are present, and low when only MeCP2 is present. This suggests that MYCN may serve as an initial but transient focus to recruit components required to mediate DNA methylation (71).

There is abundant evidence to support the use of epigenetic modifiers in neuroblastoma therapy. First, the ability of retinoids to restore differentiation indicates that despite the genetic alterations associated with high-risk tumors, the regulatory signaling pathways that control growth and differentiation are intact but dysregulated. Indeed, it is becoming increasingly evident that retinoids function to relieve epigenetic suppression. First, neuroblastoma cells

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**Table 1.** Epigenetic modifiers—current drugs and agents in development

<table>
<thead>
<tr>
<th>Class of agent</th>
<th>Target</th>
<th>Status</th>
<th>Tumor</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDAC inhibitors</td>
<td>HDAC1-3.6</td>
<td>FDA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cutaneous T-cell lymphoma</td>
<td>(86, 95, 96)</td>
</tr>
<tr>
<td>Vorinostat [SAHA, Zolinza (Merck)]</td>
<td>HDAC1-3.8</td>
<td>Ped. phase I</td>
<td>Relapsed solid tumors</td>
<td></td>
</tr>
<tr>
<td>Romidepsin [FK228, Istodax (Celgene)]</td>
<td>weak HDAC6.4</td>
<td>Ped. phase I</td>
<td>Cutaneous, peripheral T-cell lymphoma</td>
<td>(86)</td>
</tr>
<tr>
<td>Entinostat (SNDX-275), panobinostat (LBH589), belinostat (PDX101)</td>
<td>HDAC1</td>
<td>Phase I</td>
<td>Lymphoma</td>
<td></td>
</tr>
<tr>
<td>Valproic acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>HDAC1-4</td>
<td>Phase I</td>
<td>Relapsed solid tumors</td>
<td></td>
</tr>
<tr>
<td>PCI-34051</td>
<td>HDAC1-3, weak HDAC8</td>
<td>Ped. phase I</td>
<td>Refractory solid or CNS tumors</td>
<td>(86, 98)</td>
</tr>
<tr>
<td>HMT inhibitors</td>
<td>K&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Preclinical</td>
<td>Lymphoma, MYC-driven tumors</td>
<td>(75, 100)</td>
</tr>
<tr>
<td>EZH2 (SET domain)</td>
<td>EZH2</td>
<td>Preclinical</td>
<td>EZH2 mutated lymphomas</td>
<td>(101)</td>
</tr>
<tr>
<td>DZNep</td>
<td>DOT1L</td>
<td>Preclinical</td>
<td>MLL-rearranged AML and ALL</td>
<td>(102, 103)</td>
</tr>
<tr>
<td>EZH2</td>
<td>Preclinical</td>
<td>(104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPZ004777</td>
<td>Preclinical</td>
<td>(104)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histone demethylase inhibitors</td>
<td>LSD1</td>
<td>Preclinical</td>
<td>(61)</td>
<td></td>
</tr>
<tr>
<td>Monamine oxidase inhibitors</td>
<td>Pargyline, clorgyline, tranylcypromine&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Preclinical</td>
<td>(61)</td>
<td></td>
</tr>
<tr>
<td>Polyamine analogues&lt;sup&gt;c&lt;/sup&gt;</td>
<td>LSD1</td>
<td>Preclinical</td>
<td>(105)</td>
<td></td>
</tr>
<tr>
<td>Bisguanidines, biguanides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA methylation inhibitors</td>
<td>DNMT</td>
<td>FDA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>MDS, CML</td>
<td>(86)</td>
</tr>
<tr>
<td>Decitabine [Dacogen (Eisai)]</td>
<td>DNMT</td>
<td>Clinical trials</td>
<td>MDS, CML</td>
<td>(86)</td>
</tr>
<tr>
<td>Azacytidine [Vidaza (Celgene)]</td>
<td>DNMT</td>
<td>Clinical trials</td>
<td>MDS, CML</td>
<td>(86)</td>
</tr>
<tr>
<td>Zeblvarine</td>
<td>DNMT</td>
<td>Clinical trials</td>
<td>MDS, CML</td>
<td>(86)</td>
</tr>
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<sup>a</sup>Target specificity for particular HDACs based on functional data (95).
<sup>b</sup>FDA-approved for a cancer therapy indication.
<sup>c</sup>FDA-approved for noncancer indications.

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; CNS, central nervous system; DZNep, 3-deazaaneplanocin A; FDA, U.S. Food and Drug Administration; MDS, myelodysplastic syndrome; Ped., pediatric.
treated with retinoids show decreases in steady-state levels of BMI-1 (60), EZH2 (D.Y. Oh and C.J. Thiele, unpublished data), and LSD1 (61), and a recent genome-wide study indicated that retinoids reverse the methylation status of hundreds of gene promoters (72). Second, HDAC inhibitors, either alone or in combination with cis-retinoic acid, inhibit neuroblastoma growth in vitro and in vivo (73, 74). Third, reactivation of caspase 8 in drug-resistant neuroblastoma cells by exposure to DNA methylation inhibitors restores sensitivity to standard cytotoxic agents (70). Fourth, inhibition of EZH2 by 3-deazaneplanocin A (DZNep) leads to reexpression of EZH2 silenced tumor suppressor genes and results in decreased growth and differentiation of neuroblastoma cells (Fig. 4B, ref. 57).

Finally, the most aggressive neuroblastomas are driven by aberrant activation of MYCN. Recent studies showed that JQ1, a novel small-molecule inhibitor of the chromatin-modifying cofactor BRD4, dramatically inhibits proliferation and promotes terminal differentiation of acute myelogenous leukemia (AML) cells, in part by suppressing MYC function (75). Whether epigenetic therapy with JQ1 can be used to block the oncogenic activity of MYCN in neuroblastoma remains to be determined, but these findings provide an exciting new opportunity for investigation.

**Brain tumors**

Epigenetic deregulation is emerging as a fundamental process underlying the pathogenesis of pediatric brain tumors. Hypermethylation of the RASSFIA tumor suppressor gene has been identified in nearly 90% of medulloblastomas and ependymomas (76), and upregulation of PcG proteins, in particular BMI-1 and EZH2, is common and is associated with worse clinical outcomes (77, 78). Intriguingly, recent next-generation sequencing studies of pediatric medulloblastomas revealed that although tremendous genetic diversity exists between individual tumors, there is a marked overrepresentation of disruptions in genes that encode for chromatin modifiers and epigenetic regulators (14, 79). In a study by Northcott and colleagues (79), deletions in individual genes that encode H3K9 methyltransferases (i.e., EHMT1 and SMYD4) were detected in 2% of cases in a series of 212 tumors, and H3K9 demethylase genes were selectively amplified in another 2%. In total, mutations, deletions, or amplifications in genes that converge on modulating H3K9 methylation were detected in 19% of the tumors examined in that study, and an absence of nuclear staining for methylated H3K9 was confirmed in 41% of cases in an independent cohort (79). Dimethylation of H3K9 is required for silencing of proliferative genes and successful terminal differentiation of progenitor cells in the external granule layer (79). Mutations that disrupt H3K9 methylation likely contribute to malignant transformation by blocking normal differentiation (79). In a second study, inactivating mutations of the HMTs MLL2 and MLL3 were detected in 16% of primary tumors, and mutations in the SWI/SNF components SMARCA4 and ARID1A were detected in another 4% (14). Thus, mutations in epigenetic regulators figure prominently in pediatric medulloblastoma, and cumulatively these lesions contribute to tumor initiation and progression by repressing normal developmental pathways while promoting the maintenance of a more stem-like state.

Next-generation sequencing studies of gliomas have also uncovered a basis for epigenetic deregulation, with the discovery that 70% to 80% of grade II and III astrocytomas harbor a mutation in either the IDH1 or IDH2 isocitrate dehydrogenase gene (80). IDHs produce α-ketoglutarate, a necessary cosubstrate for histone demethylases and TET family proteins (25). Mutated IDH proteins produce D-2-hydroxylutarate, which acts as a competitive inhibitor of α-ketoglutarate and thereby inhibits histone and DNA demethylation (81). Thus, IDH-mutated gliomas are epigenomically abnormal and this contributes to an abnormal DNA methylator phenotype (82). Of importance, however, mutations in IDH genes are associated with younger age and an improved prognosis, indicating that epigenetically driven tumors may be less aggressive and more responsive to therapy than tumors that are characterized by genetic instability, such as those that occur in older patients (25).

**Other embryonic solid tumors**

Genome-wide studies of Wilms tumor chromatin and DNA methylation have been extremely informative and have identified global epigenetic aberrations beyond the well-established hypermethylation of H19 that contributes to loss of imprinting and overexpression of IGF2 (83). In particular, the chromatin landscape of Wilms tumors was shown to be highly related to embryonic stem cells and associated with upregulation of PcG activity, abnormal retention of bivalent marks, and silencing of genes that direct early renal differentiation (84). Although the precise cell of origin of retinoblastoma tumors remains controversial, the clinical presentation of these tumors before birth and in early infancy leaves no doubt as to their embryonic origin. Of interest, a recent study found that developmental pathways that are normally expressed in a mutually exclusive fashion during normal retinal cell development are abnormally coexpressed in single retinoblastoma cells (85). Although the mechanisms underlying this observation remain to be elucidated, it is highly probable that disruption of normal epigenetic regulation within developing retinal cells contributes to this oncogenic developmental abnormality.

**Therapeutic opportunities and challenges**

U.S. Food and Drug Administration (FDA)-approved use of epigenetic modifiers is currently limited to 2 classes of agents (Table 1). Specifically, HDAC inhibitors (vorinostat and romidepsin) and DNA methylation inhibitors [5-aza-cytidine and deoxyazacytidine (decitabine)] are approved for use in the treatment of cutaneous T-cell lymphomas and myelodysplastic syndrome, respectively, and function to reactivate aberrantly silenced genes (86). These agents, as well as other classes of HDAC inhibitors (e.g., valproate), are now being evaluated in early-phase clinical trials in adult solid tumors, in particular for patients with relapsed and
metastatic sarcomas (http://www.cancer.gov/clinicaltrials). Pediatric phase I studies for romidepsin, vorinostat, and valproic acid have been completed, and investigators report that each agent is well tolerated and results in increased acetylated-H3 histones in peripheral blood lymphocytes, a surrogate marker of drug target activity (Table 1). No objective responses were seen with the single agents, but a complete response in one neuroblastoma patient who received a combination of 13-cis retinoic acid and vorinostat in the phase I study encouraged the testing of combination treatments. Currently the New Approaches to Neuroblastoma Therapy consortium is evaluating the toxicity of vorinostat in combination with 13-cis-retinoic acid (http://www.cancer.gov/clinicaltrials; NCT01208454) or meta-iodo-benzyl-guanidine ([131I-MIBG] therapy [NCT01019850 (87)]. A phase I/II study is also under way evaluating vorinostat in combination with etoposide in pediatric patients with relapsed and refractory sarcomas as well as other solid tumors (NCT01294670). Unfortunately, a recent Children’s Oncology Group study found that dose-limiting hematologic toxicities were experienced by children with relapsed solid tumors who were treated with low-dose decitabine in combination with cytotoxic chemotherapy (88). It remains to be determined whether drug toxicity and response rates to HDAC and DNA methylation inhibitors are strictly determined by the somatic tumor genotype or whether heritable germline factors may also contribute to outcome (89). Thus, there is still much work to be done to determine the optimal dose and schedule regimens of currently available drugs, especially with regard to heavily pretreated patients. Strong consideration should be given to testing epigenetic modifiers as components of initial therapy in newly diagnosed patients with high-risk disease.

Current HDAC and DNA methylation inhibitors are very nonspecific in their action, invoking genome-wide effects and inducing cell death and differentiation through multiple different molecular mechanisms (90). Approved HDAC inhibitors broadly inhibit the activity of multiple different HDACs (Table 1), and it is hoped that as new, more target-specific agents are developed, there will be fewer dose-limiting toxicities. An improved understanding of the biology of epigenetic regulation has also contributed to the development of new classes of agents that have been designed to specifically target the cancer epigenome (Table 1). In addition, as discussed above with respect to retinoids in neuroblastoma, an improved understanding of developmental biology is uncovering epigenetic mechanisms of action for established drugs. This raises the possibility that in the future, other drugs may also be identified for repurposing as epigenetic modifiers.

Early failures with HDAC and DNA methylation inhibitors in clinical studies of adult solid tumors dampened initial enthusiasm for their potential as effective therapeutic agents. However, an improved understanding of the mechanisms of drug action and of tumor biology led to changes to drug dose and delivery schedules that have proved beneficial (86). It is now apparent that epigenetic modifiers exert their biologic effects at doses far below the maximum tolerated doses that are identified in classic phase I trials. In addition, single-agent therapy with DNMT inhibitors may lead to only transient reexpression of silenced genes as a result of continued histone deacetylation of the repressed loci (91). Therefore, chronic low doses of epigenetic modifiers (i.e., DNMT inhibitors together with HDAC inhibitors) in combination with standard cytotoxic therapy may be required to normalize the epigenetic landscape of tumor cells and induce sustainable clinical remissions (86, 91). Indeed, in a recent clinical trial, low-dose combination epigenetic therapy successfully induced clinical responses in heavily pretreated patients with non–small cell lung cancer (92). However, it is noteworthy that the responses occurred gradually over several months. This observation is consistent with studies of epigenetic modifiers in animal models, which showed that one can better determine the therapeutic efficacy of these agents by evaluating tumor-initiating potential rather than tumor shrinkage (93). Thus, standard Response Evaluation Criteria in Solid Tumors (RECIST) may not be the best initial tool to evaluate epigenetic drug efficacy. Ideally, biomarkers of drug efficacy should be used to ascertain that the agents are having the predicted biologic effect. Furthermore, it will be imperative to ensure the availability of tissues for pathologic assessment of response whenever new epigenetically targeted agents are introduced into the clinical setting.

Conclusions

Genetic mutations that result in alterations in DNA sequence have classically been considered drivers of tumorigenicity. The recent explosion of knowledge about stem cell and developmental biology combined with the advent of next-generation sequencing technologies has led to the realization that disruptions to the epigenome are at least equal contributors to the pathogenesis of human cancer (86). Thus, one might consider that tolerance and propagation of genetic lesions that initiate malignant transformation are dependent on epigenetic plasticity in the target cell (94). Nowhere is this model more supported than in the diverse array of pediatric solid tumors that, in contrast to adult malignancies, arise as a consequence of relatively few genetic mutations and progress to invasive, drug-resistant, and metastatic phenotypes despite the persistence of relatively stable genomes (14, 94). Although epigenetic therapies have not yet been extensively tested in children, the essential contribution of epigenetic deregulation to pediatric solid tumors provides a compelling rationale for their use. Continued elucidation of the contribution of epigenetic deregulation to the pathogenesis of these tumors will provide critical insights into the role of epigenomic instability as a driving force behind the malignant phenotype, and will facilitate the translation of this knowledge into effective epigenetically targeted therapies.

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No potential conflicts of interest were disclosed.
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Writing, review, and/or revision of the manuscript: E.R. Lawlor, C. J. Thiele
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Study supervision: C. J. Thiele

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2779


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Elizabeth R. Lawlor and Carol J. Thiele