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REVIEW

Epigenetic modification in chromatin machinery and its deregulation in pediatric brain tumors: Insight into epigenetic therapies

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ABSTRACT

Malignancies are characterized by the reprogramming of epigenetic patterns. This reprogramming includes gains or losses in DNA methylation and disruption of normal patterns of covalent histone modifications, which are associated with changes in chromatin remodeling processes. This review will focus on the mechanisms underlying this reprogramming and, specifically, on the role of histone modification in chromatin machinery and the modifications in epigenetic processes occurring in brain cancer, with a specific focus on epigenetic therapies for pediatric brain tumors.

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Role of epigenetics in chromatin machinery

Epigenetic background

DNA is packaged in chromatin, whose basic repeating unit, the nucleosome, consists of ~146 nucleotides wrapped around an octamer of specialized proteins called histones. Each of the 8 histone proteins has amino acid "tails" that stick out from the nucleosome. The modulation of the wrapping of DNA around this octamer constitutes the physical basis for regulation of transcription through nucleosomal DNA.[1,2] Epigenetic processes include covalent modifications of DNA (e.g., DNA cytosine methylation and hydroxymethylation) and of histone tails (such as histone lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, and lysine ubiquitination or sumoylation).[3-5] Enzymes bringing these modifications to DNA or to the histone tail are termed "writers" or "erasers," while chromatin-associated "readers" are proteins involved in the recruitment of other proteins that contain additional chromatin-modifying activities (including writers and erasers that add or remove, respectively, specific histone posttranslational modifications) (reviewed in[6]). All together, these modifications determine active and repressive chromatin states of genes and of chromosomal regions, and thus operate as switches to turn gene expression either "on" or "off," or to modulate gene expression levels (such as by enhancer methylation).[7,8]

Histone acetylation

Histone acetylation leads to an increased negative charge, which loosens the interaction between the histone and the negatively charged DNA. In addition, acetylated histones recruit specific chromatin-associated proteins that contain bromodomains as the dominant mode of recognition of acetylated lysine residues, present in 47 human proteins (reviewed in[6]). Histone acetylation also plays an important role in the regulation of RNA Polymerase II (RNAP II) activation by enhancing the search kinetics of transcriptional activators and, later, accelerating the transition of RNAP II from initiation to elongation.[9] For example, studies in living cells revealed that H3K27ac associated with active promoters and enhancers can modify downstream transcription kinetics by as much as 50%.[9]

Histone methylation

Histone methylation, by contrast, does not alter histone charge but, instead, creates a docking site for chromatin-associated proteins that contain specific methyl histone-binding domains. Histone lysine residues can be mono-, di-, or tri-methylated, whereas arginines can be monomethylated or symmetrically/asymmetrically dimethylated, with each modification having a specific biologic effect. Several lysine residues in histone H3 and H4, namely, H3K4, K9, K27, K36, K79, and H4K20, have been found to be methylated and have been extensively studied[10] (Fig. 1). Methylation of H3K4, H3K36, and H3K79 is often associated with transcriptionally active euchromatin. By contrast, methylation of H3K9, H3K27, and H4K20 helps specify transcriptionally repressed heterochromatin. Specifically, among the most studied modifications, H3K4me3 is primarily associated with active promoters; H3K4me1 is associated with active chromatin outside of promoters (e.g., enhancers),[10,11] while H3K27me3 is associated with silencing by the Polycomb Repressive Complex 2 (PRC2), specifically enhancer of zeste homolog 2 (EZH2 or KMT6) methyltransferase, the enzymatic

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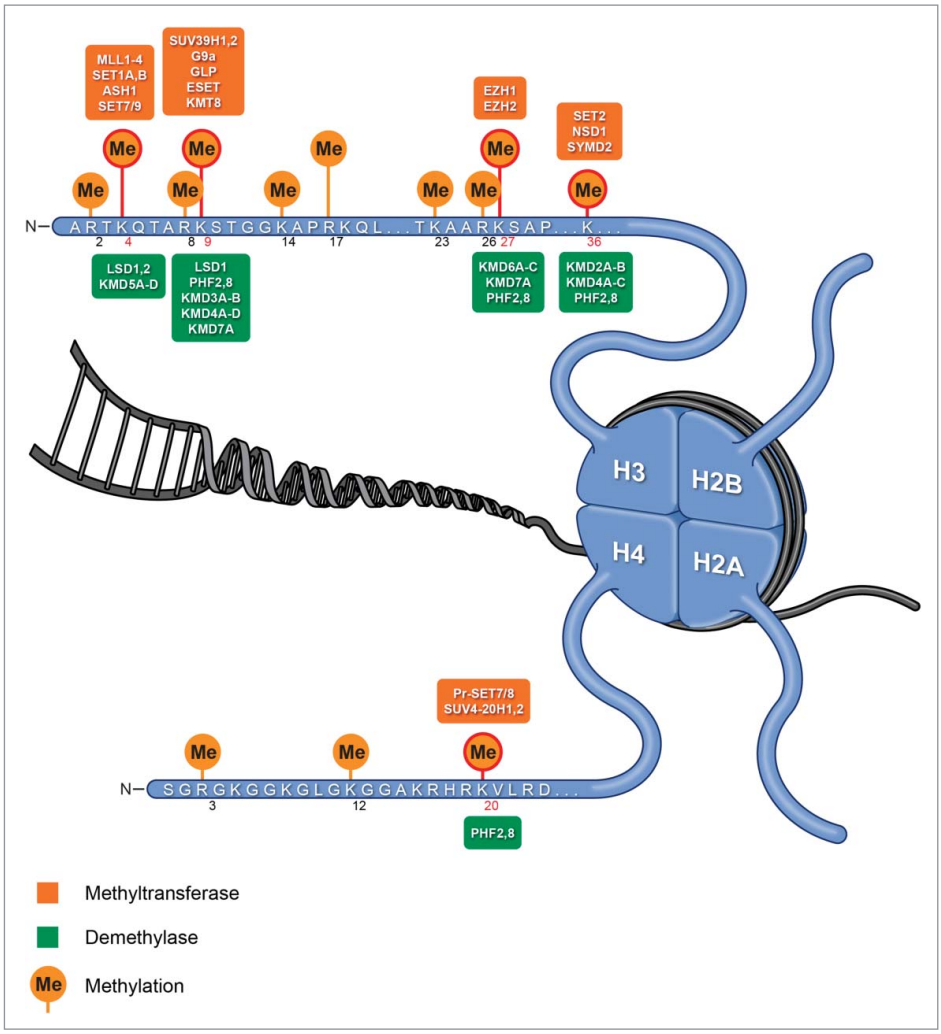

Figure 1. Histone methylases and demethylases. Histones with known lysine methylations: H3K4, K9, K27, K36, K79, and H4K20, and enzymes involved in regulation the methylation status (KMTs and KDMs) of these residues (residues extensively studied in cancer are shown in red circle).

subunit of PRC2.[12,13] Recent discoveries highlight the importance of modifications of these histone methyl marks and/or histone mutations occurring in pediatric brain cancer.

Role of histone modifications in the regulation of transcription

The distribution of histone modifications throughout the genome has been determined by genome-wide comprehensive analyses (e.g., chromatin immunoprecipitation sequencing, ChIP-seq).[14,15] These analyses helped to develop predictive models to explore the interaction between histone modifications and measures of transcription at promoters, distinguishing between modifications known to be added as a consequence of transcription (such as H3K36me3 and H3K79me2) and other categories of histone marks.[14] These models showed that activating acetylation marks (H3K27ac and H3K9ac) are roughly as informative as activating methylation marks (H3K4me3 and H3K4me2).[14] Moreover, repressive histone marks (H3K27me3 or H3K9me3) must be used to accurately predict expression. Studies also show that H3K79me2 occurs preferentially at the 5′ ends of gene bodies and H3K36me3 occurs more often at the 3′ end, supporting the previous model in which the H3K79me2 to H3K36me3 transition occurs at the first 3′ splice site.[14] Although these studies describe some relationships between histone modifications and transcription, it has remained unclear how genomic regions modified by histone methylation are specified and formed. For example, recent molecular studies showed that changes in H3K27me3 level, especially in the body of a subset of genes, are triggered by changes in transcriptional activity itself: indeed, deleting the transcription start site increases H3K27me3 level in the gene body.[16,17]

Role of histone modifications in other biologic processes

The full biologic significance of these histone modifications still needs further investigation, as these modifications are involved in a wide variety of processes. The intensities of some of these signals at enhancers can regulate tissue-specific expression patterns during development.[18] Some of these marks have been identified as a chromatin signature linked to transcriptional consistency and cell identity, and highlighting that breadth is a key component of chromatin states.[19] The regulation and

distribution of histone methylation and of related enzymes can also change during the cell cycle, affecting replication timing (reviewed in[20]). Of note, the description of these histone post-translational modifications above is not exhaustive as, for example, phosphorylation also occurs and impacts transcription.[21,22]

DNA methylation

In addition to histone modifications, DNA methylation plays a key role in coordinating gene expression and chromatin remodeling in brain tumors. DNA methylation, a process occurring at the cytosine of the CpG dinucleotide, is usually associated with gene silencing.[23] CpG is underrepresented in the genome, but clusters or "islands" are often found at the 5′ end of a gene. Surveys of DNA methylation in human tissues have established a complex landscape, including both tissue-specific and invariant methylation patterns.[24] Current models suggest that DNA methylation helps counteract chromatin disruption, as found in nucleosome displacement during RNAP elongation, for example, while CG-poor regulatory regions generally acquire a low methylation state when occupied by transcription factors (reviewed in[23]).

Epigenetic changes in brain tumors

Altered epigenetics can play an important part in the development of pediatric brain tumors. The identification of these changes has been important for prognosis and to predict the response to treatment of brain tumors. The possibility to revert epigenetic changes has been valuable to develop and further improve therapeutics, leading to the design of an appropriate therapy for these tumors. Moreover, consortia such as the Roadmap Epigenomics Consortium have extensively advanced our understanding of enhancer/gene regulation across a comprehensive spectrum of cell lines and tissues, establishing the complex cartography of the human regulatory landscape, using diseased and healthy biopsies to link these epigenomic data to the corresponding genetic information.[25,26] Comprehensive molecular profiling studies have greatly broadened our knowledge of the underlying genomic and epigenomic aberrations that are associated with the initiation and progression of these brain cancers.[27]

High-grade glioma

Pediatric high-grade gliomas are clinically and biologically distinct from adult gliomas. Approximately 80% of pediatric high-grade gliomas are restricted to the ventral pons, and are thus named diffuse intrinsic pontine gliomas (DIPGs). DIPGs primarily affect very young children, with peak incidence at 6 y of age, and have the highest mortality of all childhood solid tumors.[28] One of the obstacles to improving therapies is that the spectrum of molecular alterations is quite different in tumors from children and adults. Historically, pediatric high-grade glioma was considered similar to secondary glioblastoma multiform (GBM), an adult high-grade glioma that evolves from a less malignant precursor due to changes in gene expression, such as *OLIG1* and *OLIG2*, important neuro-developmental genes.[29]

K27M mutations in histone H3.1 or H3.3

In contrast to adult tumors, pediatric high-grade gliomas commonly have somatic oncogenic gene mutations (*H3F3A* and *HIST1H3B*), resulting in replacement of lysine 27 by methionine (K27M) in the encoded histone H3 proteins (Table 1).[27,30] Because it is subject to posttranslational histone modifications, K27 is a key residue in histone H3 variants and can be methylated or acetylated. Substitution of K27 with methionine is thought to contribute to tumorigenesis via defects in chromatin remodeling.[27,30] In a recent study aiming to further understand the impact of K27M-histone H3.3 (H3.3K27M) on tumorigenesis in DIPG, immortalized human astrocytes have been used and were transfected with N-terminally flag-tagged expression vectors containing H3.3WT, H3.3K27M, or empty vector control.[31] In this study, alterations in both expression and methylation profiles of the H3.3K27M expressing cells vs. both wild type (WT) and controls were observed. The top pathways perturbed by H3.3K27M expression corresponded to molecular and cellular functions, especially an increase in cell-to-cell signaling and a decrease in cell cycle progression. The top molecular and cellular functions affected by methylation changes in H3.3K27M cells were embryonic development, decreased cell growth and proliferation, and increased cell-to-cell signaling. H3.3K27M cells showed reduced global H3K27me3 levels compared with controls. Similarly, immunohistochemical staining of patient-derived DIPGs showed a decrease in global H3K27me3 levels in H3.3K27M-positive tumors compared with WT tumors. Global levels of H3K4me3 and H3K9ac were not altered in a mutation-dependent manner.[31]

To have a better understanding of the role of the H3.3K27M mutation in a specific cell type, another study, from Funato et al., created a model of DIPG by differentiating human

Table 1. Somatic mutations in epigenetic regulator genes or histone genes in brain cancers.

Gene mutated	Tumor	Reference
H3F3A (encoding H3.3 with K27M)	High-grade glioma	29, 30, 41, 42, 46
HIST1H3B (encoding H3.1 with K27M)	High-grade glioma	30, 41, 46
H3F3A (encoding H3.3 with G34R or G34V)	High-grade glioma	29, 41, 42, 46
NF1	GBM	46, 78
SET2 (KMT3A)	High-grade glioma	36
	GBM	46
MLL2 (MLL4, KMT2C)	Medulloblastoma	50, 95, 96, 97, 98, 99
	GBM	46
MLL3 (KMT2D)	Medulloblastoma	95
	GBM	46
EZH2 (KMT6A)	GBM	46
LSD1 (KDM1A)	Medulloblastoma	99
JMJD1A (KDM3A)	Medulloblastoma	99
JMJD2C (KDM4C)	Medulloblastoma	99
JARID1A (KDM5A)	Medulloblastoma	99
JARID1B (KDM5B)	Medulloblastoma	99
JARID1C (KDM5C)	High-grade glioma	36
UTX (KDM6A)	Medulloblastoma	50, 99
	GBM	46
JMJD1C (KDM7A)	Medulloblastoma	99
HDAC2	GBM	46
HDAC9	Medulloblastoma	99

embryonic stem cells into neural progenitor cells, and then transducing them with a viral vector carrying the gene encoding H3.3K27M.[32] Remarkably, the mutation was only oncogenic in a specific cell type, neural progenitors derived from embryonic stem cells, and not in undifferentiated embryonic stem cells or astrocytes derived from these cells. The neoplastic transformation occurred due to a synergy between H3.3K27M expression with *p53* loss and *PDGFRA* activation in these particular cells. The expression of the stem cell-associated genes (e.g., *LIN28B, PLAG1*, and *PLAGL1*) was also upregulated by H3.3K27M, and reducing expression of these genes inhibited tumor cell growth.[32] All together, these studies demonstrate the role of the K27M mutations on gene expression, methylation patterns, and transformative capacity. Of potential significance, in addition to the reduction in H3K27 methylation levels, overexpressing H3.3K27M in *Drosophila melanogaster* caused a strong increase in H3K27 acetylation levels, associated with an increased in bromodomain-containing protein 1 (BRD1) and bromodomain-containing protein 4 (BRD4) in H3.3K27M–containing nucleosomes.[33] The same observations have been made when the mutant was overexpressed in mammalian cells. Consistently, the *Drosophila melanogaster* K27M mutant models resemble PRC2 loss-of-function phenotypes, causing reduction of H3K27 methylation and derepression of PRC2 target genes. This alteration may indicate a similar molecular pathogenesis for K27M pediatric glioma models. Interestingly, these experiments in flies have been extended to other histone lysine-to-methionine mutations (i.e., H3K9M), consistently showing a depletion of methylation levels and a possible role in heterochromatic silencing.[33]

G34V/R mutation in Histone H3.3

Other pediatric high-grade gliomas involve mutations occurring at glycine at position 34 and result in replacement of glycine 34 by valine or arginine (G34V/R), which are exclusive to *H3F3A* (Table 1) (for review see[34]). G34R-mutant and G34V-mutant H3.3 are associated with global DNA hypomethylation, which is particularly pronounced in telomeric regions. These mutations can also interfere with the regulatory H3K36me3 modification, especially at the *MYCN* locus, associated with increased transcription of this gene, which drives glioma formation in neural stem cells *in vivo*.[35] H3K36me3 can be further disrupted by mutations in the H3K36 trimethyl transferase *Suppressor of variegation, Enhancer of zeste, and Trithorax 2* (*SET2* or KMT3A).[36] Besides its important role in transcription,[37] H3K36me3 is involved in recruiting DNA mismatch repair proteins, ensuring replication fidelity by correcting mismatches generated during DNA replication, thereby reducing spontaneous mutation frequency.[38] Of note, besides the impact of somatic missense mutations in histone H3 genes in pediatric brain malignancies, mutations are also found in bone malignancies. For example, glycine 34 to tryptophan/leucine (G34W/L) mutations occur in giant cell tumors of the bone, lysine 36 to methionine (K36M) mutations have been reported in ~95% of chondroblastomas, and H3K36 mutations promote sarcomagenesis.[39-42]

Associated changes in DNA methylation patterns

DNA methylation patterns have been extensively studied in adult GBM and have helped guide clinical trials, predicting the recurrence of tumors and resistance to radiotherapy. Indeed, CpG island methylator phenotype (G–CIMP) helped subdivide GBMs into glioma, G–CIMP-positive and G–CIMP-negative GBM subsets, partially predicting response to treatment. Several studies have reported promoter-associated hypermethylation of specific loci in GBM, which frequently affects the expression of genes that have known tumor suppressor functions and/or are involved in cell proliferation or radiation sensitivity (reviewed in[27]). A famous example of a clinical biomarker to predict response to temozolomide in gliomas is the inactivation by promoter hypermethylation of the *O6-methylguanine-DNA methyl-transferase*–encoding (*MGMT*) gene in samples from patients with GBM.[43-46] Importantly, part of The Cancer Genome Atlas (TCGA) studies focused on examining the genomic and epigenomic modifications occurring in GBM. These studies, applying genome-wide DNA methylation profiling in an adult patient cohort, led to the identification of a G–CIMP positive group associated with *isocitrate dehydrogenase 1* (*IDH1*) mutations, and hypermethylation at a large number of loci was linked to a less severe outcome.[47,48] Applied genome-wide DNA methylation profiling in cohorts of pediatric and adult patients also described recurrent age-specific mutations in *H3F3A*, while tumors enriched for *PDGFRA* alterations occur in patients from a wider age range.[29] These techniques also led to subgrouping DIPG patients based on CpG island methylation, identifying a subgroup with high-level amplification of *MYCN* and high-grade histology, in which targeting histones would be irrelevant.[31] Observations of DNA methylation profiles across all tumor sites in DIPG tissues was strongly associated with alterations in a specific histone 3 variant mark.[49] K27M variants recently have been shown to inhibit SET-domain-containing histone methyltransferases (Table 1),[37] possibly accounting for the specific DNA methylation pattern observed in H3.3K27M non-brainstem tumors.[29] The finding that DNA methylation profiles are associated with the K27M mutation regardless of tumor location supports its role in driving the epigenetic phenotype. Interestingly, gain-of-function mutations in *ACVR1, FGFR1*, and *PDGFRA*, encoding 3 growth factor receptors, were also associated with H3K27M variants.[49]

Medulloblastoma

Medulloblastoma, an aggressively growing pediatric brain tumor that arises in the cerebellum or medulla/brainstem, is also characterized by a reprogramming of DNA methylation patterns. Medulloblastoma is the most common malignant brain tumor in children, and shows tremendous biologic and clinical heterogeneity. Approximately 40% of children experience tumor recurrence, 30% will die from their disease, while the survivors have a significantly reduced quality of life. The genetic and epigenomic characterizations of medulloblastoma have progressed dramatically in recent years.[50] For example, H3K27ac- and BRD4-DNA interactions have been found to strongly correlate at active medulloblastoma enhancer loci.[51] Interestingly, BRD4 inhibition has been reported to decrease cell viability in medulloblastoma cell lines and xenografts.[52,53] In addition to these epigenetic changes occurring in medulloblastoma, reprogramming of DNA methylation patterns in

these tumors shows focal regions of low methylation linked to transcription-factor-binding sites, shedding light on differential transcriptional networks between subgroups. At the same time, increased methylation due to re-normalization of repressed chromatin in DNA methylation valleys was positively correlated with gene expression.[51,54] Importantly, genetic analysis showed a lack of overlap between the profile of primary tumors and that of matched samples of local or metastatic recurrence,[55] bringing another layer of complexity for the treatment of medulloblastomas. Regarding the genetic plasticity occurring in these tumors and the related resistance to treatments, the investigators proposed that a biopsy after recurrence should be mandatory for all future clinical trials for medulloblastoma to determine whether therapeutic targets are present in the dominant clones at recurrence.[55]

Ependymoma

Ependymoma is also among the most common pediatric brain tumor and remains a leading cause of cancer death in children. Over 90% of pediatric ependymomas are intracranial, with 2 thirds occurring in the posterior fossa, while many adult cases occur in the spinal cord (reviewed in[56]). Genomic abnormalities have often been observed in ependymoma, including genomic gains and losses or translocations within the ependymoma genome, but these genomic aberrations are more frequent in adult ependymomas. Methylated genes and gene expression profiles are associated with tumor location, patient age at disease onset, grade, and retrospective risk for relapse[57] (also reviewed in[56]). For example, deregulation of genes involved in neural differentiation and maintenance, particularly ion transport and synaptogenesis, highlights the importance of these events in the formation of this supratentorial ependymoma subgroup.[58] Importantly, recent studies described that poor-prognosis hindbrain ependymomas exhibit hyperactivity of PRC2 and that associated increased trimethylation of H3K27 leads to tumor suppressor gene silencing, with subsequent gene silencing by DNA CpG hypermethylation/CIMP-positive ependymomas of infancy.[59]

ATRT

Besides these tumors, atypical teratoid/rhabdoid tumor (ATRT) is a rare, high-grade embryonal brain tumor that occurs most commonly in young children and carries a very poor prognosis.[60] ATRTs are characterized by absence of the chromatin remodeling protein SNF5 (SMARCB1).[61] The *SNF5* gene encodes a subunit of ATP-dependent SWI/SNF chromatin remodeling complexes,[62] and complexes oppose epigenetic silencing by PRC2.[63] Loss of *SNF5* tumor suppressor activity leads to elevated expression of the Polycomb gene *EZH2*, and polycomb targets are broadly methylated at H3K27 and repressed in ATRT.[63] In addition, the SNF5 protein has a role in histone acetylation.[64,65] Inhibition of histone deacetylases (HDACs) has been found to specifically restore normal expression of proteins involved in cell cycle, originally altered in ATRT cells, through promoter histone H3 and H4 acetylation, recapitulating the effect of SNF5 restoration in ATRT cells.[65]

Neuroblastoma

Other extra-cranial solid tumors in children include neuroblastoma, a tumor arising from primitive neural crest cells in the sympathetic nervous system.[66] Many of these children will succumb to neuroblastoma despite intensive chemotherapy with autologous stem cell transplantation, surgery and radiation. The pathologic activation of *MYCN* plays a central role in high-risk neuroblastoma, while bromodomain-mediated inhibition of *MYCN* impaired growth and induced apoptosis in *in vivo* neuroblastoma models.[67]

Histone modifying enzymes: Role in brain cancer therapy

The identification of the functional involvement of chromatin machinery and the precise roles of the different epigenetic modifying enzymes is a necessary step to understand cancer biology, and to develop effective therapeutic strategies in human cancer. Drugs targeting epigenetic modifiers include the inhibitors of enzymatic "writers," such as DNA and histone methyltransferases, and inhibitors of enzymatic "erasers," such as histone demethylase and HDACs. Modulation of the epigenetic regulators, known as "readers," structurally diverse proteins that recognize and bind to covalent modifications of chromatin, has recently emerged as a therapeutic strategy in the treatment of brain cancer and also of other cancers (reviewed in.[6,20,68-74]).

KMTs & PRMTs

In addition to the lysine and arginine side chains of a protein, methyltransferases (MTs) can methylate DNA, RNA, and even small molecules, such as a catecholamine. The methyl acceptors for these enzymes can be N (e.g., –NH2 of a lysine), C (e.g., C5-cytosine in DNA), or O (e.g., –OH of a catecholamine) atom[69] All methyltransferases use SAM as the enzyme cofactor, with its methyl group (activated by the sulfonium) being the donor. A large family of $\geq$ 60 histone methyltransferases, including histone lysine methyltransferases (KMTs) and protein/histone arginine methyltransferases (PRMTs), were identified in humans, and the biochemical and biologic functions of many methyltransferases have been characterized.[69,75] (Fig. 1). Besides the effects on histones, some of these enzymes have other substrates[69,71] Among the non-histone proteins are the cell cycle regulator p53 and the immune response regulator NF-kB. Most KMTs contain the conserved SET domain, excepted DOLT1.[69,71] Most of these enzymes have a high degree of specificity for particular residues and the degree of methylation.

Polycomb repressive complex 2

H3K27 methylation is determined by the activity of, the enzymatic subunit of PRC2 (Fig. 1). Despite the importance of EZH2, H3K27 methylation requires other PRC2 components, including EZH1 (functional homolog of EZH2), histone core accessory proteins (EED, SUZ12, and RbAP48), and the PRC2-associated factors JARID2 and ASXL1 (for review see[76]).

EZH2 is overexpressed in a wide range of cancers, including GBM (but also advanced-stage and high-grade prostate, breast, and lung tumors) (Table 1).[70] The PRC2 subunits SUZ12 and EED are frequently deleted in malignant peripheral nerve sheath tumors, GBM, and melanomas, and data suggest that loss of PRC2 function promotes oncogenic Ras signaling.[77] Indeed, SUZ12 functions as tumor suppressor in high-grade gliomas by cooperating with mutations in *NF1* (which encodes a Ras GTPase-activating protein), and its loss drives cancer by activating Ras.[78] Importantly, however, SUZ12 inactivation also triggers an epigenetic switch that sensitizes these cancers to bromodomain inhibitors.[77] Moreover, somatic mutations and deletions of *EZH2* were also identified in malignancies.[79-82] In particular, T cell acute lymphoblastic leukemia (T-ALL) is a hematological malignancy in which H3K27 methylation is reduced by a loss-of-function *EZH2* mutation.[81,82] Mutations resulting in the replacement of a single tyrosine in the SET domain of the EZH2 protein (tyrosine Y641) can occur in diffuse large B-cell lymphomas and follicular lymphomas and reduce enzyme activity.[83] EZH2 has been described as an essential regulator for B cell activation and is involved in germinal center-derived neoplasms.[84] In myeloid neoplasia, *EZH2* is most often affected by mutations leading to direct abrogation of histone methyltransferase activity, suggesting that EZH2 acts as a tumor suppressor for myeloid malignancies.[79]

There is evidence for both increased and decreased activity of enzymes controlling H3K27 methylation in other cancers,[76,85] supporting the idea that a precise balance of this methylation is critical for normal cell growth and for a context-dependent role of polycomb proteins in oncogenesis. Besides alterations of enzymes controlling H3K27 methylation in cancer, H3K27M gain-of-function mutations often occur in highly aggressive pediatric gliomas (Table 1). These mutations are believed to sequester PRC2, which normally represses gene expression through histone methylation. While H3K27me3 is associated with transcriptionally silenced chromatin, the inactivation of PRC2 through an interaction between EZH2 and the mutant histone results in hypomethylation at H3K27, with consequent transcriptional derepression at these loci.[37,86,87] By performing ChIP-seq and whole-genome bisulfite sequencing in primary pediatric high-grade gliomas, Bender and colleagues elegantly demonstrated that reduced H3K27me3 levels and DNA hypomethylation act in concert to activate gene expression in K27M mutant pediatric high-grade gliomas.[86]

PRC2 dysfunctions have also been found in other brain tumors. As described above, disparate PRC2 H3K27me3 signatures are also found in ependymoma, a phenomenon associated with increased DNA methylation of specific genes, as well as silencing of their expression, reinforcing the rational strategy for the use of drugs targeting PRC2/EZH2, and/or HDAC inhibitors for therapy of this untreatable disease.[59] Finally, recent evidence suggests that EZH2 may also have a role in rhabdoid tumors.[63,88] Inactivation of the chromatin remodeler SWI/SNF complex component SNF5 is highly prevalent in this disease. Targeted disruption of EZH2 also strongly impairs ATRT cell growth, suppresses tumor cell self-renewal, induces apoptosis, and potently sensitizes these cells to radiation.[64]

Inhibitors of the catalytic activity of EZH2 have been developed, and suppressing global H3K27 methylation converges upon a common structural feature, a pyridone group, which is required for high affinity target binding (reviewed in[71]). These inhibitors achieve dose-dependent induction of gene expression, confirming the role of the PRC2 complex in gene silencing, although a prolonged inhibition of EZH2 catalytic activity is required to reduce H3K27me3 to levels that are sufficient to alter patterns of gene expression. As the administered dose of compound correlates with reduction in H3K27me3 levels in tumor tissue and of tumor growth, the clinical use of such inhibitors for cancers in which EZH2 is genetically altered is relevant.[88,89] Interestingly, Kim and colleagues discovered that EZH2 enhances *STAT3* activation by trimethylating lysine180 in *STAT3*, and it does so preferentially in glioma stem-like cells.[90] The use of the EZH2 inhibitor 3-deazaneplanocin A (DZNep)[91,92] and a highly selective EZH2 inhibitor GSK126[93] decreases *STAT3* activation in glioma stem-like cells. This inhibition reverses the silencing of Polycomb target genes and diminishes STAT3 activity, suggesting therapeutic strategies.[90]

The therapeutic effects of EZH2 inhibitors are also under evaluation in ATRT. Indeed, DZNep potently suppresses ATRT cell growth, leads to cell cycle alterations, increases apoptosis, potentiates the effect of radiation, decreases key cellular signal transduction pathways, and inhibits tumor sphere formation.[64] The first generation EZH2 inhibitors have recently entered phases I and II clinical trials. Tazemetostat (EPZ-6438) is a selective small molecule inhibitor of EZH2 currently being tested in clinical trials for patients having malignant tumors, including ATRT (NCT02875548, NCT02601950, including in pediatric patients NCT02601937) (Table 2).

Mixed-lineage leukemia

One of the first mutations affecting histone modification patterns was described in mixed-lineage leukemia (MLL) *KMT2A*, which encodes an H3K4me3 MT (Table 1). Indeed, more than 50 different translocations or partial tandem duplications involving MLL on chromosome 11q23 have been described and are found to be associated with poor prognosis in acute lymphoblastic leukemia or normal-karyotype acute myeloid leukemia (AML) (reviewed in[94]). Of interest, on the basis of genome-wide sequencing data, mutations of other MLLs, MLL2 (also called MLL4, KMT2D) and MLL3 (KMT2C), have been described in both GBM and medulloblastoma,[46,50,95,96,97,98,99] further increasing the interest for these MTs in cancer. Importantly, UTX (KDM6A) is part of the MLL3/4 complexes, while JMJD3 (KDM6B) associates with the common MLL complex proteins WDR5, ASH2L, and RBBP5. Interestingly, some studies suggest a role for MLL in contributing to the epigenetic heterogeneity between tumor-initiating and non-tumor-initiating cells in GBM.[100] More recently, Gallo et al. showed that MLL5 induces reorganization of chromatin structure and decreases expression of H3.3. Reduced H3.3 expression favors self-renewal properties in adult GBM cells and phenocopies pediatric GBM with H3.3 mutations, indicating potential therapeutic strategies for adult GBM.[101] The authors observe that MLL5 and H3.3 have antagonistic roles on glioma stem-like cell self-renewal and that these mechanisms are reversible. Because there are no available MLL5 inhibitors,

Table 2. Epigenetic inhibitors for brain cancer therapy.

Enzyme/ protein	Inhibitor	Mechanism	Pre-clinical studies	Clinical trials (ongoing or recently completed or terminated)
EZH2	Tazemetostat	EPZ-6438 (N-((4,6-dimethyl-2-oxo-1,2-dihydropyridin-3-yl)methyl)-5-(ethyl (tetrahydro-2H-pyran-4-yl)amino)-4-methyl-4′-(morpholinomethyl)-[1,1′-biphenyl]-3-carboxamide) is a selective small molecule inhibitor of the histone-lysine methyltransferase EZH2 (competition with S-adenosyl methionine (SAM))	Reduces intratumoral trimethylation levels of lysine 27 on histone H3 (88) Induces the apoptosis of ATRT cell-like (88)	A Phase II, Multicenter Study of the EZH2 Inhibitor Tazemetostat in Adult Subjects With INI1-Negative Tumors or Relapsed/Refractory Synovial Sarcoma (NCT02601950) Tazemetostat Rollover Study (TRuST): An Open-Label Rollover Study (NCT02875548) A Phase 1 Study of the EZH2 Inhibitor Tazemetostat in Pediatric Subjects With Relapsed or Refractory INI1-Negative Tumors or Synovial Sarcoma (NCT02601937)
EZH2	DZNep	3-deazaneplanocin A (DZNep) is a potent S-adenosylhomocysteine hydrolase inhibitors (92) It depletes EZH2 indirectly by increasing levels of adenosylhomocysteine, which in turn, inhibits EZH2 and its methyltransferase activity Has been found to suppress EZH2-mediated H3K27 methylation (91)	Suppresses self-renewal and induces radiation sensitivity in ATRT cells (64) Decreases STAT3 activation in glioma stem-like cells, which reverses the silencing of polycomb target genes (90)	
EZH2	GSK126	Potent, highly selective, S-adenosyL-methionine-competitive, small-molecule inhibitor of EZH2 methyltransferase activity (93) Decreases global H3K27me3 levels and reactivates silenced PRC2 target genes (93)	Decreases STAT3 activation in glioma stem-like cells, which reverses the silencing of polycomb target genes (90)	
MEN1	MI-2–2, MI-3	MI-2–2 and −3 belong to the thienopyrimidine class, and can block menin-MLL interaction	Inhibits tumor cell growth in vitro in H3K27M mutant cells and in xenografts in mice (32) Inhibits self-renewal of adult GBM stem-like cells, in combination with KDM inhibitors (101)	
UTX /JMJD3	GSK-J1/J4	Small-molecule catalytic site inhibitor, selective for the H3K27me3-specific JMJ subfamily (107) Chelation of the Fe(II) active site and binding of a propanoic acid side-chain to the 2OG binding site Reduces the lipopolysaccharide-induced proinflammatory response in human macrophages (107)	Increases K27me2 and K27me3 and a dose-dependent reduction in cell viability and proliferation of H3K27M mutant cells (108) Increases animal survival and reduced tumor growth in H3K27M human brainstem tumor xenografts (108)	
HDAC	Panobinostat	Hydroxamic acid, non-specific HDAC inhibitor. HDACs 1–4, 7 and 9 but less so against HDAC6 and, especially, HDAC8 (reviewed in 125)	Despite a promotion of growth arrest and apoptotic cells death in glioma models (mostly due to anti-angiogenic effects), the sample sizes in the first high-grade glioma trials were too small to understand its effects in human (120) Recent work shows that panobinostat is active against diffuse intrinsic pontine glioma (119) Increases in global H3 acetylation and H3K27 trimethylation (dose-dependant fashion) in H3K27M cells (117) Panobinostat synergized with KDM inhibitor GSK-J4 to significantly decrease cell viability in H3K27M mutant (117) Panobinostat caN-terminally differentiate ATRT tumor cells and reduce their ability to self-renew (118)	Panobinostat and Stereotactic Radiation Therapy in Treating Patients With Brain Tumors (NCT01324635) Panobinostat in Treating Younger Patients With Progressive Diffuse Intrinsic Pontine Glioma (NCT02899715) Trial of Panobinostat in Children With Diffuse Intrinsic Pontine Glioma (NCT02717455) Study of LBH589 (Panobinostat) to Treat Malignant Brain Tumors (NCT00848523)
HDAC	Vorinostat	Suberoylanilide hydroxamic acid - SAHA, non-specific HDAC inhibitor (reviewed in 125)	Despite a significant synergistic cytotoxicity between HDAC inhibitors and proteasome inhibitors in GBM cell lines, a similar combination did not provide a significant survival benefit in previous trials (121)	Study of the Combination of Vorinostat and Radiation Therapy for the Treatment of Patients With Brain Metastases (NCT00838929) Study of Suberoylanilide Hydroxamic Acid (SAHA) With Temsirolimus in Children With Diffuse Intrinsic Pontine Glioma (DIPG) (NCT02420613) Vorinostat and Temozolomide in Treating Patients With Malignant Gliomas (NCT00268385) N2007–03: Vorinostat and 131-I MIBG in Treating Patients With Resistant or Relapsed Neuroblastoma (NCT01019850)

(*Continued on next page*)

Table 2. (*Continued*)

Enzyme/ protein	Inhibitor	Mechanism	Pre-clinical studies	Clinical trials (ongoing or recently completed or terminated)
				Vorinostat and Radiation Therapy Followed by Maintenance Therapy With Vorinostat in Treating Younger Patients With Newly Diagnosed Diffuse Intrinsic Pontine Glioma (NCT01189266) Vorinostat, Temozolomide, and Radiation Therapy in Treating Patients With Newly Diagnosed Glioblastoma Multiforme (NCT00731731) Vorinostat, Temozolomide, or Bevacizumab in Combination With Radiation Therapy Followed by Bevacizumab and Temozolomide in Young Patients With Newly Diagnosed High-grade Glioma (NCT01236560) Vorinostat and Isotretinoin in Treating Patients With High-Risk Refractory or Recurrent Neuroblastoma (NCT01208454) Vorinostat, Isotretinoin and Temozolomide in Adults With Recurrent Glioblastoma Multiforme (GBM) (NCT00555399) Magnetic Resonance Spectroscopy Imaging in Predicting Response to Vorinostat and Temozolomide in Patients With Recurrent or Progressive Glioblastoma (NCT01342757) phase I/II Adaptive Randomized Trial of Bevacizumab Versus Bevacizumab Plus Vorinostat in Adults With Recurrent Glioblastoma (NCT01266031) High-Dose Vorinostat and Fractionated Stereotactic Body Radiation Therapy in Treating Patients With Recurrent Glioma (NCT01378481) Vorinostat and Bortezomib in Treating Young Patients With Refractory or Recurrent Solid Tumors, Including Central Nervous System Tumors and Lymphoma (NCT00994500)
HDAC	Belinostat	Hydroxamic acid, non-specific HDAC inhibitor. Belinostat is quite active against HDACs 1–10 (reviewed in 125)		MRSI to Predict Response to RT/TMZ ± Belinostat in GBM (NCT02137759)
HDAC	Romidepsin	FK228, Cyclic peptide, once converted to its active form (redFK) by cellular reducing activity, can inhibit HDACs 1, 2, 4 and 6 (reviewed in 125)	Induces apoptosis and suppresses cell proliferation of human glioblastoma cells in vitro and in vivo (122)	FR901228 in Treating Patients With Recurrent High-grade Gliomas (NCT00085540)
HDAC	Valproate	Short-chain fatty acids. Valproate is quite active against HDACs 1–5, 7 and 9 but less so against HDACs 6 and 10 (reviewed in 125)	Combined with radiotherapy, valproate treatement was associated with a better outcome than other HDAC inhibitors in patients with GBM (123; 124)	Valproate and Etoposide for Patients With Neuronal Tumors and Brain Metastases (NCT00513162) Stereotactic Radiosurgery With Nivolumab and Valproate in Patients With Recurrent Glioblastoma (NCT02648633) Sorafenib Tosylate, Valproic Acid, and Sildenafil Citrate in Treating Patients With Recurrent High-grade Glioma (NCT01817751) An International Clinical Program for the Diagnosis and Treatment of Children With Ependymoma (NCT02265770) phase I Study of Temozolomide, Valproic Acid and Radiation Therapy in Patients With Brain Metastases (NCT00437957) Valproic Acid and Radiation Followed by Maintenance Valproic Acid and Bevacizumab in Children With High Grade Gliomas or Diffuse Intrinsic Pontine Glioma (NCT00879437) Valproic Acid in Treating Young Patients With Recurrent or Refractory Solid Tumors or CNS Tumors (NCT00107458)
BET	OTX015	OTX015 (MK-8628) specifically targets the recognition of acetylated lysine residues of BETs (133)	Displays *in vitro* and *in vivo* antitumor effects alone and in combination with conventional therapies in glioblastoma models (133)	A Trial With Dose Optimization of OTX015 in Recurrent Glioblastoma Multiforme (GBM) Patients (NCT02296476)
BET	INCB057643	INCB057643 specifically targets the recognition of acetylated lysine residues of BETs		A Phase 1/2, Open-Label Safety and Tolerability Study of INCB057643 in Subjects With Advanced Malignancies (NCT02711137)
BET	INCB054329	INCB054329 inhibited binding of BRD2, BRD3 and BRD4 to an acetylated histone H4		An Open-Label, Dose-Escalation Study of INCB054329 in Patients With Advanced Malignancies (solid tumors of all types, including brain tumors) (NCT02431260)

(*Continued on next page*)

Table 2. (*Continued*)

Enzyme/ protein	Inhibitor	Mechanism	Pre-clinical studies	Clinical trials (ongoing or recently completed or terminated)
BET	JQ1	JQ1 specifically targets the recognition of acetylated lysine residues of BETs (127; 129)	Represses growth of orthotopic glioblastoma tumors (136)	JQ1 is not being tested in clinical trials due to its short half life.
BET	I-BET151	I-BET151 specifically targets the recognition of acetylated lysine residues of BETs (131)	Inhibits GBM cell proliferation (135)	

the authors use the TCGA database to search for alterations in other chromatin targets that may co-occur with alterations in MLL5 and H3.3 and identify the histone demethylases JMJD3/KDM6B and UTX/KDM6A and the trithorax-complex subunit Multiple Endocrine Neoplasia 1 (MEN1, menin)[101] (Table 2). Treatment of adult GBM stem-like cells with histone demethylase and MEN1 inhibitors (such as MI-2, which blocks menin-MLL interaction), recently was shown to be efficacious in preclinical models of K27M mutant gliomas (described below in the section on lysine demethylases), inhibiting the self-renewal properties of GBM cells.[101] Treatment with MEN1 inhibitors was previously found to decrease tumor cell growth *in vitro* and in mice using human embryonic stem cells to model pediatric gliomas with the H3.3K27M histone mutation.[32] Although the role of MEN1 in DIPG is unclear, these studies suggest that it may be an important therapeutic target. Further details of UTX (KDM6A) and JMJD3 (KDM6B) are described below in the section "*UTX (KDM6A) and JMJD3 (KDM6B)*."

Finally, although there is no (or very little) evidence of the involvement of other MT in brain cancer, the impact of some enzymes, including DOT1L (KMT4), NSD proteins, H3K9MT, and PRMT has been extensively studied in several cancers (reviewed in[70]).

DOT1L (KMT4)

DOT1L methylates lysine 79 on H3K79 within the globular histone domain on which DNA is wrapped, and not on the N-terminal tails. Genome-wide studies show that actively transcribed genes are enriched for H3K79 methylation.[10] Several studies highlight the impact of DOT1L in leukemia, and DOT1L inhibitors show enhanced anti-proliferative activity against MLL-rearranged leukemia cells.[102]

Nuclear receptor SET domain-containing (NSD) proteins 1, 2, and 3

Nuclear receptor SET domain-containing (NSD) MTs methylate H3K36. NSDs have been involved in some cases of AML or breast cancers,[70] and NSD2 has been reported as highly overexpressed in GBM.[103]

H3K9MTs

These enzymes have been found to be upregulated in various human tumors.[102]

Protein arginine methyltransferases. Methylation of arginines by protein arginine methyltransferases (PRMT1–9) has been associated with both positive and negative regulation of transcription. Arginine methylations have also been involved in cancer, and especially in various MLL and non-MLL. For example, PRMT1 is necessary for leukemic transformation, which requires co-recruitment of KDM4C, an H3K9 demethylase.[75,104]

Lysine demethylases

Histone methylation is also dynamically controlled by histone/protein lysine demethylases (KDMs), enzymes that remove the methyl group(s) from a methylated lysine side chain (Fig. 1). The opposite functions of MTs and DMs work to maintain balanced histone methylation levels. KDM protein families are based on the organization of their catalytic domains and the type of oxidative mechanism that underlies the demethylation reaction. The Jumonji domain-containing KDM family members utilize 2-oxoglutarate (2-OG; α-ketoglutarate) as a cofactor, while KDM1A (LSD1, BHC110, AOF2) and KDM1B (LSD2) require FAD. Histone methylation patterns can also be altered by KDMs. Mutations have been reported in the genes encoding KDM5A (JARID1A); KDM5C (JARID1C), which affects H3K4 methylation; and KDM6A (UTX), which affects H3K27 methylation.[50,81,82,98,105,106]

UTX (KDM6A) and JMJD3 (KDM6B)

Besides EZH2 methyltransferase, H3K27 methylation is also determined by the activity of demethylases, including UTX/KDM6A and JMJD3/KDM6B. Somatic loss-of-function mutations of *UTX* are found in multiple myeloma, esophageal squamous cell carcinomas, renal carcinomas, and occasionally in breast cancer, AML, T-ALL, GBM, and colorectal and bladder cancer (Table 1) (for review see.[70,81,82]), suggestive of a tumor suppressor role for UTX. Both JMJD3 and UTX function as part of a transcriptional activator complex that includes the MLL3/MLL4 H3K4 methyltransferases, indicating that these enzymes have a dual role involving removal of H3K27 methyl marks and addition of methyl groups to H3K4 (reviewed in[76]).

GSK-J1/J4 were first discovered as JMJD3 (KMD6B) and UTX (KMD6A) inhibitors, and the treatment of human primary macrophages with these pharmacological substances reduces the lipopolysaccharide-induced pro-inflammatory response[107] Remarkably, GSK-J4 recently has been used successfully to treat brainstem gliomas *in vitro* and *in vivo* ([108] and reviewed in[109-112]). The mechanism of action of GSK-J4 is chelation of the Fe(II) active site and binding of a propanoic acid side-chain to the 2OG binding site. In this study, a panel of patient-derived cell lines has been examined: 2 lines derived from patients with DIPG that harbor a heterozygous H3.3K27M dominant mutation, 2 pediatric glioblastoma lines

wild type for H3, a fifth pediatric glioblastoma line that carries the H3.3 G34V mutation, and isogenic cell lines derived from human astrocytes with and without transgene expression of the H3.3 K27M mutation.[108] Both patient-derived cell lines had global hypomethylation of H3K27 compared with the wild type models. Treatment of K27M mutant cells with GSK-J4 resulted in increased K27me2 and K27me3 and a dose-dependent reduction in cell viability and proliferation (Fig. 2) (Table 2). Treatment of 2 H3K27M human brainstem tumor xenografts with GSK-J4 increased animal survival and reduced tumor growth but did not achieve the same effect in animals with xenografts containing wild type H3.3.[108] The confirmation of the delivery of GSK-J4 into the pons using HPLC analysis of non-tumor-bearing mice further supports the use of GSK-J4 as a potentially clinically effective targeted therapy.[108] Of interest, this compound has a therapeutic potential for T-ALL, which can be driven by oncogenic activation of NOTCH1 signaling, loss-of-function mutations and deletion of the *EZH2* and *SUZ12* genes, and in which JMJD3 is more highly expressed compared with other types of leukemia.[81,113,114] Indeed, treatment of T-ALL cells with GSK-J4 also resulted in reduced cell proliferation and increased K27M methylation.[82] In this study, the antitumor activities of the GSK-J4 inhibitor was also specific for T-ALL cells, and the main action of this inhibitor in T-ALL is also thought to be channeled through the inhibition of JMJD3 activity.[82]

Histone acetyltransferases and deacetylases

Histone acetyltransferases (HATs) and HDACs (including the NAD-dependent Sirtuins) function antagonistically to control histone acetylation. Because acetylation is a histone mark for active transcription, HATs have been associated with active and HDACs with inactive genes. A recent description of genome-wide mapping of HAT and HDAC binding on chromatin has been made, finding that both are found at active genes with acetylated histones.[115] These data provide evidence that HATs and HDACs are both targeted to transcribed regions of active genes by phosphorylated RNAP II, and the majority of HDACs in the human genome function to reset chromatin by removing acetylation at active genes.[115] Besides the finding of genome-wide mapping, the most recent study revealed that HDAC mediates Ras signaling-induced silencing of the studied genes and the subsequent H3K27me3 increase, while H3K27me3 accumulation is reversed by forced activation of transcription.[16] Rare mutations have been described in tumor genomes for almost all members of this protein family (Table 1).

HDAC inhibitors work by preventing histone deacetylation, thereby facilitating an open chromatin structure and resulting in gene activation. A non-selective potent HDAC inhibitor drug, panobinostat, a hydroxamic acid (LBH589, a non-selective FDA-approved potent HDAC inhibitor), has been developed for the treatment of a various cancers.[116] A recent study aimed to screen 83 drugs selected by pediatric neuro-oncologists as either promising targeted agents for cancer or traditional chemotherapeutic agents in use for pediatric brain tumor therapy.[117] The chemical screen and subsequent validation experiments revealed patient-derived DIPG cell sensitivity to HDAC inhibitors. Indeed, Western blot analyses of cells expressing the H3.3K27M mutation demonstrated a dose-dependent increase in global H3 acetylation and H3K27 trimethylation following panobinostat treatment, suggesting that the drug produced partial rescue of the H3K27M-induced hypotrimethylation phenotype[117] (Fig. 2). Increased H3K27 trimethylation showed that polyacetylation of the H3 N-terminal tail can 'detoxify' K27M-induced inhibition of PRC2 by rescuing the methylation levels. RNA-seq performed on panobinostat- or vehicle-treated DIPG cells revealed a normalization of the K27M gene expression signature. In addition to clinical trials for brain tumors in adults (NCT01324635, NCT00848523), the side effects and best dose of panobinostat are currently being tested for the treatment of younger patients with DIPG (NCT02899715, NCT02717455). Because panobinostat was shown to reduce the expression of oncogenes in DIPG cells (e.g., *MK167, CCND1*), and to increase global H3 acetylation and H3K27 trimethylation, this drug has also been tested in combination with GSK-J4. Consistent with previous findings,[108] the study confirmed that GSK-J4 decreased cell viability in H3K27M DIPG cell cultures, and further demonstrated that panobinostat synergized with GSK-J4 to significantly decrease cell viability in H3K27M mutant DIPG cell cultures.[117]

In addition to glioma therapy, the therapeutic potential of HDAC inhibitors has also been investigated in ATRT. The rationale was that SNF5 protein loss leads to direct impairment of chromatin remodeling and changes in histone acetylation. Using mouse xenograft models, sustained low-dose panobinostat treatment caused tumor growth arrest. The use of low-dose HDAC inhibitors as a novel therapeutic approach warrants further investigation.[118] Panobinostat,[119120] and other HDAC inhibitors, including vorinostat,[121] belinostat, romidepsin[122] and valproate[123,124] (reviewed in[125]), are currently been used in clinical trials for patients with brain tumors (Table 2).

Bromodomain and extraterminal

The bromodomain and extraterminal (BET) protein BRD4 recently was found possibly to play an important role in the development of brain cancer. The involvement of BRDs has been examined not only in GBM[126] but also in medulloblastoma, where enrichment of H3K27ac and BRD4 ChIP-seq peaks strongly correlated at active medulloblastoma enhancer loci.[51] BRD4 is a "reader" of lysine acetylation, involved in initial recognition of acetylated histones followed by recruitment of Mediator, the transcription initiation cofactor, on promoter regions, leading to phosphorylation of RNAP II. BRD4 also facilitates transcription elongation. Highly selective BET bromodomain inhibitors, such as JQ-1,[127-129] I-BET151,[130,131] I-BET762,[132] INCB054329,[126] and OTX-015,[133] have been generated to specifically target the recognition of acetylated lysine residues of BETs. Competitive binding with a BET bromodomain inhibitor blocks the binding between BRD4 and acetylated histones, leading to transcriptional inactivation (Fig. 2) (Table 2).[128] These inhibitors seem to be a promising therapeutic strategy in medulloblastoma,[53,134] and GBM.[126] A number of preclinical studies has been performed in GBM models with the BRD inhibitors JQ1 and I-BET151, showing promising antitumor activity.[135,136] Moreover, OTX015 (MK-8628), a novel BET inhibitor, has been tested recently at the clinical level

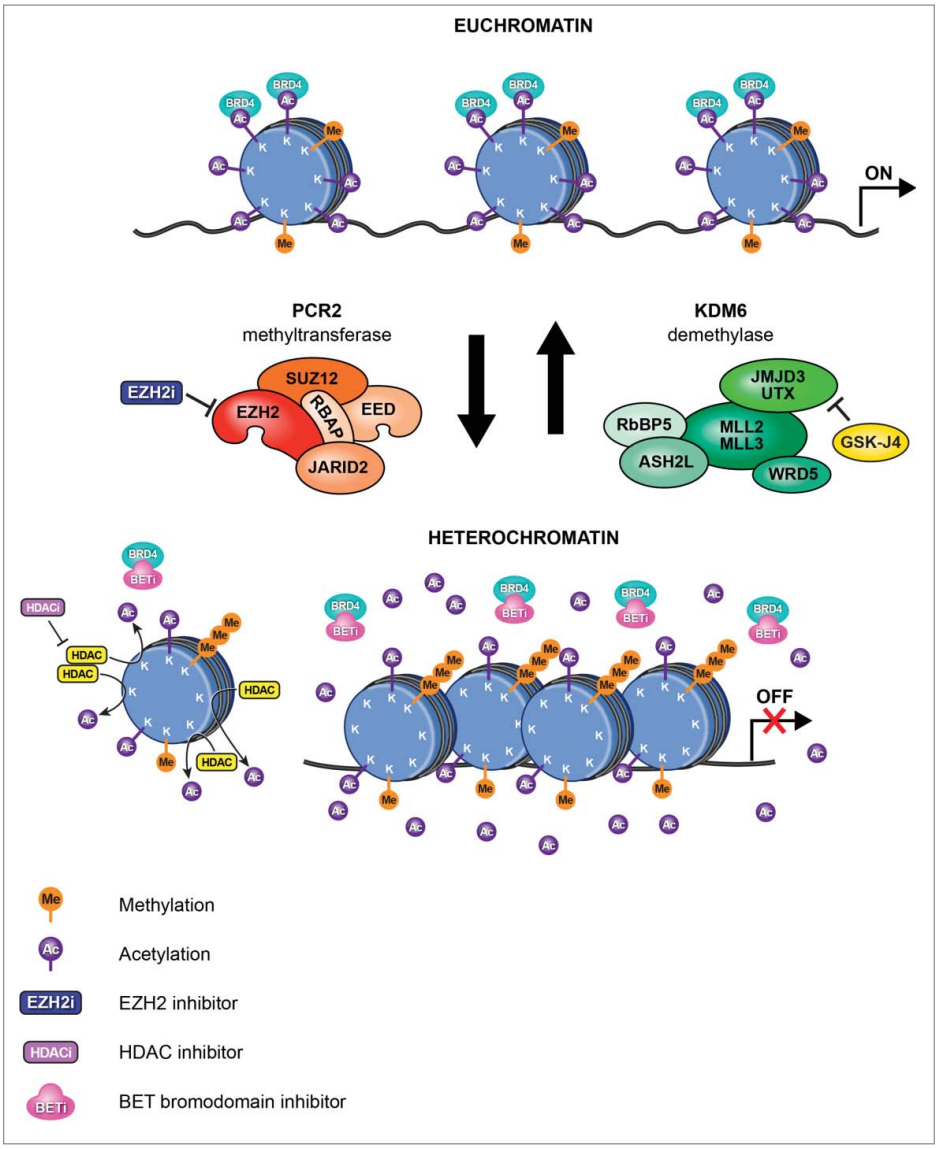

Figure 2. New epigenetic therapies for pediatric brain tumors. In DIPG, H3K27M mutations (histone H3.1 or H3.3) lead to hypomethylation of H3K27, which promotes a more accessible chromatin state characterized by H3K27 acetylation and aberrant gene expression (upper: Euchromatin). H3K27M mutations inhibit the major H3K27 methylase PRC2. Treatment of DIPG with the K27 demethylase inhibitor GSKJ4 results in increased K27me2 and K27me3 and reduced tumor growth (lower: Heterochromatin). Moreover, treatment with the non-specific HDAC inhibitor panobinostat resulted in an increase in global H3 acetylation, increasing/restoring H3K27me3 levels. In addition, competitive binding with BET bromodomain inhibitors prevents the interaction of BRD4 with acetylated histones, leading to repression of BRD4 transcriptional targets and also limiting tumor growth.

in dose-finding studies in GBM patients (NCT02296476), and displays *in vitro* and *in vivo* antitumor effects alone and in combination with conventional therapies in GBM models.[126] The safety and tolerability of another BET inhibitor, INCB057643, is also currently being tested in subjects with GBM (NCT02711137) (Table 2). Interestingly, a recent study suggests that the anti-proliferative effects of BET inhibitors in GBM might be at least partially mediated by a decrease in the tumor-promoting long noncoding RNA HOX transcript antisense RNA (HOTAIR).[137]

Safety and feasibility of epigenetic targeted therapy

Toxicological studies should confirm the accessibility of agents targeting epigenetic modifiers or the administration at the appropriate location in the CNS. The compound should be delivered in effective concentrations to inhibit the target (thereby requiring a good blood brain penetration if administered systemically), with a sufficient therapeutic window for such pharmacological inhibition and have an acceptable toxicity profile, taking into account the continuing normal development of pediatric patients. Given the important functions of histone methylation and acetylation in normal physiology, inhibition of related enzymes could cause off-target effects with systemic administration. Moreover, these treatments have an anti-proliferative activity, and the dose used plays a key role. For this purpose, the information obtained during the nonclinical safety studies needs to be used to estimate an initial safe starting dose and dose range of the pharmaceutical epigenetic treatment of the human trials and to identify parameters for clinical monitoring for potential adverse effects. In clinical trials, a wide range of toxic effects has been

reported during the systemic use of epigenetic targeted therapies.[138] For example, fatigue, thrombocytopenia, nausea, and diarrhea have been reported in GBM patients treated with vorinostat[121,139] and similar toxic side effects can be accompanied by hypophosphatemia and hemorrhage with panobinostat.[120]

In addition to the evaluation of anti-cancer activity based on tumor size, and to the evaluation of molecular effects of therapy through analysis of biopsy tissue obtained before and/or after treatment, it is relevant to have a detailed understanding of the molecular impact of a drug on healthy CNS cells as well as on peripheral organs if the drug is administered systemically. Much progress and improvement are still needed in the treatment of malignant brain tumors, both in the preclinical and clinical settings. The penetration of the various agents in development into the central nervous system remains an important issue and is being tested. As an example, OTX015 crosses the blood–brain barrier and selectively penetrates tumor tissue.[133] OTX015 tumor levels were 7–15 times higher than in normal brain tissues in several *in vivo* models.[133] As another example, GSK-J4 brain access has been assessed in murine DIPG models, after being administered to mice by intraperitoneal injection.[108] After brain resection, each brainstem was dissected from the surrounding brain. GSK-J1, the hydrolysis product and activated derivative of GSK-J4 and the most selective KDM inhibitor yet disclosed,[140] was found in all brainstem samples, indicating that the drug entered the brain and accessed the site of brainstem tumor development.[108] Extensive additional experiments confirmed the anti-tumor activity of GSK-J4 as involving inhibition of JMJD3,[108] but did not exclude the possibility of other histone demethylase activities being affected. Recent studies described that besides being a potent inhibitor of jumonji proteins, with activity toward H3K27me3/me2 (KDM6), GSK-J1 can also inhibit KDM5 activity on H3K4me3/me2 in U2OS cells *in vitro*.[141] Considering the effect of JMJD3 on several biologic functions (e.g., inflammatory response in macrophages[107]) and those of KDM5,[142] optimizing the dose and frequency of administration of such inhibitors to maximize the therapeutic window and limit toxicity will be a key factor for reaching a successful clinical outcome. The combination of agents with different mechanisms of action, as recently proposed[117] may offer a better chance for tumor control with limited toxicity.

Conclusions

Due to the recent links to brain cancer, some KDMs have come under investigation as potential therapeutic targets. The precise impact of the mutations occurring in brain cancer and related changes in chromatin machinery, as well as their complex interactions with other known oncogenes, need to be further explored. It will be also relevant to investigate the many processes that can regulate the activity of these enzymes, including phosphorylation, ubiquitination, and changes in metabolism (reviewed in[6,143]), as well as fluctuations in the availability of energy substrates, cofactors, and coenzymes[144,145] to improve specific therapies.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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