Histone methylases as novel drug targets. Focus on EZH2 inhibition.

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ABSTRACT

Posttranslational modifications of histones (so-called epigenetic modifications) play a major role in transcriptional control and normal development, and are tightly regulated. Disruption of their control is a frequent event in disease. Particularly, the methylation of lysine 27 on histone H3 (H3K27), induced by the methylase Enhancer of Zeste homolog 2 (EZH2), emerges as a key control of gene expression, and a major regulator of cell physiology. The identification of driver mutations in EZH2 has already led to new prognostic and therapeutic advances, and new classes of potent and specific inhibitors for EZH2 show promising results in preclinical trials. This review examines roles of histone lysine methylases and demethylases in cells, and focuses on the recent knowledge and developments about EZH2.

Key-terms: epigenetic, histone methylation, EZH2, cancerology, tumors, apoptosis, cell death, inhibitor, stem cells, H3K27

Histone modifications and histone code

Epigenetic has been defined as inheritable changes in gene expression that occur without a change in DNA sequence. Key components of epigenetic processes are DNA methylation, histone modifications and variants, non-histone chromatin proteins, small interfering RNA (siRNA) and micro RNA (miRNA). They induce changes in gene expression in modifying accessibility of the eukaryotic transcription machinery to specific genes. In particular, the role of histones as active participants in gene regulation has only recently been appreciated. Histones were discovered in 1884 by Albrecht Kossel. But until the early 1990s, these proteins, which are assembled into nucleosomes, forming beads around which the DNA is wrapped, were considered to be relatively inert scaffolding for packaging the genetic material. It is now known that histones play also a key-role in gene expression regulation, through post-translational modifications of histone (figure 1). In 2000, the concept of a 'histone code' emerged [1].

The histones' amino-terminal tails extend away from the central core, and are thus available for reversible acetylation, methylation, phosphorylation, ADP-ribosylation, and ubiquitination (figure 2). Histone modifications interact with DNA methylation to mark genes for silencing or transcription. By reading the combinatorial and/or sequential histone modifications that constitute the histone code (table 1), it was thought that it might be possible to predict which gene products will be transcribed and thus determine a cell's RNA repertoire and ultimately its proteome, just as reading the DNA code allows us to predict the encoded protein sequence. However, some gene loci present both histone 3 lysine 4 trimethylation (H3K4me3), associated with transcriptional activation and histone 3 lysine 27 trimethylation (H3K27me3), and linked with repression. These bivalent domains are posited to be poised for either up- or downregulation and to provide an epigenetic blueprint for lineage determination [2], and are usually found in stem cells.

These post-translational modifications (PTM) undergone by histones have a profound effect on the remodeling of chromatin. Two distinct chromatin states can be distinguished: condensed "closed" heterochromatin, and de-condensed "open" euchromatin. The change from transcriptionally silenced heterochromatin to gene expression euchromatin is mediated by posttranslational modifications of histones and uses of distinct histone variants.

Histone lysine methylation

Histone methylation is an epigenetic mark actively studied in recent years. On about 11 000 articles referenced in Pubmed since 1964, more than half of them have been published during the last four years.

The most well-characterized histone methylation appears on lysine [3,4]. Histone lysine methylation occurs primarily on histone H3 at lysines 4, 9, 14, 18, 23, 27, 36 and 79 and on histone H4 at lysine 20 [4–6]. A number of these methylation events have been linked to transcriptional regulation, including those at H3 lysines 4, 36 and 79 (associated with active transcription) and those at H3 lysines 9 and 27 (associated with gene repression and heterochromatin formation) [3,7]. Unlike acetylation and phosphorylation, which in addition to recruit proteins to chromatin can also directly affect chromatin structure by altering the histone charge, lysine methylation does not alter the charge of the residue and is therefore thought to primarily modulate chromatin structure through the recruitment of distinct reader proteins that possess the ability to facilitate transcriptional activation or repression [3,4,6,8].

Lysine residues can be modified with up to three methyl groups (mono-, di- and trimethylation) on the epsilon amine of the side-chain (figure 3). Importantly, reader domains can distinguish between the different methyl states producing distinct functional outcomes [3,4,6,8]. These observations demonstrate the complexity and fine level of control that lysine methylation contributes to chromatin function and transcriptional regulation.

Among activation marks, trimethylation at lysine 4 of histone H3 (H3K4me3) is the prominent methyllysine species at active promoter regions [9–13]. This mark plays a major role in transcription initiation, notably in recruiting the general transcription factors, or in mediating interactions with RNA polymerase associated proteins [6]. H3K36 methylation, meanwhile, primarily exists with the lower methylation states (H3K36me1 and -me2) present near 5' regions and higher methylation states (H3K36me2 and -me3) at the 3' ends of genes [11,14]. The role of H3K36 methylation is also quite diverse and has been shown to be involved in numerous functions including transcription, mRNA splicing, DNA replication and DNA repair [15,16]. Its function that has been most well defined is its role in transcription elongation. Another modification found in gene bodies is methylation of H3K79, however, unlike H3K36 methylation, its role in actively transcribed genes is less clear. It may act as a protection from silencing [6].

At opposite, histone H3 lysine 9 methylation (H3K9) has been correlated with heterochromatin formation and transcriptional repression, making the methylation state of lysine 9 an interesting marker of transcriptional activity. H3K9me3 binds heterochromatin protein 1 (HP1) to constitutive heterochromatin [17]. HP1 is responsible for transcriptional repression and the actual formation and maintenance of heterochromatin. H3K9me2 is a characteristic mark of the inactivated X chromosome (Xi) [18,19]. H3K9 methylation is also involved in cell reprogramming, or cancer. H3K27 methylation is also another epigenetic repressive mark, which plays a major role in a plethora of cellular processes, such as stem cell renewal, cell fate, reprogramming, cancer, inflammation.

Histone arginine methylation

As lysine, arginine on histone can also be methylated. The addition of one or two methyl groups on arginine residues results in three different methylation states: monomethylated, asymmetrically dimethylated or symmetrically dimethylated arginine. The methyl groups are deposited by protein arginine methyltransferases (PRMTs). Histone arginine methylation associates with both active and repressed chromatin states depending on the residue involved and the status of methylation [20]. This process is involved in several cellular processes such as transcription, RNA processing, signal transduction and DNA repair. Besides, it is now clear that there is cross-talk between arginine and lysine methylation: this has been termed “arginine/lysine-methyl/methyl switch” [21,22]

Histone methyltransferases and demethylases

There are currently more than 60 predicted lysine methyltransferases and 30 predicted lysine demethylases in the human genome [23–25].

Histone methyltransferase (HMT) activity towards lysine (and arginine) residues is found in a family of enzymes with a conserved catalytic domain called SET (Suppressor of variegation, Enhancer of Zeste, Trithorax). The human genome encodes 49 SET domain-containing proteins and the histone lysine methyltransferase DOT1L, which does not contain a SET domain (table 2). The importance of HMTs for embryonic development has been demonstrated in numerous mouse knockout studies [26]. In addition, misregulation of HMTs has been linked to diseases or cancer aggressiveness. In particular, the Polycomb group

transcriptional repressor EZH2 (methylase of H3K27), is overexpressed in many different types of cancer [27], and has been proposed as a molecular marker of some cancer progression and metastasis [28–33].

In 2004, the first histone demethylase (HDM) has been discovered, and called LSD1 (lysine-specific demethylase 1). Since, more than 20 demethylases have been identified and characterized (table 3). They belong to either the LSD family or the JmjC family, demonstrating the reversibility of all methylation states at almost all major histone lysine methylation sites (table 3). The identification of these histone demethylases (HDMs) has completely changed our initial view of histone methylation as a permanent, heritable mark [34]. The presence of both histone methyltransferases and demethylases in the same complexes permits modifying of chromatin marks and subsequently switching of transcriptional states from silenced to activated status or vice-versa. Thus, a tight regulation of the expression, activity and recruitment of HMTs and HDMs is necessary. A deregulation of their activity or expression might modify the transcriptional balance, and lead to inappropriate gene expression programs that in turn could induce human disease (table 1 in supplementary material). In particular, the histone methylase EZH2 plays a major role in cell fate and cancer development, and appear now as a promising target for treat some diseases.

Role of the lysine methyltransferase EZH2

The methyltransferase Polycomb Group (PcG) protein Enhancer of zeste homolog 2 (EZH2), also called KMT6, is the catalytic subunit of the Polycomb Repressor Complex 2 (PRC2). Its C-terminal SET domain exhibits methyltransferase activity, leading to repress gene transcription by silencing target genes through methylation of histone H3 on lysine 27 (H3K27me3) [35]. In addition to methylation of H3K27, EZH2 has been shown to methylate cellular proteins and act as a co-activator of steroid hormone receptors [36]. This function is hypothesized to be independent of PRC2 and potentially induced by phosphorylation of EZH2 [36,37]. Besides its ability of methylate H3K27, EZH2 has recently been described to methylate lysine 120 of histone H2B which competes with ubiquitination on this site [38]. EZH2 is post-translationally regulated by O-linked N-acetylglucosamine (GlcNAc) transferase (OGT)-mediated O-GlcNAcylation at S75, which stabilizes EZH2 and hence facilitates the formation of H3K27me3 [39].

Unlike other SET domains, the methylase EZH2 is inactive on its own for histone substrates. To be functional, EZH2 need to form the PRC2 complex (figure 4) by interacting with other partners, including embryonic ectoderm development (EED), suppressor of zeste 12 homologue (SUZ12), and RBAP48/RBBP4 [40–43]. Collectively, these proteins regulate vital cellular processes, such as differentiation, cell identity, stem-cell plasticity, and proliferation [44–46]. As a result, aberrations in any PRC2 component can have powerful physiologic consequences on the cell.

EZH2, stem cells and reprogramming

EZH2 plays a central role in stem cells. Recent report showed that Ezh2 is important for establishing ES cell lines from blastocysts [47–49]. Additionally, Ezh2 is required for efficient somatic cell reprogramming by cell fusion and nuclear transfer [49,50]. Ezh2 is abundantly expressed in iPS cells (at a similar level as in ES cells), and Ezh2 knockdown severely impaired iPS cell generation. Proper differentiation of iPS cells and reprogramming require, thus, Ezh2 [51]. However, once pluripotency is established, Ezh2 knockdown leaves the pluripotent phenotype of iPS cells unaffected [52]. All this indicates that Ezh2 is critical for induction of pluripotency, but once pluripotency is established, Ezh2 is not required anymore. The mechanism of EZH2 in reprogramming is still poorly known, but it has been recently found that Ezh2 impacts on iPS cell generation at least in part through repression of the CDK inhibitor Ink4a/Arf, which represents a major roadblock for iPS cell generation [52]. Furthermore, c-Myc, one of the iPS cell inducing factors, was recently shown to directly regulate the Ezh2 expression and to be required for maintaining high Ezh2 expression in ES cells [53].

The role of EZH2 in reprogramming is, however, unclear. Indeed, in a recent paper[54], Fragola et al. generated iPS cells from MEF with a conditional *Ezh2* knockout allele for the deletion of the catalytic Ezh2 SET domain [54]. Ezh2-deficient iPS cells, obtained using a cell-permeable TAT-Cre recombinase, exhibited a global loss of H3K27me3, and presented a typical iPS cell phenotype, including ES cell-like morphology, growth, and differentiation potential. This result on Ezh2-deficient iPS cells contrasts other papers which showed essential role of EZH2 in reprogramming [51,52]. It might be explain by used methodology, in that Ezh2 inactivation could have occurred after reprogramming.

EZH2 and cell fate

Enhancer of zeste homolog 2 also regulates expression of tissue-specific genes involved in cellular differentiation and developmental programs [35,55–58]. It is involved in differentiation of embryonic and adult stem cells into several cell lineages (myogenesis, adipogenesis, osteogenesis, neurogenesis, hematopoiesis, lymphopoiesis, epidermal differentiation and hepatogenesis) [59].

For instance, EZH2 was clearly shown to act as a negative regulator of skeletal muscle differentiation favoring the proliferation of myogenic precursors [60–62]. This function results from an EZH2-dependent direct repression of genes related to myogenic differentiation [60] through the H3K27me3 mark deposition on the promoters of myogenic genes [60,63]. EZH2 is expressed early in the myotomal compartment of developing somites and in proliferation satellite cells and is down-regulated in terminally differentiated muscle cells [60]. In skeletal muscle progenitors, EZH2 is, thus, highly expressed and prevents an unscheduled differentiation by repressing muscle-specific gene expression. During the course of their differentiation, EZH2 is downregulated, favoring the expression of muscle specific genes, such as muscle creatine kinase (mCK), myogenin (MyoG), myh, or MyoD [64,65]. Furthermore, the key-role of EZH2 in control of self-renewal and safeguard of the transcriptional identity of skeletal muscle stem cells has been shown using mice with conditional ablation of *Ezh2* in satellite cells. These mice have reduced muscle mass and fail to appropriately regenerate. These defects were associated with derepression of genes expressed in nonmuscle cell lineages [66]. Besides, in humans, abnormal expression of *Ezh2* is observed in the muscular disorder Duchenne muscular dystrophy [67].

EZH2 was also found to be involved in commitment of mesenchymal stem cells towards osteoblast lineage [68]. Suppression of *Ezh2* activity promotes differentiation of human mesenchymal stem cells into osteoblasts. The mechanism might be linked to Runx2 regulation since a striking decrease in *Ezh2* mRNA levels has been found to be correlated to a increased Runx2 binding, suggesting that the transcription of *Ezh2* is potentially negatively regulated by Runx2 [69]. At contrary, deletion of *Ezh2* inhibits adipogenesis, by eliminating H3K27me3 on Wnt promoters and derepressing Wnt expression, which leads to activation of Wnt/ β -catenin signaling [70]. These data show that *Ezh2* facilitates adipogenesis whereas it suppresses osteogenesis.

EZH2 and immune system

EZH2 plays also a role in immune system, for both T and B cell development. Ezh2 is most abundant at sites of embryonic lymphopoiesis, such as fetal liver and thymus [71].

In B cell progenitors, Ezh2 expression is downregulated during differentiation. It is the highest in pro-B cells and very low in mature recirculating B cells (Su et al., 2002). Up-regulation of Ezh2 in proliferating human germinal center B cells (centroblasts) [72] and mitogen-stimulated lymphocytes [73] suggested an important role for this histone methylase in B cell division and activation. This is further supported by the association of EZH2 with Vav, one of the key regulators of the receptor-mediated signaling in lymphocytes [74]. But the major proof of a critical role for Ezh2 in early B cell development and rearrangement of the immunoglobulin heavy chain gene (Igh) has been established, in 2002, using Cre-mediated conditional mutagenesis. Ezh2 deficiency leads to diminished generation of pre-B cells and immature B cells in the bone marrow. Defective B cell development cannot be restored by the presence of the wild-type cells in the mixed bone marrow chimeras. The requirement for Ezh2 is development stage-specific: Ezh2 is a key regulator of histone H3 methylation in early B cell progenitors [75].

EZH2 is a master regulator of the germinal center (GC) B-cell phenotype [76]. It represses genes involved in proliferation checkpoints (*e.g.* *CDKN1A*) and in exit from the GC and terminal differentiation (*e.g.* *IRF4* and *PRDM1*). This function is aberrantly reinforced by mutant EZH2^{Y146N} lymphoma disease alleles [76]. EZH2 also established bivalent chromatin domains at key regulatory loci to transiently suppress GC B-cell differentiation. Beside, EZH2 cooperates with BCL2 to generate GC derived lymphomas [76].

A recent study also established a functional link between this histone methyltransferase EZH2 and transcriptional regulation of lineage-specifying genes in terminally differentiated CD4(+) T cells. EZH2 inactivation specifically enhanced T helper 1 (Th1) and Th2 cell differentiation and plasticity. Ezh2 directly binds Tbx21 and Gata3 genes, leading to substantial trimethylation at lysine 27 of histone 3 (H3K27me3) at these locus, thereby facilitating correct expression of these primordial genes in differentiating Th1 and Th2 cells. Additionally, Ezh2 deficiency leads to spontaneous generation of a small IFN- γ and Th2 cytokine-producing populations in non-polarizing cultures, and under these conditions, IFN- γ expression was largely dependent on increased expression of the transcription factor

Eomesodermin. Besides, *in vivo*, in a model of allergic asthma, *Ezh2* loss results in exacerbated pathology with a progressive accumulation of memory phenotype Th2 cells [77].

EZH2 and cancer

Among EZH2 roles, its implication in cancer is the most studied: more than 70% of articles referenced in Pubmed for “*Ezh2*” term, are related to cancer. Alterations in *EZH2* were first discovered in breast and prostate cancer, where amplification and overexpression first implied it may function as an oncogene [28,31]. Since, increasing evidence demonstrates that EZH2 is not only aberrantly expressed in several types of human cancers, but often behaves as a molecular biomarker of poor prognosis [27,28,31,78–84]. The role of EZH2 in cancer development was initially validated both *in vitro* and *in vivo*, with *EZH2* overexpression proving sufficient to drive proliferation in cancer cells and transform primary fibroblasts [27,85].

Overexpression of EZH2 has now been found in a number of human cancers, such as prostate cancers, gastric cancers, breast cancer, renal cancer, colorectal cancer, non small cell lung cancer, squamous cell carcinomas, urothelial carcinomas in addition to synovial sarcomas, chondrosarcoma, lymphomas and melanomas [31,86–91]. EZH2 expression is correlated with aggressiveness, metastasis, and poor prognosis in most of these cancers. Elevated expression of EZH2 has, also, been identified as a marker for breast cancer initiating cells, possibly reflecting its role in maintaining “stemness” [31,92].

In addition, several mutations, located the most often in SET domain leading to increased trimethylation efficiency, have been associated to cancers (table 4) [93–98]. Recurrent mutations of EZH2 have been found in germinal center B-cell like diffuse large B-cell lymphoma, follicular lymphoma, and melanoma [99]. The mutated residues alter the substrate specificity of EZH2 and facilitate the conversion from a dimethylated to a trimethylated state, thus resulting in significantly elevated global H3K27me3 levels [93,98]. The most frequent identified mutation appears on Y641 (mutations Y/F, Y/N, Y/H, Y/C) [98,100,101]. Another mutation has been identified (A677G and A687V) though these mutants are less prevalent in [93,102].

Together this data suggests a causative role for elevated catalytic activity of EZH2 in the development of cancer. The functional consequence of increased EZH2 (either by overexpression or mutations) in cancer tissues includes the silencing of genes that promote differentiation and restrain proliferation.

Nonetheless, a high expression of EZH2 and trimethylation of histone H3 at lysine 27 were sometimes associated with improvements in survival. Thus, increased EZH2 expression is correlated to better overall survival in diffuse large B-cell lymphoma and lung cancer [103,104]. In the same way, a recent report showed that EZH2 serves as a tumor suppressor in myelodysplastic syndromes, which was evidenced by *EZH2* deletions, missense and frameshift mutations [105]. Besides, enhanced trimethylation of H3K27me3 has been correlated with longer overall survival and better prognosis in non-small cell lung cancer, breast, ovarian and pancreatic cancers [106,107].

Mechanistically, EZH2 is usually believed to function predominately as a transcriptional repressor that silences an array of target genes, including more than 200 tumor suppressors [88,108]. EZH2 is identified as a downstream mediator of the retinoblastoma protein (pRB) pathway-E2F pathway which controls multiple key cell-cycle regulators during cell proliferation in normal and cancer cells [27]. Additionally, EZH2 represses the p16, p19 and p15 directly or indirectly which activates the cyclin D-CDK4/6 complex and promotes progression through G1 phase and cell proliferation [109,110]. Furthermore, enforced expression of EZH2 increases cancer cell proliferation, epithelial-mesenchymal transition, metastatic spreading and other oncogenic properties, whereas its depletion inhibited cell proliferation, migration and invasion and induced cell apoptosis and senescence both in vitro and in vivo [87,111,112]. Besides, EZH2 could cause a rise in cell migration and invasion in cancer cells by regulating E-cadherin and MMP [113]. Increasing evidence also suggests that aberrant overexpression of EZH2 could contribute to acquired chemotherapeutic resistance in multiple cancers [114–116].

In addition to its role as a transcriptional repressor, several studies have shown that EZH2 may also function in target gene activation [36,117,118]. Recently, Xu *et al* reported that EZH2 plays an important role in castration-resistant prostate cancer, and its oncogenic function does not depend on silencing but rather on transcriptional induction of its target

genes [36]. Many of these genes were downregulated upon EZH2 knockdown, suggesting that the role of EZH2 as an activator was independent of the PRC2 complex. This function is hypothesized to be induced by phosphorylation of EZH2 [36,37].

Antagonistic relationship between PRC2 and SWI/SNF

Accumulating evidence has suggested that SWI/SNF (SWitch/Sucrose NonFermentable) chromatin-remodeling complex oppose epigenetic silencing by PcG proteins, and functions as a tumor suppressor in some cancers. This SWI/SNF complex is a multi-subunit chromatin remodeling complex that uses the energy of ATP hydrolysis to reposition nucleosomes, thereby regulating access to the DNA and modulating transcription and DNA replication/repair [119].

The activity of SWI/SNF complex can be counteracted by PcG proteins [120,121]. This antagonistic relationship between SWI/SNF components and PcG proteins were first uncovered via genetic studies in *Drosophila*. In 1988, mutations in core components of the SWI/SNF complex were found to suppress defects in body segment identity conferred by mutations in PcG proteins [122]. Latter, in 90's year, it was discovered that the SWI/SNF complex promotes *Hox* gene activation during embryogenesis, while PcG proteins maintain their repression [123,124]. SWI/SNF is also capable of displacing PcG proteins from the INK4a/ARF locus [125].

Furthermore, there seems to be a balanced function between SWI/SNF and PcG. Accumulating evidence raises the possibility that the antagonistic relationship between these two complexes plays important roles in preventing tumor formation in mammals. Intriguingly, while PcG proteins are frequently overexpressed in cancers, specific inactivating mutations of SWI/SNF complex have been identified in several human cancers [126]. The most compelling case has been that of *SMARCB1* (SNF5), which was discovered to be homozygously inactivated in nearly all rhabdoid tumors (a rare pediatric malignancy) [127]. Interestingly, *SMARCB1*-heterozygous mice develop sarcomas that closely resemble human rhabdoid tumors [128]. Tumorigenesis can be completely suppressed by tissue-specific codeletion of *EZH2*, suggesting an antagonistic interaction between PRC2 and SWI/SNF [129].

EZH2 inhibitors

As described above, most findings have established that EZH2 functions as an important oncogenic biomarker for cancer initiation and progression, thus leading to the hypothesis that blocking EZH2 expression/activity and its downstream signaling cascade may represent a promising strategy for novel anticancer treatment. That's why several groups have developed small-molecule inhibitors of EZH2 [130]. Over the past few years, several potent inhibitors of EZH2, with various selectivities, have been discovered and demonstrated promising preclinical results (figure 5, table 5).

DZNep as an indirect inhibitor

The first EZH2 inhibitor which was described is a cyclopentanyl analog of 3-deazaadenosine, called 3-Deazaneplanocin A (DZNep). It is a cyclopentanyl analog of 3-deazaadenosine that potently inhibits the activity of S-adenosylhomocysteine hydrolase (SAH), resulting in cellular accumulation of (SAH) which in turn represses the S-adenosyl-L-methionine-dependent histone lysine methyltransferase activities [143] (figure 5). Initially studied for its antiviral properties, recent findings indicate that DZNep is a chromatin-remodeling compound that induces degradation of cellular PRC2 proteins including EZH2 and concomitant removal of H3K27me3 mark [79,132].

Disruption of EZH2 by DZNep leads to the reactivation of the epigenetically silenced targets. This induces apoptosis, inhibits cell invasion and enhances chemotherapeutic sensitivity in tumoral cells, but not in normal and untransformed cells at tumor-inhibiting doses [79]. As DZNep has minimal toxicity *in vivo* [144], it may be a promising drug candidate for anti-cancer treatment. That's why, it has been widely examined as a possible epigenetic therapeutic agent for the treatment of various cancers, including lung cancer [145], gastric cancer [146], myeloma [133], acute myeloid leukemia [132], lymphoma [147], but also chondrosarcoma [91]. DZNep-induced inhibition of EZH2 dramatically diminished the number and self-renewal capacity of cancer cells with tumor-initiating properties and significantly decreased tumor xenograft growth and improved survival [134,148].

DZNep selectively induced apoptosis in cancer cells but not in normal cells by preferential reactivation of genes repressed by PRC2 including the apoptosis effector FBOX32 [79]. EZH2

depletion induced not only cell cycle arrest and apoptosis, but also cell senescence. EZH2 decrease triggered simultaneous remarkable gains of two senescence-associated regulators p16 and p21. These data suggest that DZNep exerts its anticancer roles partially through inducing cell apoptosis and senescence and inhibiting cell proliferation [149]. Interestingly, DZNep also reduces tumoral cell migration and invasion, in part through upregulating E-cadherin [150].

These findings suggested DZNep may be a promising therapeutic agent for cancer treatment through multiple mechanisms. Besides its antitumoral role, DZNep has been reported to modulate allogeneic T cell responses and may represent a novel therapeutic approach for treatment of graft *versus* host disease [151]. DZNep also promotes erythroid differentiation of K562 cells, presumably through a mechanism that is not directly related to EZH2 inhibition [152], suggesting that this inhibitor may also be exploited for therapeutic applications for hematological diseases, including anemia.

SAM-competitive inhibitor

Because DZNep is not totally specific to EZH2, significant efforts have been made over the past few years to obtain compounds that are potent and highly selective for EZH2 (table 6) [99,138,140,141,153]. To identify inhibitors of EZH2 methyltransferase activity, high-throughput biochemical screening experiments have been performed. Although the structure of the EZH2 active site has not yet been determined, the conserved SET domain architecture predicts two essential binding pockets: one for the SAM methyl donor and another for the Lys27 substrate. Because more than 50 SET domain proteins have been identified in humans thus far, the selectivity of the inhibitors is crucial for minimizing off-target effects [154]. From the end of 2012, several SAM-competitive inhibitors were announced with promising preclinical results [153] (figure 5, table 6).

The compound EPZ005687 has a K_i value of 24 nmol/L and is over 500-fold more selective for EZH2 versus 15 other PMTs and 50-fold more selective for EZH2 versus the closely related enzyme EZH1 [138]. Interestingly, EPZ005687 can also inhibit H3K27 methylation induced by the EZH2 mutants Y646 and A682, and it has been shown to selectively kill lymphoma cells that are heterozygous for one of these EZH2 mutations, with minimal effect on the proliferation of wild-type cells [138]. Another EZH2 inhibitor developed by Epizyme Inc. is EPZ-6438 (also called E7438). It shares similar *in vitro* properties (i.e. mechanism of action,

specificity, and cellular activity) as EPZ005687, but it demonstrates significantly improved pharmacokinetic properties, including good oral bioavailability in animals. Interestingly, oral dosing of EPZ-6438 leads to potent *in vivo* target inhibition and antitumor activity in a SMARCB1-deleted malignant rhabdoid tumor xenograft model (21). The ability of EPZ-6438 to reduce global H3K27Me3 levels was further demonstrated in several other human lymphoma cell lines, including lines expressing either wild-type or mutant EZH2. This compound is currently under study in a phase 1/2 trial as a single agent in subjects with advanced solid tumors or with B cell lymphomas. The primary goal of the phase 1 trial is to establish the safety and define the maximal tolerated dose of the drug.

EI1, another inhibitor of EZH2, was developed by Novartis [140] and shows very good selectivity with a low K_i value (approximately 13 nmol/L). Loss of the H3K27 methylation function and activation of PRC2 target genes have been observed in EI1-treated cells. EI1 is equally active against both wild type and the Y646 mutant form of EZH2, and the inhibition of the EZH2 Y646 mutant in B-cell lymphomas decreases the H3K27 methylation level genome-wide and activates PRC2 target genes, leading to decreased proliferation, cell cycle arrest, and apoptosis [140].

Another EZH2 inhibitor is GSK126 (developed by GlaxoSmithKline), which has a K_i of 0.5–3 nmol/L [99]. The selectivity of GSK126 for EZH2 is more than 1000-fold higher than its selectivity for 20 other human methyltransferases containing SET or non-SET domains, and it is over 150-fold more selective for EZH2 than for EZH1. McCabe *et al.* showed that the compound GSK126 decreased global methylation at H3K27 and reactivated silenced PRC2 target genes in EZH2-mutant diffuse large B-cell lymphoma (DLBCL) cell lines [99]. Furthermore, this compound effectively inhibited the proliferation of the EZH2-mutant DLBCL cells, and suppressed tumor growth in a mouse xenograft model.

UNC1999, an analogue of GSK126, is the first orally bioavailable inhibitor that has high *in vitro* potency against wild type and mutant EZH2 over a broad range of epigenetic and non-epigenetic targets. As GSK126, UNC1999 potently reduced H3K27me3 levels in cells (IC_{50} <50 nmol/L) and selectively killed DLBCL cell lines harboring the Y646N mutation [141]. However, UNC1999 shows less selectivity for EZH1 than the inhibitors mentioned above.

Stabilized α -helix of EZH2 (SAH-EZH2)

Most recently, Kim *et al.* developed a peptide called stabilized α -helix of EZH2 (SAH-EZH2), which inhibits EZH2 inhibition by a different mechanism from previous inhibitors [142]. SAH-EZH2 selectively disrupts the contact between EZH2 and EED, another subunit in the PRC2 complex, whereas the other EZH2 inhibitors target the HMT catalytic domain (figure 5). As in the case of GSK126, SAH-EZH2 decreases the H3K27 trimethylation level, resulting in growth arrest of PRC2-dependent MLL-AF9 leukemia cells (table 6).

Future perspective

Due to frequent activation of *EZH2* in cancers, these new targeted therapies hold exciting promise in the clinic. Indeed, as discussed above, several reports have shown that genetic silencing and pharmacologic inhibition of EZH2 induced cell apoptosis, inhibited cell invasion and tumor angiogenesis, ultimately suppressed cancer growth and progression [155,156]. More importantly, given the advantages of specific chemical compounds including convenient to use and reversible nature of epigenetic modifications behind carcinogenesis, administration of small molecules targeting EZH2 seems to be a plausible and appealing way as a novel anti-cancer strategy [157]. However, the down-regulation of the EZH2 causes the hepatocytes to become more susceptible to lipid accumulation and inflammation. Significantly, from a translational point of view, because EZH2 inhibitors are potential and promising drugs useful in the treatment of various types of cancer, the patients who will be eventually treated with them should be monitored for the induction of non-alcoholic fatty liver disease (NAFLD) as a potential side effect [158].

Executive summary

- Histone modifications and histone code: Post-translational modifications of histone play a major role in transcriptional control and normal development, and are tightly regulated (histone code).
- Role of the lysine methyltransferase EZH2:
 - * H3K27 methylation is a major epigenetic mark, related to gene silencing, and its control by HMTs (EZH2) and HDMs (JMJD3 and UTX) is a major regulator of cell physiology (reprogramming, cell differentiation, immune system, cancers...).
 - * EZH2 is overexpressed or mutated in numerous types of cancers.

- EZH2 inhibitors: EZH2 inhibitors are promising anticancer drugs

Defined key-terms

- 1) A current search of the PubMed database for the term '**epigenetic**' returns more than 33 000 papers, with about half of them published during the past 4 years, marking an explosion of research efforts on this topic. Striking is the diversity of biological processes that are described in these articles, including fundamental aspects of development, cell fate or reprogramming in diverse organisms, as well as basic mechanisms of transcriptional control or DNA damage repair. Thus, epigenetic, through the modulation of genetic information, plays roles in fundamental life processes, such as cell proliferation, cell development, cell fate, or decision between cell survival and cell death.
- 2) **EZH2** (Enhancer of Zeste Drosophila Homolog 2) was initially cloned in 1996. This genes located on human chromosome 21 encodes a histone methyltransferase and constitutes the catalytic component of the polycomb repressive complex-2 (PRC2). EZH2 specifically methylates the histone H3 at lysine-27 (H3K27). It plays a major role in a plethora of biological processes, including development, cell fate or reprogramming, as well as regulation of immune system or cancers.

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Figures

Figure 1: Schema of nucleosome organization

The fundamental DNA packing unit is known as a nucleosome. Each nucleosome is about 11nm in diameter. The DNA double helix wraps around a central core of eight histone protein molecules (an octamer containing 2 H2A, 2 H2B, 2 H3 and 2 H4) to form a single nucleosome. The N-terminal “tail” of these histones can undergo post-translational modifications (acetylation, methylation or phosphorylation).

Figure 2: Schematic representation of major histone modifications

Histone modifications mainly occur on the N-terminal tails of histones but also on the C-terminal tails and globular domains. The major modifications shown include acetylation (A), methylation (M), phosphorylation (P) and ubiquitination (U).

Figure 3: Methyl group transfer reaction on lysine

The lysine amino group of the substrate histone polypeptide engages in a $\text{S}_\text{N}2$ reaction with the activated co-factor S-adenosyl-L-methionine (SAM), resulting in the formation of an N-methylated lysine and S-adenosyl-L-homocysteine (SAH).

Figure 4: Schematic representation of PRC2 complex.

(A) Domain organizations of each subunit in the human PRC2 complex.

Domain “1”, binding region for PHF1 in human cells; domain “2”, binding region for SUZ12; CXC, cysteine-rich domain; SANT, domain that allows chromatin remodeling protein to interact with histones; SET, catalytic domain of EZH2; VEFS, VRN2-EMF2-FIS2-SUZ12 domain; WD, WD-40 domain; WDB, WD-40 binding domain; Zn, Zn-finger region.

(B) The subunits of human PRC2 complexes, their interactions, and schematic function of PRC2 are shown.

Figure 5: Modes of inhibition of PRC2.

Three types of inhibitors are indicated: DZNep as an SAH hydrolase inhibitor, SAM competitive inhibitors, and SAH-EZH2 peptides as disrupters of the contact between EZH2 and EED.

Tables

Table 1: The histone code.¹

	Methylation			Acetylation	Ubiquitination
	Mono-methylation	di-methylation	tri-methylation		
H2AK119	-	-	-	-	repression
H2BK5	activation	-	repression	-	-
H3K4	activation	activation	activation	-	-
H3K9	activation	repression	repression	activation	-
H3K14	-	-	-	activation	-
H3K18	-	-	-	activation	-
H3K27	activation	repression	repression	activation	-
H3K36	repression	activation	activation	-	-
H3K56	-	-	-	activation	-
H3K79	activation	activation	activation repression	-	-
H4K12	-	-	-	activation	-
H4K20	activation		repression	-	-

¹ For each post-translational modification, the known functional association on gene transcription is shown. By reading the combinatorial and/or sequential histone modifications that constitute the histone code, it may be possible to predict which gene products will be transcribed. However, this code is controversial, since some gene loci present marks both associated with transcriptional activation and linked with repression. These bivalent domains are posited to be poised for either up- or downregulation and to provide an epigenetic blueprint for lineage determination, and are usually found in stem cells.

Table 2: Histone target substrates and domain structure of histone lysine methyltransferases.²

	Synonyms	Protein structure	Histone substrates
With SET domain	MLL, KMT2A		H3K4me1/2/3
	MLL2, KMT2D		H3K4me1/2/3
	SETD1A, SET1A, KMT2F		H3K4me1/2/3
	SETD1D1B, SET1B, KMT2G		H3K4me1/2/3
	MLL4, KMT2B		H3K4me1/2/3
	MLL3, HALR, KMT2C		H3K4me1/2/3
	EZH2, KTM6A, KTM6		H3K27me2/3
	EZH1, KTM6B		H3K27me2/3
	NSD2, WHSC1, MMSET		H3K36me3
	NSD3, WHSC1L
	NSD1, KMT3B		H3K36me2/3
	SET2, HYPB, SETD2		H3K36me3
	ASH1L		H3K4me3
	SUV39H1, KTM1A		H3K9me2/3
	SUV39H2, KTM1B		H3K9me2/3
	EHMT2, G9A		H3K9me1/2
	EHMT1, GLP1		H3K9me1/2
	SETDB1, ESET		H3K9me2/3
	SETDB2, CLL8
	SETMAR
	SETD8, PR-SET7		H4K20me1
	SMYD4
	MLL5, KMT2E
	SETD5

² For each protein, the official name as well as the most commonly used synonyms, the histone target substrates and domain structure are provided.

SET: Suppressor of variegation, Enhancer of Zeste, Trithorax domain; pre/post-SET: cysteine-rich motifs found adjacent to a subset of SET domains; PHD: plant homeodomain zinc finger; ANK: ankyrin repeats; AT hook: A/T DNA binding motif; C2H2 Znf: C2H2-type zinc finger; HMG: high mobility group; SANT: SWI3, ADA2, N-CoR and TFIIIB DNA-binding domain; CxxC: CxxC zinc finger; MBD: methyl CpG binding domain.

	SETD7, SET7/9		H3K4me1
	SETD4		
	SUV4-20H1, KMT5B		H4K20me2/3
	SUV4-20H2, KMT5C		H4K20me2/3
	SMYD5		
	SETD3		
	SETD6		
	SMYD1, KMT3D		
	SMYD2, KMT3C		H3K36me2
			H3K4
	SMYD3, KMT3E		H3K4me2/3
	PRDM1, BLIMP1		
	PRDM14		
	PRDM9, MEISETZ		H3K4me3
	PRDM11, PFM8		
	PRDM4, PFM1		
	PRDM15, PFM15		
	PRDM6, PFM3		
	PRDM12, PFM9		
	PRDM5, PFM2		
	PRDM8, PFM5		
	PRDM13, PFM10		
	PRDM3, PDS1-EVI1		
	PRDM6, MEL1, PFM3		
	PRDM2, RIZ1, KMT8		H3K9
Without SET domain	DOT1L, KMT4		H3K79

SET	bromo	TUDOR	C2H2 Znf	CxxC
pre-SET	chromo	ANK	HMG	MBD
pre-SET	PHD	AT hook	SANT	DOT

Table 3: Histone target substrates and domain structure of histone lysine demethyltransferases. ³

	Synonyms	Protein structure	Histone substrates	Other substrates
LSD demethylases	LSD1, KDM1A, AOF2, BHC110		H3K4me1, H3K4me2 H3K9me1, H3K9me2	p53, E2F1, DNMT1
	LSD2, KDM1B, AOF1		H3K4me1, H3K4me2	
JMJC demethylases	JMJD7
	HIF1AN
	HSPBAP1
	JMJD5, KDM8		H3K36me2	
	JMJD4
	JMJD6, PSR, PTDSR		H3R2 H4R3	
	JMJD8
	FBXL10, JHDM1B, KDM2B		H3K36me1, H3K36me2 H3K4me3	
	FBXL11, JHDM1A, KDM2A		H3K36me1, H3K36me2	NFkB (p65)
	KIAA1718, JHDM1D		H3K9me1, H3K9me2 H3K27me1, H3K27me2	
	PHF8, JHDM1F		H3K9me1, H3K9me2	

³ For each protein, the official name as well as the most commonly used synonyms, the histone target specificities and domain structure are provided. Structural domains are annotated.

ARID: AT-rich interacting domain; amine oxidase: amine oxidase domain; C5HC2: C5HC2 zinc-finger domain; CXXC: CXXC zinc-finger domain; DNMT1: DNA methyltransferase 1; FBOX: F-box domain; FBXL: F-box and Leu-rich repeat protein; HIF1AN: hypoxia-inducible factor 1A inhibitor; HR: hairless domain; HSPBAP1: heat shock protein-associated protein 1; JARID: Jumonji domain-ARID-containing protein; JMJC: Jumonji C domain; LRR: Leu-rich repeat domain; LSD: Lys-specific demethylase; MINA: MYC induced nuclear antigen; NFkB: nuclear factor kB; NO66: nucleolar protein 66; PHD: plant homeodomain; SWIRM: Swi3p Rsc8p and Moira domain; TPR: tetratricopeptide domain; TUDOR: Tudor domain; UTX: ubiquitously transcribed X chromosome tetratricopeptide repeat protein; UTY: ubiquitously transcribed Y chromosome tetratricopeptide repeat protein.

		H4K20me1	
PHF2, JHDM1E		H3K9me2	ARID5B
HR
KDM3B
JMJD1A, JHDM2A, TSGA, KDM3A		H3K9me1, H3K9me2	
JMJD1C
JMJD3, KDM6B		H3K27me2, H3K27me3	
UTX, KDM6A		H3K27me2, H3K27me3	
UTY
JMJD2A, JHDM3A, KDM4A		H3K9me2, H3K9me3	
		H3K36me2, H3K36me3	
		H1.4K26me2, H1.4K26me3	
JMJD2C, JHDM3C, GASC1, KDM4C		H3K9me2, H3K9me3	
		H3K36me2, H3K36me3	
		H1.4K26me2, H1.4K26me3	
JMJD2B, JHDM3B, KDM4B		H3K9me2, H3K9me3	
		H3K36me2, H3K36me3	
		H1.4K26me2, H1.4K26me3	
JMJD2D, JHDM3D, KDM4D		H3K9me2, H3K9me3	
		H3K36me2, H3K36me3	
		H1.4K26me2, H1.4K26me3	
JARID1B, PLU1, KDM5B		H3K4me2, H3K4me3	
JARID1C, SMCX, KDM5C		H3K4me2, H3K4me3	
JARID1D, SMCY, KDM5D		H3K4me2, H3K4me3	
JARID1A, RBP2, KDM5A		H3K4me2, H3K4me3	
JARID2
MINA
NO66		H3K4me2, H3K4me3	
		H3K36me2, H3K36me3	

Table 4: Association between EZH2 mutations and disease.⁴

Mutated domain	Mutation	Phenotype
CXC domain (503-605)	H530N	Acute myeloid leukemia
	C547fs	Acute myeloid leukemia
	Q553X	Acute myeloid leukemia
	C571Y	Myelofibrosis
	C576W	Myelodysplastic syndrome, myeloproliferative neoplasms
	P577L	Early T-cell precursor acute lymphoblastic leukaemia
	R583X	Chronic myelomonocytic leukemia
SET domain (612-727)	V626M	Werner syndrome
	K639E	Werner syndrome
	Y646N, H, F, C	Diffuse large B-cell lymphoma
	I651F	Early T-cell precursor acute lymphoblastic leukaemia
	V662fs	Myelodysplastic syndrome
	D644E	Atypical Chronic Myeloid Leukemia, Myelodysplastic syndrome, myeloproliferative neoplasms
	D664V	Werner syndrome
	D664fs	Acute megakaryoblastic leukemia
	N673S	Chronic myelomonocytic leukemia
	L647V	Myelodysplastic syndrome, Acute myeloid leukemia
	N675K	Refractory Cytopenia with Multilineage Dysplasia
	V679E	Myelofibrosis
	A682G	Lymphoma,
	A682T	Werner syndrome, neuroblastoma
	A682V	Acute myeloid leukemia
	R684C	Werner syndrome, Myelofibrosis
	R684H	Early T-cell precursor acute lymphoblastic leukaemia
	K685fs	Chronic myelomonocytic leukemia
	R690H	Refractory Cytopenia with Multilineage Dysplasia, Chronic myelomonocytic leukemia
	R690C	Myelodysplastic syndrome

⁴ EZH2 mutations identified in association with disease are annotated below with the disease-associated with each mutation, the nature of the mutation, and the structural domain involved. The sequence is numbered in accordance with EZH2 isoform A and the numbering for some mutations has been transposed from the original references so that all mutations can be referred to relative to the same sequence. (Abbreviations: fs, frameshift; X, nonsense).

	A692V	Diffuse large B-cell lymphoma
	N693T	Acute Myelomonocytic Leukemia
	N693Y	Early T-cell precursor acute lymphoblastic leukaemia, Myelofibrosis
	H694Y	Werner syndrome
	H694R	Chronic myelomonocytic leukemia
	S695L	Werner syndrome, Early T-cell precursor acute lymphoblastic leukaemia
	I727fs	Myelodysplastic/myeloproliferative neoplasm, unclassifiable
Other domain	F728fs	Early T-cell precursor acute lymphoblastic leukaemia
	Y731X	Chronic myelomonocytic leukemia
	Y733fs	Myelodysplastic syndrome
	Y733X	Werner syndrome
	Y741C	Werner syndrome
	V742ins	Acute myeloid leukemia
	V742D	Early T-cell precursor acute lymphoblastic leukaemia
	I744fs	Acute myeloid leukemia
	E745K	Werner syndrome, lymphoma
	E745fs	Acute myeloid leukemia

Table 5: Chemical structures and biochemical data for small-molecule inhibitors of EZH2

Structure	Compound	Mechanism and potency	Selectivity toward EZH2	Highest clinical status	References
DZNep	SAH hydrolase inhibitor	non selective	preclinical	[79,131–134]
GSK126	SAM-competitive inhibitor of PRC2, $K_i = 0,5-3$ nM	> 1000-fold over 20 other HMTs; over EZH1	preclinical	[99,135]
GSK343	SAM-competitive inhibitor of PRC2, $K_i = 0,5-3$ nM	$IC_{50} = 4$ nM and is over 1000-fold selective for other HMTs except EZH1 (60-fold selectivity)	preclinical	[136,137]
EPZ005687	SAM-competitive inhibitor of PRC2, $K_i = 24$ nM	> 500-fold over 15 other HMTs; about 50-fold over EZH1	preclinical	[138]
EPZ-6438	SAM-competitive inhibitor of PRC2, $K_i = 0,5-3$ nM $IC_{50} = 11$ nM	35-fold selectivity versus EZH1 ; >4,500-fold selectivity relative to 14 other HMTs	phase I/II	[139]

EI1	SAM-competitive inhibitor of PRC2 IC ₅₀ =15 nM; K _i =13 nM		preclinical	[140]
UNC1999	SAM-competitive inhibitor of PRC2 IC ₅₀ =2-15 nM; K _i =13 nM	over 1000-fold selective for other HMTs except EZH1 (22-fold selectivity).	preclinical	[141]
Peptide: FSSNRXKILXRTQILNQEWKQRRIQPV	stabilized α-helix of EZH2 peptide (SAH- EZH2)	Hydrocarbon-stapled peptide that mimics the α-helical EED-binding domain of EZH2, disrupting the EZH2–EED complex	not selective for EZH1	preclinical	[142]

1 Supplementary material

2 Table 1: List of histone lysine methyltransferases and demethylases linked to disease.¹

	Gene	histone substrate	Disease	Genetic/epigenetic aberration	Aberrant expression	References
histone lysine methyl transferase	MLL	H3K4	Leukemia (AML, ALL, MLL)	> 50 different MLL fusions		[1–7]
			Acute myeloid leukemia (AML)	MLL-PTD		[8–10]
			Wiedemann-Steiner syndrome	intragenic mutations		[11–13]
	MLL2	H3K4	Hepatocellular carcinoma (HCC)	Hepatitis B virus integration into MLL2 : HBx-MLL2 fusion		[14]
			Acute myeloid leukemia (AML) (mice)	NUP98-JARID1A		[15]
			Kabuki syndrome 1 (KABUK1)	intragenic mutations		[16,17]
	MLL3	H3K4	Leukemia			[18,19]
			Colorectal cancer	Intragenic mutations		[19–22]
	DOT1L	H3K79	Leukemia (AML, ALL)	MLL-AF10 fusion, MLL-AF4 fusion		[23,24]
			T cell acute lymphoblastic leukemia (T-ALL)	CALM-AF10 fusion, SET-NUP214 fusion		[25,26]
			Osteoarthritis	intragenic mutations		[27,28]
	EZH2	H3K27	Bladder carcinoma		overexpression	[29–36]
			Breast cancer		overexpression	[29,30,37,38]
			Colorectal cancer		overexpression	[29,39–45]
			Gastric cancer		overexpression	[46–53]
			Hepatocellular carcinoma		overexpression	[54–59]
			Lymphoma	Intragenic mutations	overexpression downregulation	[60–82] [64]
			Myeloproliferative neoplasms	Intragenic mutations		[83–91]
			Rhabdoid tumors	Intragenic mutations; Mutations of opposing chromatin modifying complex SWI-SNF		[92–94] [95]
			Melanoma		overexpression	[38,96–99]
			Prostate cancer		overexpression	[100–121]
			chondrosarcoma		overexpression	[122]
			lung cancer		downregulation	[123]
			Various other cancers		overexpression	[29,38]
			Weaver syndrome	Intragenic mutations		[124–126]

¹ However, in many cases the molecular mechanisms of disease development are not well understood yet, and it remains to be shown whether misregulation of these HMTs contributes to disease initiation or progression.

	NSD1	H3K36	Acute myeloid leukemia (AML)	t(5;11)(q32;p15,5); translocation: NUP98- NSD1 fusion		[127–138]
			Myelodysplastic syndrome	t(5,11)(q35;p15,5)		[131,139]
			Beckwith-Wiedemann syndrome	intragenic mutations		[140]
			Sotos syndrome	intragenic mutations; 5q35 microdeletions		[140–173]
	NSD2	H3K36	glioblastoma multiform (GBM)		overexpression	[174]
			hepatocellular carcinoma (HCC)		overexpression	[175]
			leukemia		overexpression	[176]
			multiple myeloma (MM)	t(4;14)(p16;q32): altered expression of FGFR3, NSD2		[177,178]
			Various other cancers			[179]
			Wolf-Hirschhorn syndrome			[180]
	NSD3		acute myeloid leukemia (AML)	t(8;11)(p11,2;p15); translocation: NUP98- NSD3 fusion		[181]
	EHMT1	H3K9	9q subtelomeric deletion syndrome / Kleeftstra syndrome	haploinsufficiency of EHMT1; microdeletion of 9q34.3: intragenic mutation	downregulation	[175,182–185]
			breast cancer	intragenic mutations		[186]
			medulloblastoma		downregulation	[187]
	SETDB1	H3K9	Huntington's disease (HD)		overexpression	[188]
	SETDB2		asthma	mutation		[189]
	SMYD2	H3K4 H3K36	pediatric acute lymphoblastic leukemia		overexpression	[190]
			esophageal squamous cell carcinoma		overexpression	[191]
			hepatocellular carcinoma (HCC)		overexpression	[192]
	SMYD3	H3K4	breast cancer		overexpression	[193,194]
			colorectal cancer		overexpression	[195]
			hepatocellular carcinoma (HCC)		overexpression	[196]
	SMYD4		breast cancer		downregulation	[197]
			medulloblastoma		downregulation	[187]
	PRDM1		lymphoma	mutations in PRDM1 gene, epigenetic silencing		[198–207]
			lupus erythematosus		overexpression	[208–210]
			Crohn's disease	intragenic mutation		[211–213]
	PRDM2	H3K9	breast cancer	mutations in PRDM2 gene; promoter DNA methylation		[186,214,215]
			colorectal cancer (CRC)	mutations in PRDM2 gene		[216–218]

			gastric cancer	mutations in PRDM2 genes, promoter DNA methylation		[216,218–221]
			hepatocellular carcinoma (HCC)	promoter DNA methylation	downregulation	[222–226]
			lung cancer		downregulation	[215,227]
			neuroblastoma		downregulation	[228,229]
	PRDM5		breast cancer	promoter DNA methylation	downregulation	[230]
			colorectal and gastric cancer	promoter DNA methylation	downregulation	[231]
			liver cancer	promoter DNA methylation	downregulation	[230]
	PRDM8		lafora disease	mutation		[232]
	PRDM9	H3K4	azoospermia, infertility	mutation		[233–235]
	PRDM12		chronic myeloid leukemia (CML)	9q microdeletions encompassing RRP4 and PRDM12		[236]
	PRDM14		breast cancer		overexpression	[237]
Histone demethylase	LSD1	H3K4 H3K9	neuroblastoma		overexpression	[238]
			chondrosarcoma, Ewing's sarcoma, osteosarcoma		overexpression	[239]
			leukemia		overexpression	[238,240–243]
			others cancers		overexpression	[238,244–263]
			hypertension		downregulation	[264,265]
	JMJD5	H3K36	tumors		overexpression	[266]
	KDM2B	H3K36 H3K4	leukemias		overexpression	[267]
	PHF8	H3K9 H4K20	X-linked mental retardation	mutation		[268–272]
	JMJD1A	H3K9	Ewing sarcoma		overexpression	[273]
			kidney cancer		overexpression	[274]
	JMJD3	H3K27	hodgkin's lymphoma		overexpression	[275]
			renal cell carcinoma		overexpression	[276]
	UTX	H3K27	Kabuki syndrome	mutation		[277–280]
			cancers	mutation		[276,281–293]
	JMJD2A	H3K9 H3K36 H1.4K26	cancers		overexpression	[258,294–300]
	JMJD2C	H3K9 H3K36 H1.4K26	cancers		overexpression	[301–304]
	JMJD2B	H3K9 H3K36 H1.4K26	cancers		overexpression	[305–310]
	KDM5B	H3K4	melanoma		overexpression	[311–315]

		prostate cancer		overexpression	[316]
		breast cancer		overexpression	[317–322]
	KDM5C	H3K4	non-syndromic X-linked mental retardation	mutation	[323–333]
	KDM5A	H3K4	breast cancer	overexpression	[334]

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