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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/*b*-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 identify (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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References

1. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature*. 403(6765), 41–45 (2000).).** This article is one of the first paper about the concept "histone code".
2. Marks H, Kalkan T, Menafra R, *et al*. The Transcriptional and Epigenomic Foundations of Ground State Pluripotency. *Cell*. 149(3), 590–604 (2012).

3. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res.* 21(3), 381–395 (2011).
4. Black JC, Van Rechem C, Whetstone JR. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol. Cell.* 48(4), 491–507 (2012). * This is an excellent review about the discovery, characterization and regulation of the KMTs and KDMs.
5. Tan M, Luo H, Lee S, *et al.* Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. *Cell.* 146(6), 1016–1028 (2011).
6. Wozniak GG, Strahl BD. Hitting the “mark”: Interpreting lysine methylation in the context of active transcription. *Biochim. Biophys. Acta BBA - Gene Regul. Mech.* [Internet]. Available from: <http://www.sciencedirect.com/science/article/pii/S1874939914000534>.
7. Berger SL. The complex language of chromatin regulation during transcription. *Nature.* 447(7143), 407–412 (2007).
8. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. *Nat. Struct. Mol. Biol.* 14(11), 1025–1040 (2007).
9. Bernstein BE, Kamal M, Lindblad-Toh K, *et al.* Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell.* 120(2), 169–181 (2005).
10. Schubeler D, MacAlpine DM, Scalzo D, *et al.* The histone modification pattern of active genes revealed through genome-wide chromatin analysis of a higher eukaryote. *Genes Dev.* 18(11), 1263–1271 (2004).
11. Pokholok DK, Harbison CT, Levine S, *et al.* Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell.* 122(4), 517–527 (2005).
12. Santos-Rosa H, Schneider R, Bannister AJ, *et al.* Active genes are tri-methylated at K4 of histone H3. *Nature.* 419(6905), 407–411 (2002).
13. Strahl BD, Ohba R, Cook RG, Allis CD. Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in Tetrahymena. *Proc. Natl. Acad. Sci. U. S. A.* 96(26), 14967–14972 (1999).
14. Rao B, Shibata Y, Strahl BD, Lieb JD. Dimethylation of Histone H3 at Lysine 36 Demarcates Regulatory and Nonregulatory Chromatin Genome-Wide. *Mol. Cell. Biol.* 25(21), 9447–9459 (2005).
15. Wagner EJ, Carpenter PB. Understanding the language of Lys36 methylation at histone H3. *Nat. Rev. Mol. Cell Biol.* 13(2), 115–126 (2012).
16. Lee J-S, Shilatifard A. A site to remember: H3K36 methylation a mark for histone deacetylation. *Mutat. Res.* 618(1-2), 130–134 (2007).

17. Lehnertz B, Ueda Y, Derijck AAHA, *et al.* Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr. Biol. CB.* 13(14), 1192–1200 (2003).
18. Rougeulle C, Chaumeil J, Sarma K, *et al.* Differential histone H3 Lys-9 and Lys-27 methylation profiles on the X chromosome. *Mol. Cell. Biol.* 24(12), 5475–5484 (2004).
19. Escamilla-Del-Arenal M, da Rocha ST, Spruijt CG, *et al.* Cdy1, a new partner of the inactive X chromosome and potential reader of H3K27me3 and H3K9me2. *Mol. Cell. Biol.* 33(24), 5005–5020 (2013).
20. Molina-Serrano D, Schiza V, Kirmizis A. Cross-talk among epigenetic modifications: lessons from histone arginine methylation. *Biochem. Soc. Trans.* 41(3), 751–759 (2013).
21. Migliori V, Phalke S, Bezzi M, Guccione E. Arginine/lysine-methyl/methyl switches: biochemical role of histone arginine methylation in transcriptional regulation. *Epigenomics.* 2(1), 119–137 (2010).
22. Lorenzo AD, Bedford MT. Histone Arginine Methylation. *FEBS Lett.* 585(13), 2024–2031 (2011).
23. Petrossian TC, Clarke SG. Uncovering the Human Methyltransferasome. *Mol. Cell. Proteomics.* 10(1), M110.000976 (2011).
24. Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat. Rev. Genet.* 13(5), 343–357 (2012).
25. Kooistra SM, Helin K. Molecular mechanisms and potential functions of histone demethylases. *Nat. Rev. Mol. Cell Biol.* 13(5), 297–311 (2012).
26. Hublitz P, Albert M, Peters AHFM. Mechanisms of transcriptional repression by histone lysine methylation. *Int. J. Dev. Biol.* 53(2-3), 335–354 (2009).
27. Bracken AP, Pasini D, Capra M, Prosperini E, Colli E, Helin K. EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.* 22(20), 5323–5335 (2003).
28. Varambally S, Dhanasekaran SM, Zhou M, *et al.* The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature.* 419(6907), 624–629 (2002). ** This paper is the first to identify the role of EZH2 in cancer.
29. Collett K, Eide GE, Arnes J, *et al.* Expression of enhancer of zeste homologue 2 is significantly associated with increased tumor cell proliferation and is a marker of aggressive breast cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 12(4), 1168–1174 (2006).
30. Bachmann IM, Halvorsen OJ, Collett K, *et al.* EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and

- cancers of the endometrium, prostate, and breast. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* 24(2), 268–273 (2006).
31. Kleer CG, Cao Q, Varambally S, *et al.* EZH2 is a marker of aggressive breast cancer and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 100(20), 11606–11611 (2003).
 32. Weikert S, Christoph F, Köllermann J, *et al.* Expression levels of the EZH2 polycomb transcriptional repressor correlate with aggressiveness and invasive potential of bladder carcinomas. *Int. J. Mol. Med.* 16(2), 349–353 (2005).
 33. Albert M, Helin K. Histone methyltransferases in cancer. *Semin. Cell Dev. Biol.* 21(2), 209–220 (2010).
 34. Agger K, Christensen J, Cloos PAC, Helin K. The emerging functions of histone demethylases. *Curr. Opin. Genet. Dev.* 18(2), 159–168 (2008).
 35. Kirmizis A, Bartley SM, Kuzmichev A, *et al.* Silencing of human polycomb target genes is associated with methylation of histone H3 Lys 27. *Genes Dev.* 18(13), 1592–1605 (2004).
 36. Xu K, Wu ZJ, Groner AC, *et al.* EZH2 Oncogenic Activity in Castration Resistant Prostate Cancer Cells is Polycomb-Independent. *Science.* 338(6113), 1465–1469 (2012).
 37. Kim E, Kim M, Woo D-H, *et al.* Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell.* 23(6), 839–852 (2013).
 38. Kogure M, Takawa M, Saloura V, *et al.* The Oncogenic Polycomb Histone Methyltransferase EZH2 Methylates Lysine 120 on Histone H2B and Competes Ubiquitination. *Neoplasia N. Y. N.* 15(11), 1251–1261 (2013).
 39. Chu C-S, Lo P-W, Yeh Y-H, *et al.* O-GlcNAcylation regulates EZH2 protein stability and function. *Proc. Natl. Acad. Sci. U. S. A.* 111(4), 1355–1360 (2014).
 40. Cao R, Zhang Y. SUZ12 is required for both the histone methyltransferase activity and the silencing function of the EED-EZH2 complex. *Mol. Cell.* 15(1), 57–67 (2004).
 41. Pasini D, Bracken AP, Jensen MR, Lazzerini Denchi E, Helin K. Suz12 is essential for mouse development and for EZH2 histone methyltransferase activity. *EMBO J.* 23(20), 4061–4071 (2004).
 42. Sewalt RG, van der Vlag J, Gunster MJ, *et al.* Characterization of interactions between the mammalian polycomb-group proteins Enx1/EZH2 and EED suggests the existence of different mammalian polycomb-group protein complexes. *Mol. Cell. Biol.* 18(6), 3586–3595 (1998).
 43. Denisenko O, Shnyreva M, Suzuki H, Bomsztyk K. Point mutations in the WD40 domain of Eed block its interaction with Ezh2. *Mol. Cell. Biol.* 18(10), 5634–5642 (1998).

44. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 469(7330), 343–349 (2011).
45. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic regulators in myeloid malignancies. *Nat. Rev. Cancer*. 12(9), 599–612 (2012).
46. Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat. Rev. Cancer*. 6(11), 846–856 (2006).
47. O’Carroll D, Erhardt S, Pagani M, Barton SC, Surani MA, Jenuwein T. The polycomb-group gene *Ezh2* is required for early mouse development. *Mol. Cell. Biol.* 21(13), 4330–4336 (2001).
48. Shen X, Liu Y, Hsu Y-J, *et al.* EZH1 mediates methylation on histone H3 lysine 27 and complements EZH2 in maintaining stem cell identity and executing pluripotency. *Mol. Cell*. 32(4), 491–502 (2008).
49. Pereira CF, Piccolo FM, Tsubouchi T, *et al.* ESCs require PRC2 to direct the successful reprogramming of differentiated cells toward pluripotency. *Cell Stem Cell*. 6(6), 547–556 (2010).
50. Zhang M, Wang F, Kou Z, Zhang Y, Gao S. Defective chromatin structure in somatic cell cloned mouse embryos. *J. Biol. Chem.* 284(37), 24981–24987 (2009).
51. Villasante A, Piazzolla D, Li H, Gomez-Lopez G, Djabali M, Serrano M. Epigenetic regulation of Nanog expression by *Ezh2* in pluripotent stem cells. *Cell Cycle Georget. Tex.* 10(9), 1488–1498 (2011).
52. Ding X, Wang X, Sontag S, *et al.* The Polycomb Protein *Ezh2* Impacts on iPS Cell Generation. *Stem Cells Dev.* , 131210220315000 (2013).
53. Neri F, Zippo A, Krepelova A, Cherubini A, Rocchigiani M, Oliviero S. Myc regulates the transcription of the PRC2 gene to control the expression of developmental genes in embryonic stem cells. *Mol. Cell. Biol.* 32(4), 840–851 (2012).
54. Fragola G, Germain P-L, Laise P, *et al.* Cell reprogramming requires silencing of a core subset of polycomb targets. *PLoS Genet.* 9(2), e1003292 (2013).
55. Müller J, Hart CM, Francis NJ, *et al.* Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. *Cell*. 111(2), 197–208 (2002).
56. Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell*. 111(2), 185–196 (2002).
57. Cao R, Wang L, Wang H, *et al.* Role of histone H3 lysine 27 methylation in Polycomb-group silencing. *Science*. 298(5595), 1039–1043 (2002). ** This paper shows that polycomb and H3K27me3 is associated to gene silencing.

58. Kuzmichev A, Jenuwein T, Tempst P, Reinberg D. Different EZH2-containing complexes target methylation of histone H1 or nucleosomal histone H3. *Mol. Cell.* 14(2), 183–193 (2004).
59. Chen Y-H, Hung M-C, Li L-Y. EZH2: a pivotal regulator in controlling cell differentiation. *Am. J. Transl. Res.* 4(4), 364–375 (2012).
60. Caretti G, Di Padova M, Micales B, Lyons GE, Sartorelli V. The Polycomb Ezh2 methyltransferase regulates muscle gene expression and skeletal muscle differentiation. *Genes Dev.* 18(21), 2627–2638 (2004).
61. Juan AH, Kumar RM, Marx JG, Young RA, Sartorelli V. Mir-214-dependent regulation of the polycomb protein Ezh2 in skeletal muscle and embryonic stem cells. *Mol. Cell.* 36(1), 61–74 (2009).
62. Wong CF, Tellam RL. MicroRNA-26a targets the histone methyltransferase Enhancer of Zeste homolog 2 during myogenesis. *J. Biol. Chem.* 283(15), 9836–9843 (2008).
63. Palacios D, Mozzetta C, Consalvi S, *et al.* TNF/ α /polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell.* 7(4), 455–469 (2010).
64. Marchesi I, Fiorentino FP, Rizzolio F, Giordano A, Bagella L. The ablation of EZH2 uncovers its crucial role in rhabdomyosarcoma formation. *Cell Cycle Georget. Tex.* 11(20), 3828–3836 (2012).
65. Woodhouse S, Pugazhendhi D, Brien P, Pell JM. Ezh2 maintains a key phase of muscle satellite cell expansion but does not regulate terminal differentiation. *J. Cell Sci.* 126(Pt 2), 565–579 (2013).
66. Juan AH, Derfoul A, Feng X, *et al.* Polycomb EZH2 controls self-renewal and safeguards the transcriptional identity of skeletal muscle stem cells. *Genes Dev.* 25(8), 789–794 (2011).
67. Acharyya S, Sharma SM, Cheng AS, *et al.* TNF Inhibits Notch-1 in Skeletal Muscle Cells by Ezh2 and DNA Methylation Mediated Repression: Implications in Duchenne Muscular Dystrophy. *PLoS ONE.* 5(8), e12479 (2010).
68. Wei Y, Chen Y-H, Li L-Y, *et al.* CDK1-dependent phosphorylation of EZH2 suppresses methylation of H3K27 and promotes osteogenic differentiation of human mesenchymal stem cells. *Nat. Cell Biol.* 13(1), 87–94 (2011).
69. Wu H, Whitfield TW, Gordon JA, *et al.* Genomic occupancy of Runx2 with global expression profiling identifies a novel dimension to control of osteoblastogenesis. *Genome Biol.* 15(3), R52 (2014).
70. Wang L, Jin Q, Lee J-E, Su I, Ge K. Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 107(16), 7317–7322 (2010).

71. Hobert O, Sures I, Ciossek T, Fuchs M, Ullrich A. Isolation and developmental expression analysis of Enx-1, a novel mouse Polycomb group gene. *Mech. Dev.* 55(2), 171–184 (1996).
72. Raaphorst FM, van Kemenade FJ, Blokzijl T, *et al.* Coexpression of BMI-1 and EZH2 polycomb group genes in Reed-Sternberg cells of Hodgkin's disease. *Am. J. Pathol.* 157(3), 709–715 (2000).
73. Fukuyama T, Otsuka T, Shigematsu H, *et al.* Proliferative involvement of ENX-1, a putative human polycomb group gene, in haematopoietic cells. *Br. J. Haematol.* 108(4), 842–847 (2000).
74. Hobert O, Jallal B, Ullrich A. Interaction of Vav with ENX-1, a putative transcriptional regulator of homeobox gene expression. *Mol. Cell. Biol.* 16(6), 3066–3073 (1996).
75. Su I-H, Basavaraj A, Krutchinsky AN, *et al.* Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. *Nat. Immunol.* 4(2), 124–131 (2003).
76. Beguelin W, Popovic R, Teater M, *et al.* EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell.* 23(5), 677–692 (2013).
77. Tumes DJ, Onodera A, Suzuki A, *et al.* The polycomb protein Ezh2 regulates differentiation and plasticity of CD4(+) T helper type 1 and type 2 cells. *Immunity.* 39(5), 819–832 (2013).
78. Raaphorst FM, Meijer CJLM, Fieret E, *et al.* Poorly differentiated breast carcinoma is associated with increased expression of the human polycomb group EZH2 gene. *Neoplasia N. Y. N.* 5(6), 481–488 (2003).
79. Tan J, Yang X, Zhuang L, *et al.* Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. *Genes Dev.* 21(9), 1050–1063 (2007). ** This article is the first to demonstrate that DZNep, an inhibitor of EZH2, induces apoptosis in tumoral cells.
80. Suvà M-L, Riggi N, Janiszewska M, *et al.* EZH2 Is Essential for Glioblastoma Cancer Stem Cell Maintenance. *Cancer Res.* 69(24), 9211–9218 (2009).
81. Kodach LL, Jacobs RJ, Heijmans J, *et al.* The role of EZH2 and DNA methylation in the silencing of the tumour suppressor RUNX3 in colorectal cancer. *Carcinogenesis.* 31(9), 1567–1575 (2010).
82. Takawa M, Masuda K, Kunizaki M, *et al.* Validation of the histone methyltransferase EZH2 as a therapeutic target for various types of human cancer and as a prognostic marker. *Cancer Sci.* 102(7), 1298–1305 (2011).
83. Varambally S, Cao Q, Mani R-S, *et al.* Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science.* 322(5908), 1695–1699 (2008).

84. Wagener N, Macher-Goeppinger S, Pritsch M, *et al.* Enhancer of zeste homolog 2 (EZH2) expression is an independent prognostic factor in renal cell carcinoma. *BMC Cancer*. 10, 524 (2010).
85. Croonquist PA, Van Ness B. The polycomb group protein enhancer of zeste homolog 2 (EZH 2) is an oncogene that influences myeloma cell growth and the mutant ras phenotype. *Oncogene*. 24(41), 6269–6280 (2005).
86. Yu J, Yu J, Rhodes DR, *et al.* A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. *Cancer Res*. 67(22), 10657–10663 (2007).
87. Chase A, Cross NCP. Aberrations of EZH2 in Cancer. *Clin. Cancer Res*. 17(9), 2613–2618 (2011).
88. Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat. Res*. 647(1-2), 21–29 (2008).
89. Velichutina I, Shaknovich R, Geng H, *et al.* EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. *Blood*. 116(24), 5247–5255 (2010).
90. Sellers WR, Loda M. The EZH2 polycomb transcriptional repressor--a marker or mover of metastatic prostate cancer? *Cancer Cell*. 2(5), 349–350 (2002).
91. Girard N, Bazille C, Lhuissier E, *et al.* 3-Deazaneplanocin A (DZNep), an Inhibitor of the Histone Methyltransferase EZH2, Induces Apoptosis and Reduces Cell Migration in Chondrosarcoma Cells. *PLoS ONE* [Internet]. 9(5) (2014). Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4031152/>.
92. Kunju LP, Cookingham C, Toy KA, Chen W, Sabel MS, Kleer CG. EZH2 and ALDH-1 mark breast epithelium at risk for breast cancer development. *Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc*. 24(6), 786–793 (2011).
93. McCabe MT, Graves AP, Ganji G, *et al.* Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on lysine 27 (H3K27). *Proc. Natl. Acad. Sci. U. S. A.* 109(8), 2989–2994 (2012).
94. Morin RD, Mendez-Lago M, Mungall AJ, *et al.* Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*. 476(7360), 298–303 (2011).
95. Pasqualucci L, Trifonov V, Fabbri G, *et al.* Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat. Genet*. 43(9), 830–837 (2011).
96. Sneeringer CJ, Scott MP, Kuntz KW, *et al.* Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc. Natl. Acad. Sci. U. S. A.* 107(49), 20980–20985 (2010).

97. Wigle TJ, Knutson SK, Jin L, *et al.* The Y641C mutation of EZH2 alters substrate specificity for histone H3 lysine 27 methylation states. *FEBS Lett.* 585(19), 3011–3014 (2011).
98. Yap DB, Chu J, Berg T, *et al.* Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. *Blood.* 117(8), 2451–2459 (2011).
99. McCabe MT, Ott HM, Ganji G, *et al.* EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature.* 492(7427), 108–112 (2012).
100. Bödör C, Grossmann V, Popov N, *et al.* EZH2 mutations are frequent and represent an early event in follicular lymphoma. *Blood.* , blood–2013–04–496893 (2013).
101. Bödör C, O’Riain C, Wrench D, *et al.* EZH2 Y641 mutations in follicular lymphoma. *Leukemia.* 25(4), 726–729 (2011).
102. Majer CR, Jin L, Scott MP, *et al.* A687V EZH2 is a gain-of-function mutation found in lymphoma patients. *FEBS Lett.* 586(19), 3448–3451 (2012).
103. Lee HJ, Shin DH, Kim KB, *et al.* Polycomb protein EZH2 expression in diffuse large B-cell lymphoma is associated with better prognosis in patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone. *Leuk. Lymphoma.* (2014).
104. Li Z, Xu L, Tang N, *et al.* The polycomb group protein EZH2 inhibits lung cancer cell growth by repressing the transcription factor Nrf2. *FEBS Lett.* (2014).
105. Nikoloski G, Langemeijer SMC, Kuiper RP, *et al.* Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat. Genet.* 42(8), 665–667 (2010).
106. Chen X, Song N, Matsumoto K, *et al.* High expression of trimethylated histone H3 at lysine 27 predicts better prognosis in non-small cell lung cancer. *Int. J. Oncol.* 43(5), 1467–1480 (2013).
107. Wei Y, Xia W, Zhang Z, *et al.* Loss of Trimethylation at Lysine 27 of Histone H3 Is a Predictor of Poor Outcome in Breast, Ovarian, and Pancreatic Cancers. *Mol. Carcinog.* 47(9), 701–706 (2008).
108. Chang C-J, Hung M-C. The role of EZH2 in tumour progression. *Br. J. Cancer.* 106(2), 243–247 (2012).
109. Zhong J, Min L, Huang H, *et al.* EZH2 regulates the expression of p16 in the nasopharyngeal cancer cells. *Technol. Cancer Res. Treat.* 12(3), 269–274 (2013).
110. Kheradmand Kia S, Solaimani Kartalaei P, Farahbakhshian E, Pourfarzad F, von Lindern M, Verrijzer CP. EZH2-dependent chromatin looping controls INK4a and INK4b, but not ARF, during human progenitor cell differentiation and cellular senescence. *Epigenetics Chromatin.* 2(1), 16 (2009).

111. Ferraro A, Mourtzoukou D, Kosmidou V, *et al.* EZH2 is regulated by ERK/AKT and targets integrin alpha2 gene to control Epithelial-Mesenchymal Transition and anoikis in colon cancer cells. *Int. J. Biochem. Cell Biol.* 45(2), 243–254 (2013).
112. Smits M, Nilsson J, Mir SE, *et al.* miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. *Oncotarget.* 1(8), 710–720 (2011).
113. Shin YJ, Kim J-H. The role of EZH2 in the regulation of the activity of matrix metalloproteinases in prostate cancer cells. *PloS One.* 7(1), e30393 (2012).
114. Ougolkov AV, Bilim VN, Billadeau DD. Regulation of Pancreatic Tumor Cell Proliferation and Chemoresistance by the Histone Methyltransferase EZH2. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 14(21), 6790–6796 (2008).
115. Zhang Y, Liu G, Lin C, Liao G, Tang B. Silencing the EZH2 gene by RNA interference reverses the drug resistance of human hepatic multidrug-resistant cancer cells to 5-Fu. *Life Sci.* 92(17-19), 896–902 (2013).
116. Hu S, Yu L, Li Z, *et al.* Overexpression of EZH2 contributes to acquired cisplatin resistance in ovarian cancer cells in vitro and in vivo. *Cancer Biol. Ther.* 10(8), 788–795 (2010).
117. Lee ST, Li Z, Wu Z, *et al.* Context-specific regulation of NF- κ B target gene expression by EZH2 in breast cancers. *Mol. Cell.* 43(5), 798–810 (2011).
118. Shi B, Liang J, Yang X, *et al.* Integration of estrogen and Wnt signaling circuits by the polycomb group protein EZH2 in breast cancer cells. *Mol. Cell. Biol.* 27(14), 5105–5119 (2007).
119. Wilson BG, Roberts CWM. SWI/SNF nucleosome remodellers and cancer. *Nat. Rev. Cancer.* 11(7), 481–492 (2011).
120. Shao Z, Raible F, Mollaaghababa R, *et al.* Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell.* 98(1), 37–46 (1999).
121. Francis NJ, Saurin AJ, Shao Z, Kingston RE. Reconstitution of a functional core polycomb repressive complex. *Mol. Cell.* 8(3), 545–556 (2001).
122. Kennison JA, Tamkun JW. Dosage-dependent modifiers of polycomb and antennapedia mutations in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 85(21), 8136–8140 (1988).
123. Tamkun JW, Deuring R, Scott MP, *et al.* brahma: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell.* 68(3), 561–572 (1992).
124. Kennison JA. The Polycomb and trithorax group proteins of *Drosophila*: trans-regulators of homeotic gene function. *Annu. Rev. Genet.* 29, 289–303 (1995).

125. Kia SK, Gorski MM, Giannakopoulos S, Verrijzer CP. SWI/SNF mediates polycomb eviction and epigenetic reprogramming of the INK4b-ARF-INK4a locus. *Mol. Cell. Biol.* 28(10), 3457–3464 (2008).
126. Shain AH, Pollack JR. The spectrum of SWI/SNF mutations, ubiquitous in human cancers. *PLoS One.* 8(1), e55119 (2013).
127. Versteeg I, Sévenet N, Lange J, *et al.* Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature.* 394(6689), 203–206 (1998).
128. Roberts CW, Galusha SA, McMenamin ME, Fletcher CD, Orkin SH. Haploinsufficiency of Snf5 (integrator 1) predisposes to malignant rhabdoid tumors in mice. *Proc. Natl. Acad. Sci. U. S. A.* 97(25), 13796–13800 (2000).
129. Wilson BG, Wang X, Shen X, *et al.* Epigenetic antagonism between polycomb and SWI/SNF complexes during oncogenic transformation. *Cancer Cell.* 18(4), 316–328 (2010).
130. Verma SK, Knight SD. Recent progress in the discovery of small-molecule inhibitors of the HMT EZH2 for the treatment of cancer. *Future Med. Chem.* 5(14), 1661–1670 (2013).
131. Miranda TB, Cortez CC, Yoo CB, *et al.* DZNep Is a Global Histone Methylation Inhibitor that Reactivates Developmental Genes Not Silenced by DNA Methylation. *Mol. Cancer Ther.* 8(6), 1579–1588 (2009).
132. Fiskus W, Wang Y, Sreekumar A, *et al.* Combined epigenetic therapy with the histone methyltransferase EZH2 inhibitor 3-deazaneplanocin A and the histone deacetylase inhibitor panobinostat against human AML cells. *Blood.* 114(13), 2733–2743 (2009).
133. Xie Z, Bi C, Cheong LL, *et al.* Determinants of Sensitivity to DZNep Induced Apoptosis in Multiple Myeloma Cells. *PLoS ONE* [Internet]. 6(6) (2011). Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3123372/>.
134. Chiba T, Suzuki E, Negishi M, *et al.* 3-Deazaneplanocin A is a promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells. *Int. J. Cancer.* 130(11), 2557–2567 (2012).
135. Van Aller GS, Pappalardi MB, Ott HM, *et al.* Long residence time inhibition of EZH2 in activated polycomb repressive complex 2. *ACS Chem. Biol.* 9(3), 622–629 (2014).
136. Amatangelo MD, Garipov A, Li H, Conejo-Garcia JR, Speicher DW, Zhang R. Three-dimensional culture sensitizes epithelial ovarian cancer cells to EZH2 methyltransferase inhibition. *Cell Cycle Georget. Tex.* 12(13), 2113–2119 (2013).
137. Verma SK, Tian X, LaFrance LV, *et al.* Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. *ACS Med. Chem. Lett.* 3(12), 1091–1096 (2012).

138. Knutson SK, Wigle TJ, Warholc NM, *et al.* A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat. Chem. Biol.* 8(11), 890–896 (2012).
139. Knutson SK, Kawano S, Minoshima Y, *et al.* Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. *Mol. Cancer Ther.* 13(4), 842–854 (2014).
140. Qi W, Chan H, Teng L, *et al.* Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. *Proc. Natl. Acad. Sci. U. S. A.* 109(52), 21360–21365 (2012).
141. Konze KD, Ma A, Li F, *et al.* An orally bioavailable chemical probe of the Lysine Methyltransferases EZH2 and EZH1. *ACS Chem. Biol.* 8(6), 1324–1334 (2013).
142. Kim W, Bird GH, Neff T, *et al.* Targeted disruption of the EZH2-EED complex inhibits EZH2-dependent cancer. *Nat. Chem. Biol.* 9(10), 643–650 (2013).
143. Glazer RI, Hartman KD, Knode MC, *et al.* 3-Deazaneplanocin: a new and potent inhibitor of S-adenosylhomocysteine hydrolase and its effects on human promyelocytic leukemia cell line HL-60. *Biochem. Biophys. Res. Commun.* 135(2), 688–694 (1986).
144. Bray M, Driscoll J, Huggins JW. Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. *Antiviral Res.* 45(2), 135–147 (2000).
145. Kikuchi J, Takashina T, Kinoshita I, *et al.* Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non-small cell lung cancer cells. *Lung Cancer Amst. Neth.* 78(2), 138–143 (2012).
146. Cheng LL, Itahana Y, Lei ZD, *et al.* TP53 genomic status regulates sensitivity of gastric cancer cells to the histone methylation inhibitor 3-deazaneplanocin A (DZNep). *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 18(15), 4201–4212 (2012).
147. Fiskus W, Rao R, Balusu R, *et al.* Superior Efficacy of a Combined Epigenetic Therapy against Human Mantle Cell Lymphoma Cells. *Clin. Cancer Res.* 18(22), 6227–6238 (2012).
148. Benoit YD, Witherspoon MS, Laursen KB, *et al.* Pharmacological inhibition of polycomb repressive complex-2 activity induces apoptosis in human colon cancer stem cells. *Exp. Cell Res.* 319(10), 1463–1470 (2013).
149. Li Z, Wang Y, Qiu J, *et al.* The polycomb group protein EZH2 is a novel therapeutic target in tongue cancer. *Oncotarget.* 4(12), 2532–2549 (2013).
150. Liu L, Xu Z, Zhong L, *et al.* EZH2 promotes tumor cell migration and invasion via epigenetic repression of E-cadherin in renal cell carcinoma. *BJU Int.* (2014).

151. He S, Wang J, Kato K, *et al.* Inhibition of histone methylation arrests ongoing graft-versus-host disease in mice by selectively inducing apoptosis of alloreactive effector T cells. *Blood*. 119(5), 1274–1282 (2012).
152. Fujiwara T, Saitoh H, Inoue A, *et al.* 3-Deazaneplanocin A (DZNep), an Inhibitor of S-Adenosylmethionine-dependent Methyltransferase, Promotes Erythroid Differentiation. *J. Biol. Chem.* 289(12), 8121–8134 (2014).
153. Tan J, Yan Y, Wang X, Jiang Y, Xu HE. EZH2: biology, disease, and structure-based drug discovery. *Acta Pharmacol. Sin.* 35(2), 161–174 (2014).
154. Copeland RA, Solomon ME, Richon VM. Protein methyltransferases as a target class for drug discovery. *Nat. Rev. Drug Discov.* 8(9), 724–732 (2009).
155. Crea F, Hurt EM, Mathews LA, *et al.* Pharmacologic disruption of Polycomb Repressive Complex 2 inhibits tumorigenicity and tumor progression in prostate cancer. *Mol. Cancer*. 10, 40 (2011).
156. Chen Y, Lin MC, Yao H, *et al.* Lentivirus-mediated RNA interference targeting enhancer of zeste homolog 2 inhibits hepatocellular carcinoma growth through down-regulation of stathmin. *Hepatol. Baltim. Md.* 46(1), 200–208 (2007).
157. Crea F, Fornaro L, Bocci G, *et al.* EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. *Cancer Metastasis Rev.* 31(3-4), 753–761 (2012).
158. Vella S, Gnani D, Crudele A, *et al.* EZH2 down-regulation exacerbates lipid accumulation and inflammation in in vitro and in vivo NAFLD. *Int. J. Mol. Sci.* 14(12), 24154–24168 (2013).

Figures

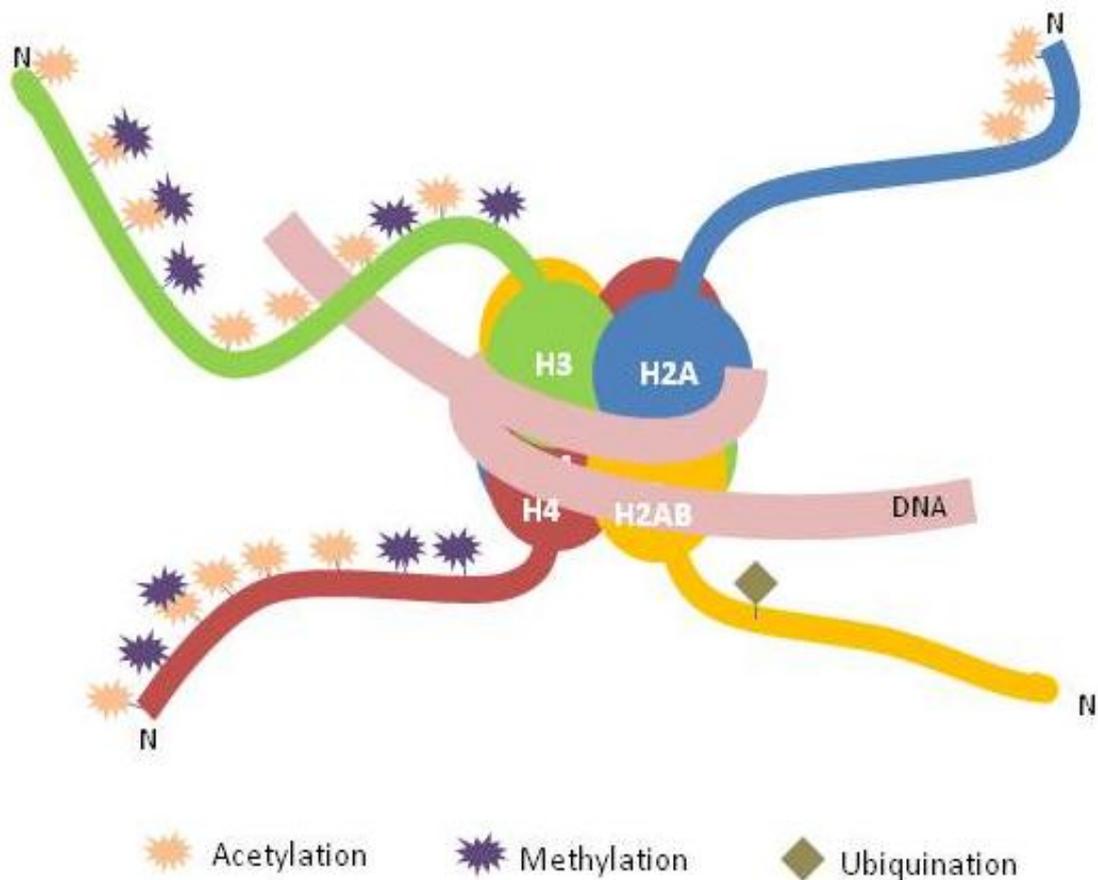


Figure 1: Schema of nucleosome organization

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).

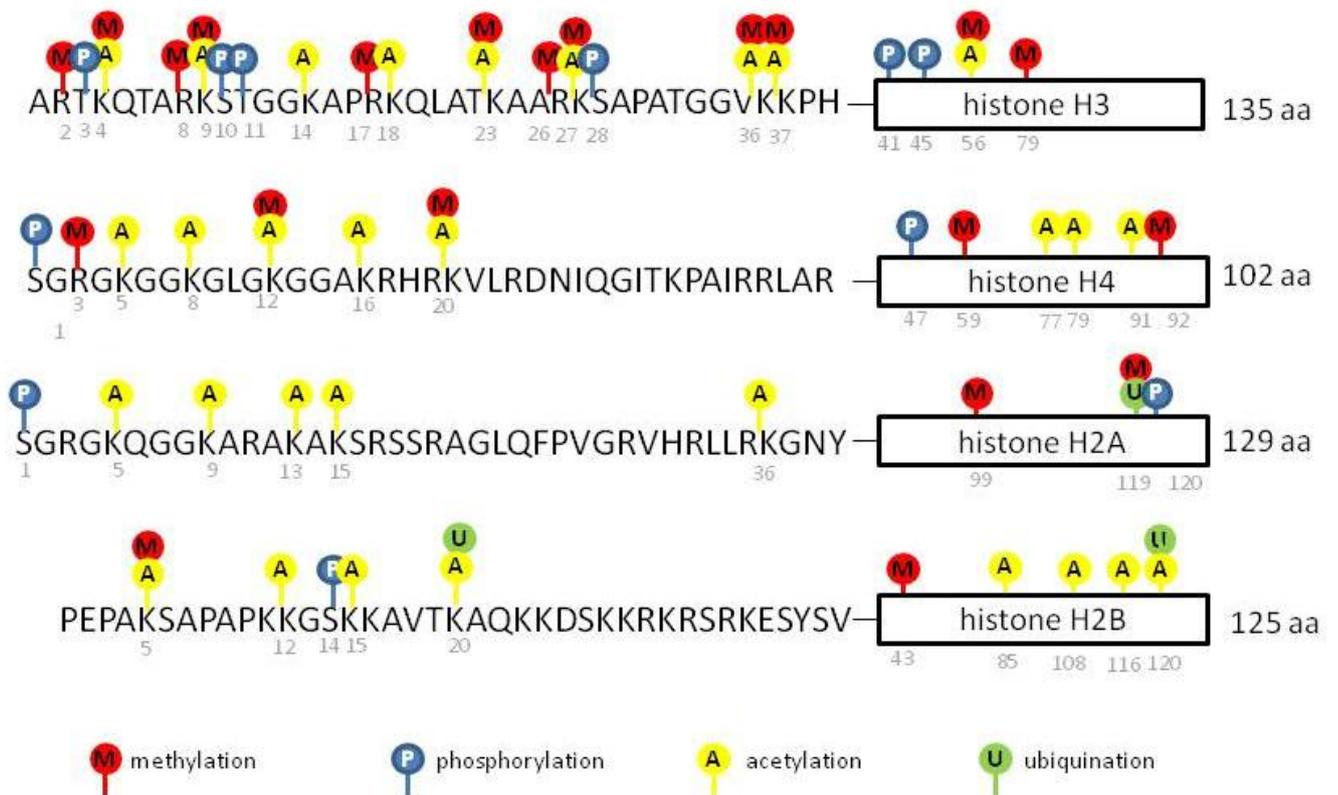


Figure 2: Schematic representation of major histone modifications

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).

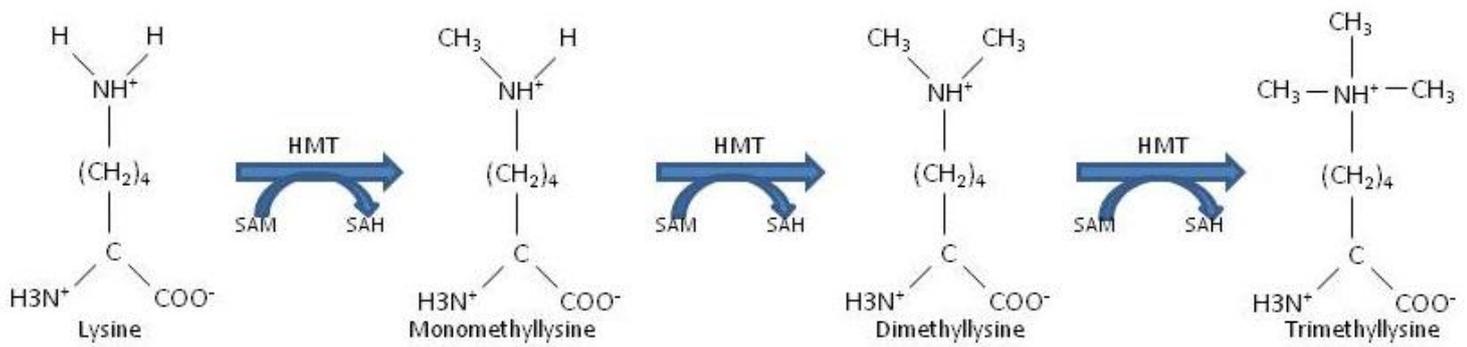


Figure 3: Methyl group transfer reaction on lysine

The lysine amino group of the substrate histone polypeptide engages in a SN₂ 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).

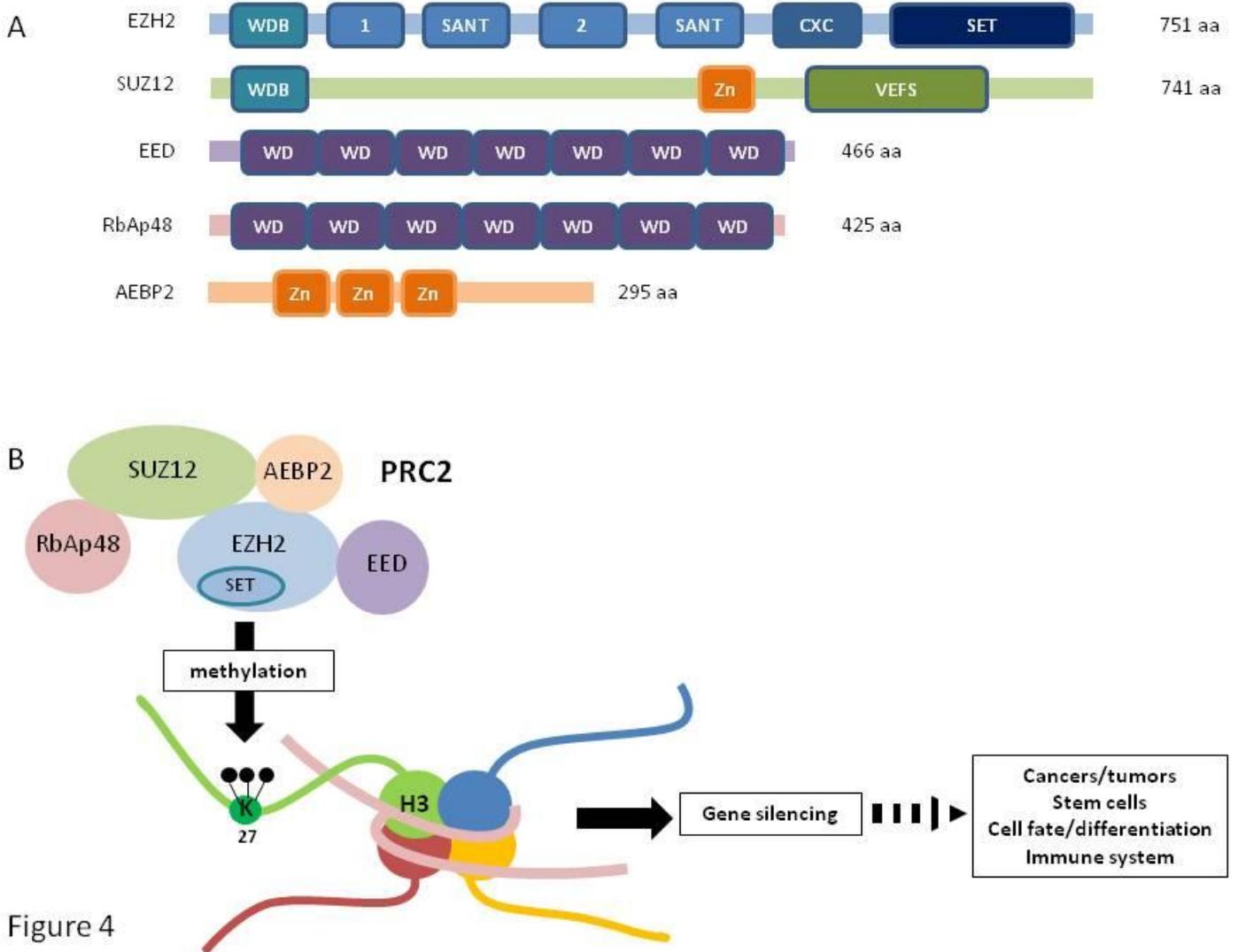


Figure 4: Schematic representation of PRC2 complex.

(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.

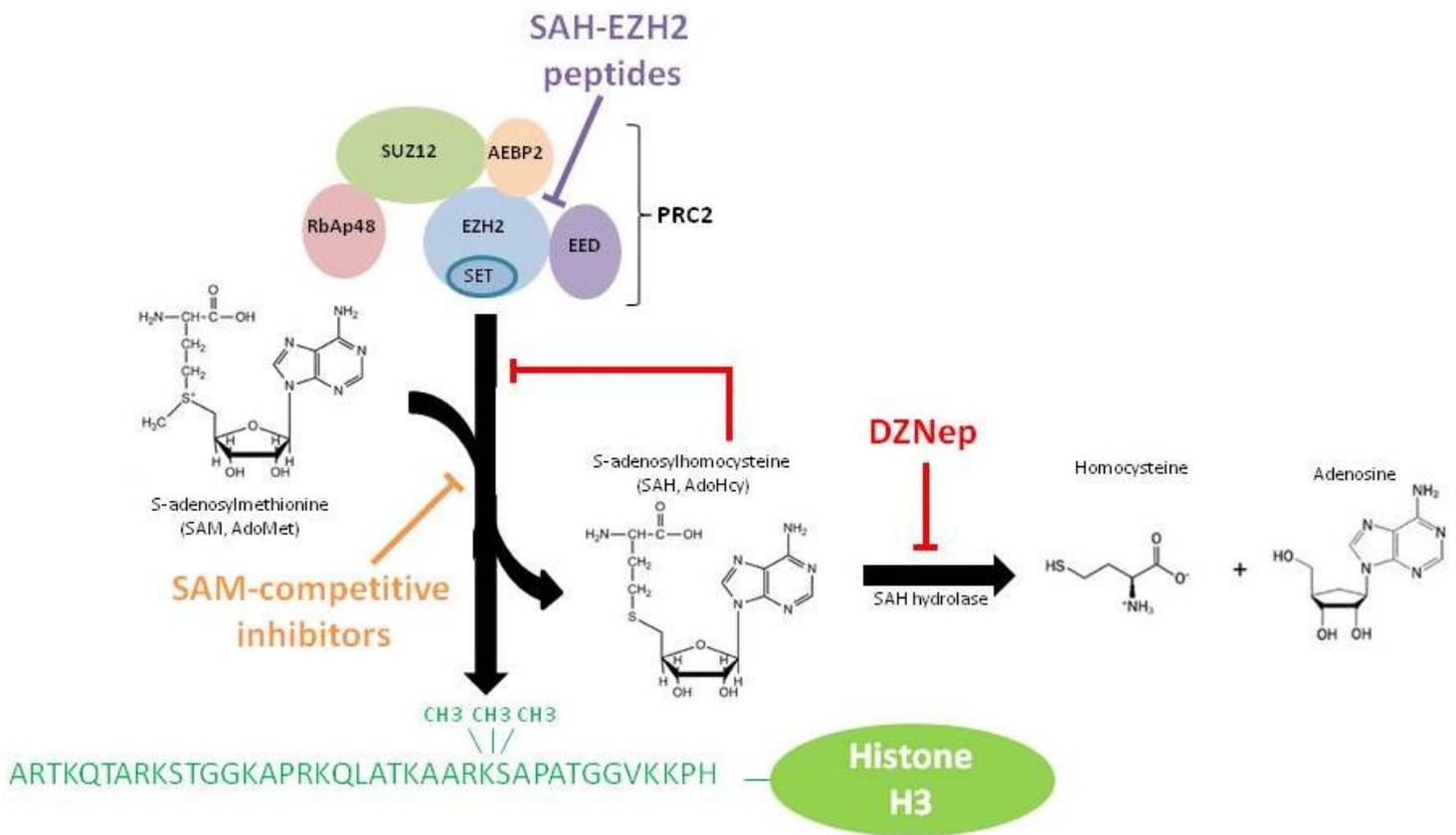


Figure 5: Modes of inhibition of PRC2.

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
	SET1D1B, 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 TFIIB 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-EV11		
PRDM6, MEL1, PFM3		
PRDM2, RIZ1, KMT8		H3K9
Without SET domain		
DOT1L, KMT4		H3K79

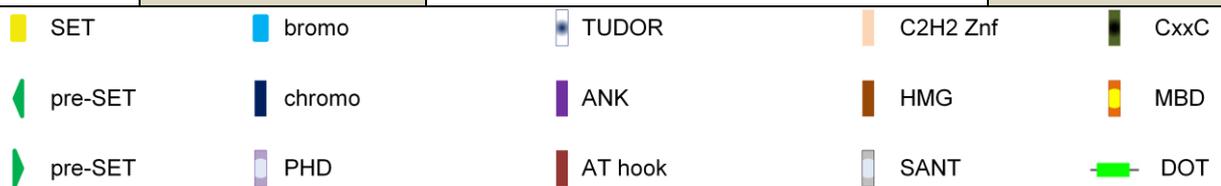
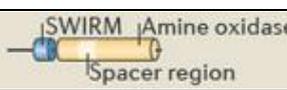
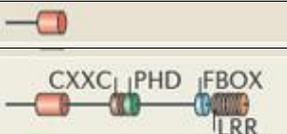
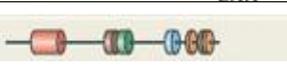
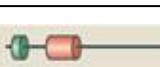
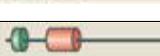


Table 3: Histone target substrates and domain structure of histone lysine demethyltransferases. ³

	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.

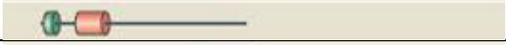
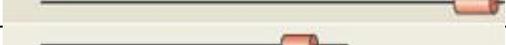
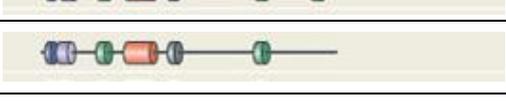
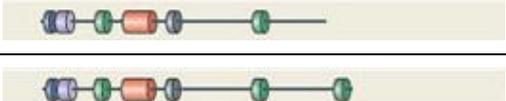
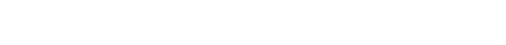
		H4K20me1	
PHF2, JHDM1E		H3K9me2	ARID5B
HR			
KDM3B			
JMJD1A, JHDM2A, TSGA, KDM3A		H3K9me1, H3K9me2	
JMJD1C			
JMJD3, KDM6B		H3K27me2, H3K27me3	
UTX, KDM6A		H3K27me2, H3K27me3	
UTY			
JMJD2A, JHDM3A, KDM4A		H3K9me2, H3K9me3	
		H3K36me2, H3K36me3	
		H1.4K26me2, H1.4K26me3	
JMJD2C, JHDM3C, GASC1, KDM4C		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	

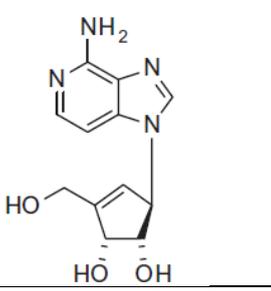
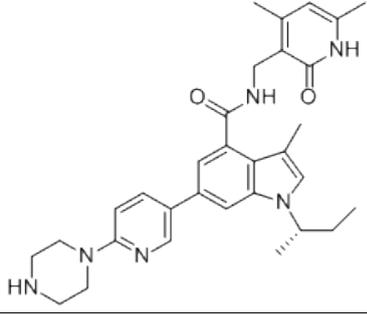
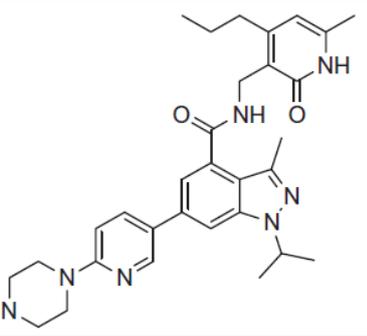
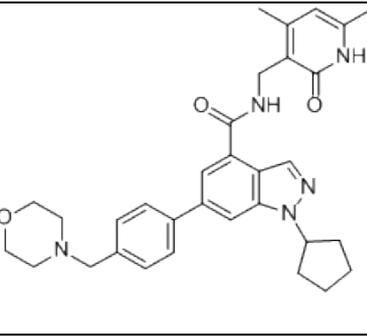
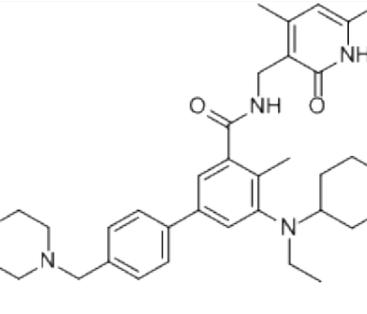
Table 4: Association between EZH2 mutations and disease.⁴

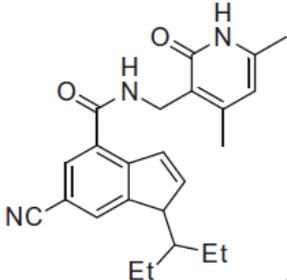
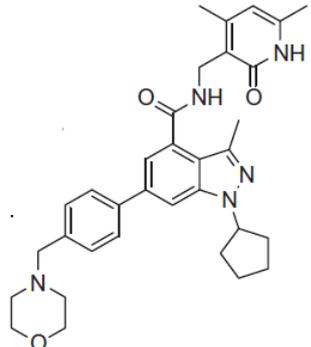
Mutated domain	Mutation	Phenotype
CXC domain (503-605)	H530N	Acute myeloid leukemia
	C547fs	Acute myeloid leukemia
	Q553X	Acute myeloid leukemia
	C571Y	Myelofibrosis
	C576W	Myelodysplastic syndrome, myeloproliferative neoplasms
	P577L	Early T-cell precursor acute lymphoblastic leukaemia
	R583X	Chronic 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]

	<p>EI1</p>	<p>SAM-competitive inhibitor of PRC2 IC₅₀=15 nM; K_i=13 nM</p>		<p>preclinical</p>	<p>[140]</p>
	<p>UNC1999</p>	<p>SAM-competitive inhibitor of PRC2 IC₅₀=2-15 nM; K_i=13 nM</p>	<p>over 1000-fold selective for other HMTs except EZH1 (22-fold selectivity).</p>	<p>preclinical</p>	<p>[141]</p>
<p>Peptide: FSSNRXKILXRTQILNQEWKQRRIQPV</p>	<p>stabilized α-helix of EZH2 peptide (SAH-EZH2)</p>	<p>Hydrocarbon-stapled peptide that mimics the α-helical EED-binding domain of EZH2, disrupting the EZH2–EED complex</p>	<p>not selective for EZH1</p>	<p>preclinical</p>	<p>[142]</p>

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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5 References

- 6 1. Rapin N, Porse BT. Oncogenic fusion proteins expressed in immature hematopoietic
7 cells fail to recapitulate the transcriptional changes observed in human AML.
8 *Oncogenesis*. 3, e106 (2014).
- 9 2. Gao W, Wang T, Wu Y, Liu HX, Li YC, Chen WM. Mixed lineage leukemia-septin 5
10 fusion transcript in de novo adult acute myeloid leukemia with t(11;22)(q23;q11.2): A
11 case report. *Oncol. Lett.* 7(6), 1930–1932 (2014).
- 12 3. Krumbholz M, Jung R, Bradtke J, Reinhardt D, Stachel D, Metzler M. Response
13 monitoring of infant acute myeloid leukemia treatment by quantification of the tumor
14 specific MLL-FNBP1 fusion gene. *Leuk. Lymphoma.* , 1–10 (2014).
- 15 4. Sakamoto K, Imamura T, Yano M, *et al.* Sensitivity of MLL-rearranged AML cells to all-
16 trans retinoic acid is associated with the level of H3K4me2 in the RAR α promoter
17 region. *Blood Cancer J.* 4, e205 (2014).
- 18 5. Van der Linden MH, Willekes M, van Roon E, *et al.* MLL fusion-driven activation of
19 CDK6 potentiates proliferation in MLL-rearranged infant ALL. *Cell Cycle Georget. Tex.*
20 13(5), 834–844 (2014).
- 21 6. Placke T, Faber K, Nonami A, *et al.* Requirement for CDK6 in MLL-rearranged acute
22 myeloid leukemia. *Blood.* (2014).
- 23 7. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia
24 stem-cell development. *Nat. Rev. Cancer.* 7(11), 823–833 (2007).
- 25 8. Dorrance AM, Liu S, Chong A, *et al.* The Mll partial tandem duplication: differential,
26 tissue-specific activity in the presence or absence of the wild-type allele. *Blood.*
27 112(6), 2508–2511 (2008).
- 28 9. Dorrance AM, Liu S, Yuan W, *et al.* Mll partial tandem duplication induces aberrant
29 Hox expression in vivo via specific epigenetic alterations. *J. Clin. Invest.* 116(10), 2707–
30 2716 (2006).
- 31 10. Whitman SP, Hackanson B, Liyanarachchi S, *et al.* DNA hypermethylation and
32 epigenetic silencing of the tumor suppressor gene, SLC5A8, in acute myeloid leukemia
33 with the MLL partial tandem duplication. *Blood.* 112(5), 2013–2016 (2008).

- 34 11. Strom SP, Lozano R, Lee H, *et al.* De Novo variants in the KMT2A (MLL) gene causing
35 atypical Wiedemann-Steiner syndrome in two unrelated individuals identified by
36 clinical exome sequencing. *BMC Med. Genet.* 15(1), 49 (2014).
- 37 12. Mendelsohn BA, Pronold M, Long R, Smaoui N, Slavotinek AM. Advanced bone age in
38 a girl with Wiedemann-Steiner syndrome and an exonic deletion in KMT2A (MLL). *Am.*
39 *J. Med. Genet. A.* (2014).
- 40 13. Jones WD, Dafou D, McEntagart M, *et al.* De novo mutations in MLL cause
41 Wiedemann-Steiner syndrome. *Am. J. Hum. Genet.* 91(2), 358–364 (2012).
- 42 14. Saigo K, Yoshida K, Ikeda R, *et al.* Integration of hepatitis B virus DNA into the
43 myeloid/lymphoid or mixed-lineage leukemia (MLL4) gene and rearrangements of
44 MLL4 in human hepatocellular carcinoma. *Hum. Mutat.* 29(5), 703–708 (2008).
- 45 15. Wang GG, Song J, Wang Z, *et al.* Haematopoietic malignancies caused by dysregulation
46 of a chromatin-binding PHD finger. *Nature.* 459(7248), 847–851 (2009).
- 47 16. Hannibal MC, Buckingham KJ, Ng SB, *et al.* Spectrum of MLL2 (ALR) mutations in 110
48 cases of Kabuki syndrome. *Am. J. Med. Genet. A.* 155A(7), 1511–1516 (2011).
- 49 17. Ng SB, Bigham AW, Buckingham KJ, *et al.* Exome sequencing identifies MLL2 mutations
50 as a cause of Kabuki syndrome. *Nat. Genet.* 42(9), 790–793 (2010).
- 51 18. Chen C, Liu Y, Rappaport AR, *et al.* MLL3 is a haploinsufficient 7q tumor suppressor in
52 acute myeloid leukemia. *Cancer Cell.* 25(5), 652–665 (2014).
- 53 19. Li W-D, Li Q-R, Xu S-N, *et al.* Exome sequencing identifies an MLL3 gene germ line
54 mutation in a pedigree of colorectal cancer and acute myeloid leukemia. *Blood.*
55 121(8), 1478–1479 (2013).
- 56 20. Sjöblom T, Jones S, Wood LD, *et al.* The consensus coding sequences of human breast
57 and colorectal cancers. *Science.* 314(5797), 268–274 (2006).
- 58 21. Je EM, Lee SH, Yoo NJ, Lee SH. Mutational and expressional analysis of MLL genes in
59 gastric and colorectal cancers with microsatellite instability. *Neoplasma.* 60(2), 188–
60 195 (2013).
- 61 22. Watanabe Y, Castoro RJ, Kim HS, *et al.* Frequent alteration of MLL3 frameshift
62 mutations in microsatellite deficient colorectal cancer. *PloS One.* 6(8), e23320 (2011).
- 63 23. Okada Y, Feng Q, Lin Y, *et al.* hDOT1L links histone methylation to leukemogenesis.
64 *Cell.* 121(2), 167–178 (2005).
- 65 24. Krivtsov AV, Feng Z, Lemieux ME, *et al.* H3K79 methylation profiles define murine and
66 human MLL-AF4 leukemias. *Cancer Cell.* 14(5), 355–368 (2008).
- 67 25. Okada Y, Jiang Q, Lemieux M, Jeannotte L, Su L, Zhang Y. Leukaemic transformation by
68 CALM-AF10 involves upregulation of Hoxa5 by hDOT1L. *Nat. Cell Biol.* 8(9), 1017–1024
69 (2006).

- 70 26. Van Vlierberghe P, van Grotel M, Tchinda J, *et al.* The recurrent SET-NUP214 fusion as
71 a new HOXA activation mechanism in pediatric T-cell acute lymphoblastic leukemia.
72 *Blood.* 111(9), 4668–4680 (2008).
- 73 27. Evangelou E, Valdes AM, Castano-Betancourt MC, *et al.* The DOT1L rs12982744
74 polymorphism is associated with osteoarthritis of the hip with genome-wide statistical
75 significance in males. *Ann. Rheum. Dis.* 72(7), 1264–1265 (2013).
- 76 28. Castaño Betancourt MC, Cailotto F, Kerkhof HJ, *et al.* Genome-wide association and
77 functional studies identify the DOT1L gene to be involved in cartilage thickness and
78 hip osteoarthritis. *Proc. Natl. Acad. Sci. U. S. A.* 109(21), 8218–8223 (2012).
- 79 29. Bracken AP, Pasini D, Capra M, Prosperini E, Colli E, Helin K. EZH2 is downstream of
80 the pRB-E2F pathway, essential for proliferation and amplified in cancer. *EMBO J.*
81 22(20), 5323–5335 (2003).
- 82 30. Collett K, Eide GE, Arnes J, *et al.* Expression of enhancer of zeste homologue 2 is
83 significantly associated with increased tumor cell proliferation and is a marker of
84 aggressive breast cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 12(4), 1168–
85 1174 (2006).
- 86 31. Friedman JM, Jones PA, Liang G. The tumor suppressor microRNA-101 becomes an
87 epigenetic player by targeting the polycomb group protein EZH2 in cancer. *Cell Cycle*
88 *Georget. Tex.* 8(15), 2313–2314 (2009).
- 89 32. Arisan S, Buyuktuncer ED, Palavan-Unsal N, Caşkurly T, Cakir OO, Ergenekon E.
90 Increased expression of EZH2, a polycomb group protein, in bladder carcinoma. *Urol.*
91 *Int.* 75(3), 252–257 (2005).
- 92 33. Raman JD, Mongan NP, Tickoo SK, Boorjian SA, Scherr DS, Gudas LJ. Increased
93 expression of the polycomb group gene, EZH2, in transitional cell carcinoma of the
94 bladder. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 11(24 Pt 1), 8570–8576 (2005).
- 95 34. Wang H, Albadine R, Magheli A, *et al.* Increased EZH2 protein expression is associated
96 with invasive urothelial carcinoma of the bladder. *Urol. Oncol.* 30(4), 428–433 (2012).
- 97 35. Hinz S, Kempkensteffen C, Christoph F, *et al.* Expression of the polycomb group
98 protein EZH2 and its relation to outcome in patients with urothelial carcinoma of the
99 bladder. *J. Cancer Res. Clin. Oncol.* 134(3), 331–336 (2008).
- 100 36. Weikert S, Christoph F, Köllermann J, *et al.* Expression levels of the EZH2 polycomb
101 transcriptional repressor correlate with aggressiveness and invasive potential of
102 bladder carcinomas. *Int. J. Mol. Med.* 16(2), 349–353 (2005).
- 103 37. Klier CG, Cao Q, Varambally S, *et al.* EZH2 is a marker of aggressive breast cancer and
104 promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci. U.*
105 *S. A.* 100(20), 11606–11611 (2003).

- 106 38. Bachmann IM, Halvorsen OJ, Collett K, *et al.* EZH2 expression is associated with high
107 proliferation rate and aggressive tumor subgroups in cutaneous melanoma and
108 cancers of the endometrium, prostate, and breast. *J. Clin. Oncol. Off. J. Am. Soc. Clin.*
109 *Oncol.* 24(2), 268–273 (2006).
- 110 39. Mimori K, Ogawa K, Okamoto M, Sudo T, Inoue H, Mori M. Clinical significance of
111 enhancer of zeste homolog 2 expression in colorectal cancer cases. *Eur. J. Surg. Oncol.*
112 *J. Eur. Soc. Surg. Oncol. Br. Assoc. Surg. Oncol.* 31(4), 376–380 (2005).
- 113 40. Wang J, Ma Z-B, Li K, Guo G-H. Association between EZH2 polymorphisms and
114 colorectal cancer risk in Han Chinese population. *Med. Oncol. Northwood Lond. Engl.*
115 31(3), 874 (2014).
- 116 41. Tamagawa H, Oshima T, Numata M, *et al.* Global histone modification of H3K27
117 correlates with the outcomes in patients with metachronous liver metastasis of
118 colorectal cancer. *Eur. J. Surg. Oncol. J. Eur. Soc. Surg. Oncol. Br. Assoc. Surg. Oncol.*
119 39(6), 655–661 (2013).
- 120 42. Fornaro L, Crea F, Masi G, *et al.* EZH2 polymorphism and benefit from bevacizumab in
121 colorectal cancer: another piece to the puzzle. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.*
122 *ESMO.* 23(5), 1370–1371 (2012).
- 123 43. Crea F, Fornaro L, Paolicchi E, *et al.* An EZH2 polymorphism is associated with clinical
124 outcome in metastatic colorectal cancer patients. *Ann. Oncol. Off. J. Eur. Soc. Med.*
125 *Oncol. ESMO.* 23(5), 1207–1213 (2012).
- 126 44. Wang C-G, Ye Y-J, Yuan J, Liu F-F, Zhang H, Wang S. EZH2 and STAT6 expression
127 profiles are correlated with colorectal cancer stage and prognosis. *World J.*
128 *Gastroenterol. WJG.* 16(19), 2421–2427 (2010).
- 129 45. Fluge Ø, Gravdal K, Carlsen E, *et al.* Expression of EZH2 and Ki-67 in colorectal cancer
130 and associations with treatment response and prognosis. *Br. J. Cancer.* 101(8), 1282–
131 1289 (2009).
- 132 46. Guo L, Yang T-F, Liang S-C, Guo J-X, Wang Q. Role of EZH2 protein expression in gastric
133 carcinogenesis among Asians: a meta-analysis. *Tumour Biol. J. Int. Soc.*
134 *Oncodevelopmental Biol. Med.* (2014).
- 135 47. He L-J, Cai M-Y, Xu G-L, *et al.* Prognostic significance of overexpression of EZH2 and
136 H3k27me3 proteins in gastric cancer. *Asian Pac. J. Cancer Prev. APJCP.* 13(7), 3173–
137 3178 (2012).
- 138 48. Lee H, Yoon SO, Jeong WY, Kim HK, Kim A, Kim B. Immunohistochemical analysis of
139 polycomb group protein expression in advanced gastric cancer. *Hum. Pathol.* 43(10),
140 1704–1710 (2012).
- 141 49. Zhou Y, Du W-D, Wu Q, *et al.* EZH2 genetic variants affect risk of gastric cancer in the
142 Chinese Han population. *Mol. Carcinog.* (2012).

- 143 50. Cai GH, Wang K, Miao Q, Peng YS, Chen XY. Expression of polycomb protein EZH2 in
144 multi-stage tissues of gastric carcinogenesis. *J. Dig. Dis.* 11(2), 88–93 (2010).
- 145 51. Choi JH, Song YS, Yoon JS, Song KW, Lee YY. Enhancer of zeste homolog 2 expression is
146 associated with tumor cell proliferation and metastasis in gastric cancer. *APMIS Acta*
147 *Pathol. Microbiol. Immunol. Scand.* 118(3), 196–202 (2010).
- 148 52. Mattioli E, Vogiatzi P, Sun A, *et al.* Immunohistochemical analysis of pRb2/p130, VEGF,
149 EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric
150 cancer. *J. Cell. Physiol.* 210(1), 183–191 (2007).
- 151 53. Matsukawa Y, Semba S, Kato H, Ito A, Yanagihara K, Yokozaki H. Expression of the
152 enhancer of zeste homolog 2 is correlated with poor prognosis in human gastric
153 cancer. *Cancer Sci.* 97(6), 484–491 (2006).
- 154 54. Hung S-Y, Lin H-H, Yeh K-T, Chang J-G. Histone-modifying genes as biomarkers in
155 hepatocellular carcinoma. *Int. J. Clin. Exp. Pathol.* 7(5), 2496–2507 (2014).
- 156 55. Yu Y-L, Su K-J, Hsieh Y-H, *et al.* Effects of EZH2 polymorphisms on susceptibility to and
157 pathological development of hepatocellular carcinoma. *PLoS One.* 8(9), e74870 (2013).
- 158 56. Hajósi-Kalcakosz S, Dezső K, Bugyik E, *et al.* Enhancer of zeste homologue 2 (EZH2) is a
159 reliable immunohistochemical marker to differentiate malignant and benign hepatic
160 tumors. *Diagn. Pathol.* 7, 86 (2012).
- 161 57. Cai M-Y, Tong Z-T, Zheng F, *et al.* EZH2 protein: a promising immunomarker for the
162 detection of hepatocellular carcinomas in liver needle biopsies. *Gut.* 60(7), 967–976
163 (2011).
- 164 58. Cai M-Y, Hou J-H, Rao H-L, *et al.* High expression of H3K27me3 in human
165 hepatocellular carcinomas correlates closely with vascular invasion and predicts worse
166 prognosis in patients. *Mol. Med. Camb. Mass.* 17(1-2), 12–20 (2011).
- 167 59. Sudo T, Utsunomiya T, Mimori K, *et al.* Clinicopathological significance of EZH2 mRNA
168 expression in patients with hepatocellular carcinoma. *Br. J. Cancer.* 92(9), 1754–1758
169 (2005).
- 170 60. Berg T, Thoene S, Yap D, *et al.* A transgenic mouse model demonstrating the
171 oncogenic role of mutations in the polycomb-group gene EZH2 in lymphomagenesis.
172 *Blood.* 123(25), 3914–3924 (2014).
- 173 61. Guo S, Chan JKC, Iqbal J, *et al.* EZH2 Mutations in Follicular Lymphoma from Different
174 Ethnic Groups and Associated Gene Expression Alterations. *Clin. Cancer Res. Off. J.*
175 *Am. Assoc. Cancer Res.* 20(12), 3078–3086 (2014).
- 176 62. Okosun J, Bödör C, Wang J, *et al.* Integrated genomic analysis identifies recurrent
177 mutations and evolution patterns driving the initiation and progression of follicular
178 lymphoma. *Nat. Genet.* 46(2), 176–181 (2014).

- 179 63. Parry M, Rose-Zerilli MJ, Gibson J, *et al.* Whole exome sequencing identifies novel
180 recurrently mutated genes in patients with splenic marginal zone lymphoma. *PLoS*
181 *One.* 8(12), e83244 (2013).
- 182 64. Lee HJ, Shin DH, Kim KB, *et al.* Polycomb protein EZH2 expression in diffuse large B-cell
183 lymphoma is associated with better prognosis in patients treated with rituximab,
184 cyclophosphamide, doxorubicin, vincristine and prednisone. *Leuk. Lymphoma.* (2014).
- 185 65. Shioyama S, Yoshida S, Soga D, Motohashi H, Shintani S. Aberrant expression of EZH2
186 is associated with pathological findings and P53 alteration. *Anticancer Res.* 33(10),
187 4309–4317 (2013).
- 188 66. Bödör C, Grossmann V, Popov N, *et al.* EZH2 mutations are frequent and represent an
189 early event in follicular lymphoma. *Blood.* , blood–2013–04–496893 (2013).
- 190 67. Abd Al Kader L, Oka T, Takata K, *et al.* In aggressive variants of non-Hodgkin
191 lymphomas, Ezh2 is strongly expressed and polycomb repressive complex PRC1.4
192 dominates over PRC1.2. *Virchows Arch. Int. J. Pathol.* 463(5), 697–711 (2013).
- 193 68. Heyn H, Esteller M. EZH2: an epigenetic gatekeeper promoting lymphomagenesis.
194 *Cancer Cell.* 23(5), 563–565 (2013).
- 195 69. Saieg MA, Geddie WR, Boerner SL, Bailey D, Crump M, da Cunha Santos G. EZH2 and
196 CD79B mutational status over time in B-cell non-Hodgkin lymphomas detected by
197 high-throughput sequencing using minimal samples. *Cancer Cytopathol.* 121(7), 377–
198 386 (2013).
- 199 70. Guo S-Q, Zhang Y-Z. Overexpression of enhancer of zests homolog 2 in lymphoma.
200 *Chin. Med. J. (Engl.).* 125(20), 3735–3739 (2012).
- 201 71. Chen J, Li J, Han Q, *et al.* Enhancer of zeste homolog 2 is overexpressed and
202 contributes to epigenetic inactivation of p21 and phosphatase and tensin homolog in
203 B-cell acute lymphoblastic leukemia. *Exp. Biol. Med. Maywood NJ.* 237(9), 1110–1116
204 (2012).
- 205 72. Majer CR, Jin L, Scott MP, *et al.* A687V EZH2 is a gain-of-function mutation found in
206 lymphoma patients. *FEBS Lett.* 586(19), 3448–3451 (2012).
- 207 73. Simon C, Chagraoui J, Kros J, *et al.* A key role for EZH2 and associated genes in mouse
208 and human adult T-cell acute leukemia. *Genes Dev.* 26(7), 651–656 (2012).
- 209 74. McCabe MT, Graves AP, Ganji G, *et al.* Mutation of A677 in histone methyltransferase
210 EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on
211 lysine 27 (H3K27). *Proc. Natl. Acad. Sci. U. S. A.* 109(8), 2989–2994 (2012).
- 212 75. Ntziachristos P, Tzirigos A, Van Vlierberghe P, *et al.* Genetic inactivation of the
213 polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. *Nat. Med.*
214 18(2), 298–301 (2012).

- 215 76. Ryan RJH, Nitta M, Borger D, *et al.* EZH2 codon 641 mutations are common in BCL2-
216 rearranged germinal center B cell lymphomas. *PLoS One.* 6(12), e28585 (2011).
- 217 77. Capello D, Gloghini A, Martini M, *et al.* Mutations of CD79A, CD79B and EZH2 genes in
218 immunodeficiency-related non-Hodgkin lymphomas. *Br. J. Haematol.* 152(6), 777–780
219 (2011).
- 220 78. Bödör C, O’Riain C, Wrench D, *et al.* EZH2 Y641 mutations in follicular lymphoma.
221 *Leukemia.* 25(4), 726–729 (2011).
- 222 79. Sasaki D, Imaizumi Y, Hasegawa H, *et al.* Overexpression of Enhancer of zeste homolog
223 2 with trimethylation of lysine 27 on histone H3 in adult T-cell leukemia/lymphoma as
224 a target for epigenetic therapy. *Haematologica.* 96(5), 712–719 (2011).
- 225 80. Park SW, Chung NG, Eom HS, Yoo NJ, Lee SH. Mutational analysis of EZH2 codon 641 in
226 non-Hodgkin lymphomas and leukemias. *Leuk. Res.* 35(1), e6–7 (2011).
- 227 81. Morin RD, Johnson NA, Severson TM, *et al.* Somatic mutations altering EZH2 (Tyr641)
228 in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat. Genet.*
229 42(2), 181–185 (2010).
- 230 82. Visser HP, Gunster MJ, Kluijn-Nelemans HC, *et al.* The Polycomb group protein EZH2 is
231 upregulated in proliferating, cultured human mantle cell lymphoma. *Br. J. Haematol.*
232 112(4), 950–958 (2001).
- 233 83. Muto T, Sashida G, Oshima M, *et al.* Concurrent loss of Ezh2 and Tet2 cooperates in
234 the pathogenesis of myelodysplastic disorders. *J. Exp. Med.* 210(12), 2627–2639
235 (2013).
- 236 84. Wang J, Ai X, Gale RP, *et al.* TET2, ASXL1 and EZH2 mutations in Chinese with
237 myelodysplastic syndromes. *Leuk. Res.* 37(3), 305–311 (2013).
- 238 85. Shih AH, Abdel-Wahab O, Patel JP, Levine RL. The role of mutations in epigenetic
239 regulators in myeloid malignancies. *Nat. Rev. Cancer.* 12(9), 599–612 (2012).
- 240 86. Puda A, Milosevic JD, Berg T, *et al.* Frequent deletions of JARID2 in leukemic
241 transformation of chronic myeloid malignancies. *Am. J. Hematol.* 87(3), 245–250
242 (2012).
- 243 87. Zhang S-J, Abdel-Wahab O. Disordered epigenetic regulation in the pathophysiology of
244 myeloproliferative neoplasms. *Curr. Hematol. Malig. Rep.* 7(1), 34–42 (2012).
- 245 88. Reuther GW. Recurring mutations in myeloproliferative neoplasms alter epigenetic
246 regulation of gene expression. *Am. J. Cancer Res.* 1(6), 752–762 (2011).
- 247 89. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and
248 pathogenesis of myeloproliferative neoplasms. *Blood.* 118(7), 1723–1735 (2011).
- 249 90. Abdel-Wahab O. Genetics of the myeloproliferative neoplasms. *Curr. Opin. Hematol.*
250 18(2), 117–123 (2011).

- 251 91. Tefferi A, Abdel-Wahab O, Cervantes F, *et al.* Mutations with epigenetic effects in
252 myeloproliferative neoplasms and recent progress in treatment: Proceedings from the
253 5th International Post-ASH Symposium. *Blood Cancer J.* 1, e7 (2011).
- 254 92. Venneti S, Le P, Martinez D, *et al.* Malignant rhabdoid tumors express stem cell
255 factors, which relate to the expression of EZH2 and Id proteins. *Am. J. Surg. Pathol.*
256 35(10), 1463–1472 (2011).
- 257 93. Alimova I, Birks DK, Harris PS, *et al.* Inhibition of EZH2 suppresses self-renewal and
258 induces radiation sensitivity in atypical rhabdoid teratoid tumor cells. *Neuro-Oncol.*
259 (2012).
- 260 94. Knutson SK, Warholic NM, Wigle TJ, *et al.* Durable tumor regression in genetically
261 altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc.*
262 *Natl. Acad. Sci. U. S. A.* 110(19), 7922–7927 (2013).
- 263 95. Wilson BG, Wang X, Shen X, *et al.* Epigenetic antagonism between polycomb and
264 SWI/SNF complexes during oncogenic transformation. *Cancer Cell.* 18(4), 316–328
265 (2010).
- 266 96. Tiffen J, Gallagher SJ, Hersey P. EZH2: An emerging role in melanoma biology and
267 strategies for targeted therapy. *Pigment Cell Melanoma Res.* (2014).
- 268 97. Asangani IA, Harms PW, Dodson L, *et al.* Genetic and epigenetic loss of microRNA-31
269 leads to feed-forward expression of EZH2 in melanoma. *Oncotarget.* 3(9), 1011–1025
270 (2012).
- 271 98. Holling TM, Bergevoet MWT, Wilson L, *et al.* A role for EZH2 in silencing of IFN-gamma
272 inducible MHC2TA transcription in uveal melanoma. *J. Immunol. Baltim. Md 1950.*
273 179(8), 5317–5325 (2007).
- 274 99. McHugh JB, Fullen DR, Ma L, Kleer CG, Su LD. Expression of polycomb group protein
275 EZH2 in nevi and melanoma. *J. Cutan. Pathol.* 34(8), 597–600 (2007).
- 276 100. Vieira FQ, Costa-Pinheiro P, Ramalho-Carvalho J, *et al.* Deregulated expression of
277 selected histone methylases and demethylases in prostate carcinoma. *Endocr. Relat.*
278 *Cancer.* 21(1), 51–61 (2014).
- 279 101. Li K, Liu C, Zhou B, *et al.* Role of EZH2 in the Growth of Prostate Cancer Stem Cells
280 Isolated from LNCaP Cells. *Int. J. Mol. Sci.* 14(6), 11981–11993 (2013).
- 281 102. Deb G, Thakur VS, Gupta S. Multifaceted role of EZH2 in breast and prostate
282 tumorigenesis: epigenetics and beyond. *Epigenetics Off. J. DNA Methylation Soc.* 8(5),
283 464–476 (2013).
- 284 103. Yang YA, Yu J. EZH2, an epigenetic driver of prostate cancer. *Protein Cell.* 4(5), 331–
285 341 (2013).
- 286 104. Xu K, Wu ZJ, Groner AC, *et al.* EZH2 Oncogenic Activity in Castration Resistant Prostate
287 Cancer Cells is Polycomb-Independent. *Science.* 338(6113), 1465–1469 (2012).

- 288 105. Ribarska T, Bastian K-M, Koch A, Schulz WA. Specific changes in the expression of
289 imprinted genes in prostate cancer--implications for cancer progression and
290 epigenetic regulation. *Asian J. Androl.* 14(3), 436–450 (2012).
- 291 106. Ugolkov AV, Eisengart LJ, Luan C, Yang XJ. Expression analysis of putative stem cell
292 markers in human benign and malignant prostate. *The Prostate.* 71(1), 18–25 (2011).
- 293 107. Karanikolas BDW, Figueiredo ML, Wu L. Comprehensive evaluation of the role of EZH2
294 in the growth, invasion, and aggression of a panel of prostate cancer cell lines. *The*
295 *Prostate.* 70(6), 675–688 (2010).
- 296 108. Karanikolas BDW, Figueiredo ML, Wu L. Polycomb group protein enhancer of zeste 2 is
297 an oncogene that promotes the neoplastic transformation of a benign prostatic
298 epithelial cell line. *Mol. Cancer Res. MCR.* 7(9), 1456–1465 (2009).
- 299 109. Hoffmann MJ, Engers R, Florl AR, Otte AP, Muller M, Schulz WA. Expression changes in
300 EZH2, but not in BMI-1, SIRT1, DNMT1 or DNMT3B are associated with DNA
301 methylation changes in prostate cancer. *Cancer Biol. Ther.* 6(9), 1403–1412 (2007).
- 302 110. Cho KS, Oh HY, Lee EJ, Hong SJ. Identification of enhancer of zeste homolog 2
303 expression in peripheral circulating tumor cells in metastatic prostate cancer patients:
304 a preliminary study. *Yonsei Med. J.* 48(6), 1009–1014 (2007).
- 305 111. Yu J, Yu J, Rhodes DR, *et al.* A polycomb repression signature in metastatic prostate
306 cancer predicts cancer outcome. *Cancer Res.* 67(22), 10657–10663 (2007).
- 307 112. Cooper CS, Campbell C, Jhavar S. Mechanisms of Disease: biomarkers and molecular
308 targets from microarray gene expression studies in prostate cancer. *Nat. Clin. Pract.*
309 *Urol.* 4(12), 677–687 (2007).
- 310 113. Laitinen S, Martikainen PM, Tolonen T, Isola J, Tammela TLJ, Visakorpi T. EZH2, Ki-67
311 and MCM7 are prognostic markers in prostatectomy treated patients. *Int. J. Cancer J.*
312 *Int. Cancer.* 122(3), 595–602 (2008).
- 313 114. Bryant RJ, Cross NA, Eaton CL, Hamdy FC, Cunliffe VT. EZH2 promotes proliferation
314 and invasiveness of prostate cancer cells. *The Prostate.* 67(5), 547–556 (2007).
- 315 115. Van Leenders GJLH, Dukers D, Hessels D, *et al.* Polycomb-group oncogenes EZH2,
316 BMI1, and RING1 are overexpressed in prostate cancer with adverse pathologic and
317 clinical features. *Eur. Urol.* 52(2), 455–463 (2007).
- 318 116. Berezovska OP, Glinskii AB, Yang Z, Li X-M, Hoffman RM, Glinsky GV. Essential role for
319 activation of the Polycomb group (PcG) protein chromatin silencing pathway in
320 metastatic prostate cancer. *Cell Cycle Georget. Tex.* 5(16), 1886–1901 (2006).
- 321 117. Saramäki OR, Tammela TLJ, Martikainen PM, Vessella RL, Visakorpi T. The gene for
322 polycomb group protein enhancer of zeste homolog 2 (EZH2) is amplified in late-stage
323 prostate cancer. *Genes. Chromosomes Cancer.* 45(7), 639–645 (2006).

- 324 118. Bachmann N, Hoegel J, Haeusler J, *et al.* Mutation screen and association study of
325 EZH2 as a susceptibility gene for aggressive prostate cancer. *The Prostate*. 65(3), 252–
326 259 (2005).
- 327 119. Ogata R, Matsueda S, Yao A, Noguchi M, Itoh K, Harada M. Identification of polycomb
328 group protein enhancer of zeste homolog 2 (EZH2)-derived peptides immunogenic in
329 HLA-A24+ prostate cancer patients. *The Prostate*. 60(4), 273–281 (2004).
- 330 120. Sellers WR, Loda M. The EZH2 polycomb transcriptional repressor--a marker or mover
331 of metastatic prostate cancer? *Cancer Cell*. 2(5), 349–350 (2002).
- 332 121. Varambally S, Dhanasekaran SM, Zhou M, *et al.* The polycomb group protein EZH2 is
333 involved in progression of prostate cancer. *Nature*. 419(6907), 624–629 (2002).
- 334 122. Girard N, Bazille C, Lhuissier E, *et al.* 3-Deazaneplanocin A (DZNep), an Inhibitor of the
335 Histone Methyltransferase EZH2, Induces Apoptosis and Reduces Cell Migration in
336 Chondrosarcoma Cells. *PLoS ONE* [Internet]. 9(5) (2014). Available from:
337 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4031152/>.
- 338 123. Li Z, Xu L, Tang N, *et al.* The polycomb group protein EZH2 inhibits lung cancer cell
339 growth by repressing the transcription factor Nrf2. *FEBS Lett.* (2014).
- 340 124. Tatton-Brown K, Murray A, Hanks S, *et al.* Weaver syndrome and EZH2 mutations:
341 Clarifying the clinical phenotype. *Am. J. Med. Genet. A*. 161A(12), 2972–2980 (2013).
- 342 125. Tatton-Brown K, Hanks S, Ruark E, *et al.* Germline mutations in the oncogene EZH2
343 cause Weaver syndrome and increased human height. *Oncotarget*. 2(12), 1127–1133
344 (2011).
- 345 126. Gibson WT, Hood RL, Zhan SH, *et al.* Mutations in EZH2 cause Weaver syndrome. *Am.*
346 *J. Hum. Genet.* 90(1), 110–118 (2012).
- 347 127. Thanasopoulou A, Tzankov A, Schwaller J. Potent cooperation between the NUP98-
348 NSD1 fusion and FLT3-ITD mutation in acute myeloid leukemia induction.
349 *Haematologica*. (2014).
- 350 128. Akiki S, Dyer SA, Grimwade D, *et al.* NUP98-NSD1 fusion in association with FLT3-ITD
351 mutation identifies a prognostically relevant subgroup of pediatric acute myeloid
352 leukemia patients suitable for monitoring by real time quantitative PCR. *Genes.*
353 *Chromosomes Cancer*. 52(11), 1053–1064 (2013).
- 354 129. Shiba N, Ichikawa H, Taki T, *et al.* NUP98-NSD1 gene fusion and its related gene
355 expression signature are strongly associated with a poor prognosis in pediatric acute
356 myeloid leukemia. *Genes. Chromosomes Cancer*. 52(7), 683–693 (2013).
- 357 130. Fasan A, Haferlach C, Alpermann T, Kern W, Haferlach T, Schnittger S. A rare but
358 specific subset of adult AML patients can be defined by the cytogenetically cryptic
359 NUP98-NSD1 fusion gene. *Leukemia*. 27(1), 245–248 (2013).

- 360 131. Thol F, Kölking B, Hollink IH, *et al.* Analysis of NUP98/NSD1 translocations in adult
361 AML and MDS patients. *Leukemia*. 27(3), 750–754 (2013).
- 362 132. Hollink IHIM, van den Heuvel-Eibrink MM, Arentsen-Peters STCJM, *et al.* NUP98/NSD1
363 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct
364 HOX gene expression pattern. *Blood*. 118(13), 3645–3656 (2011).
- 365 133. Petit A, Radford I, Waill M-C, Romana S, Berger R. NUP98-NSD1 fusion by insertion in
366 acute myeloblastic leukemia. *Cancer Genet. Cytogenet.* 180(1), 43–46 (2008).
- 367 134. Wang T-F, Horsley SW, Lee K-F, Chu S-C, Li C-C, Kao R-H. Translocation between
368 chromosome 5q35 and chromosome 11q13-- an unusual cytogenetic finding in a
369 primary refractory acute myeloid leukemia. *Clin. Lab. Haematol.* 28(3), 160–163
370 (2006).
- 371 135. Cerveira N, Correia C, Dória S, *et al.* Frequency of NUP98-NSD1 fusion transcript in
372 childhood acute myeloid leukaemia. *Leukemia*. 17(11), 2244–2247 (2003).
- 373 136. Casas S, Aventín A, Nomdedéu J, Sierra J. Cryptic t(5;11)(q35;p15.5) in adult de novo
374 acute myelocytic leukemia with normal karyotype. *Cancer Genet. Cytogenet.* 145(2),
375 183 (2003).
- 376 137. Panarello C, Rosanda C, Morerio C. Cryptic translocation t(5;11)(q35;p15.5) with
377 involvement of the NSD1 and NUP98 genes without 5q deletion in childhood acute
378 myeloid leukemia. *Genes. Chromosomes Cancer.* 35(3), 277–281 (2002).
- 379 138. Jaju RJ, Fidler C, Haas OA, *et al.* A novel gene, NSD1, is fused to NUP98 in the
380 t(5;11)(q35;p15.5) in de novo childhood acute myeloid leukemia. *Blood*. 98(4), 1264–
381 1267 (2001).
- 382 139. Ishikawa M, Yagasaki F, Okamura D, *et al.* A novel gene, ANKRD28 on 3p25, is fused
383 with NUP98 on 11p15 in a cryptic 3-way translocation of t(3;5;11)(p25;q35;p15) in an
384 adult patient with myelodysplastic syndrome/acute myelogenous leukemia. *Int. J.*
385 *Hematol.* 86(3), 238–245 (2007).
- 386 140. Baujat G, Rio M, Rossignol S, *et al.* Paradoxical NSD1 mutations in Beckwith-
387 Wiedemann syndrome and 11p15 anomalies in Sotos syndrome. *Am. J. Hum. Genet.*
388 74(4), 715–720 (2004).
- 389 141. Park SH, Lee JE, Sohn YB, Ko JM. First identified Korean family with Sotos syndrome
390 caused by a novel intragenic mutation in NSD1. *Ann. Clin. Lab. Sci.* 44(2), 228–231
391 (2014).
- 392 142. Kılıç E, Utine GE, Boduroğlu K. A case of Sotos syndrome with 5q35 microdeletion and
393 novel clinical findings. *Turk. J. Pediatr.* 55(2), 207–209 (2013).
- 394 143. Dikow N, Maas B, Gaspar H, *et al.* The phenotypic spectrum of duplication 5q35.2-
395 q35.3 encompassing NSD1: is it really a reversed Sotos syndrome? *Am. J. Med. Genet.*
396 A. 161(9), 2158–2166 (2013).

- 397 144. Rosenfeld JA, Kim KH, Angle B, *et al.* Further Evidence of Contrasting Phenotypes
398 Caused by Reciprocal Deletions and Duplications: Duplication of NSD1 Causes Growth
399 Retardation and Microcephaly. *Mol. Syndromol.* 3(6), 247–254 (2013).
- 400 145. Sohn YB, Lee CG, Ko JM, *et al.* Clinical and genetic spectrum of 18 unrelated Korean
401 patients with Sotos syndrome: frequent 5q35 microdeletion and identification of four
402 novel NSD1 mutations. *J. Hum. Genet.* 58(2), 73–77 (2013).
- 403 146. Hirai N, Matsune K, Ohashi H. Craniofacial and oral features of Sotos syndrome:
404 differences in patients with submicroscopic deletion and mutation of NSD1 gene. *Am.*
405 *J. Med. Genet. A.* 155A(12), 2933–2939 (2011).
- 406 147. Kasnauskiene J, Cimbalistiene L, Ciuladaite Z, *et al.* De novo 5q35.5 duplication with
407 clinical presentation of Sotos syndrome. *Am. J. Med. Genet. A.* 155A(10), 2501–2507
408 (2011).
- 409 148. Fickie MR, Lapunzina P, Gentile JK, *et al.* Adults with Sotos syndrome: review of 21
410 adults with molecularly confirmed NSD1 alterations, including a detailed case report
411 of the oldest person. *Am. J. Med. Genet. A.* 155A(9), 2105–2111 (2011).
- 412 149. Nicita F, Tarani L, Spalice A, *et al.* Novel missense mutation (L1917P) involving sac-
413 domain of NSD1 gene in a patient with Sotos syndrome. *J. Genet.* 90(1), 147–150
414 (2011).
- 415 150. Piccione M, Consiglio V, Di Fiore A, *et al.* Deletion of NSD1 exon 14 in Sotos syndrome:
416 first description. *J. Genet.* 90(1), 119–123 (2011).
- 417 151. Fryssira H, Drossatou P, Sklavou R, Barambouti F, Manolaki N. Two cases of Sotos
418 syndrome with novel mutations of the NSD1 gene. *Genet. Couns. Geneva Switz.* 21(1),
419 53–59 (2010).
- 420 152. Berdasco M, Ropero S, Setien F, *et al.* Epigenetic inactivation of the Sotos overgrowth
421 syndrome gene histone methyltransferase NSD1 in human neuroblastoma and glioma.
422 *Proc. Natl. Acad. Sci. U. S. A.* 106(51), 21830–21835 (2009).
- 423 153. Kotilainen J, Pohjola P, Pirinen S, Arte S, Nieminen P. Premolar hypodontia is a
424 common feature in Sotos syndrome with a mutation in the NSD1 gene. *Am. J. Med.*
425 *Genet. A.* 149A(11), 2409–2414 (2009).
- 426 154. Fagali C, Kok F, Nicola P, *et al.* MLPA analysis in 30 Sotos syndrome patients revealed
427 one total NSD1 deletion and two partial deletions not previously reported. *Eur. J. Med.*
428 *Genet.* 52(5), 333–336 (2009).
- 429 155. Zechner U, Kohlschmidt N, Kempf O, *et al.* Familial Sotos syndrome caused by a novel
430 missense mutation, C2175S, in NSD1 and associated with normal intelligence, insulin
431 dependent diabetes, bronchial asthma, and lipedema. *Eur. J. Med. Genet.* 52(5), 306–
432 310 (2009).

- 433 156. Ellison J. Gene symbol: NSD1. Disease: Sotos syndrome. *Hum. Genet.* 124(3), 311
434 (2008).
- 435 157. Mochizuki J, Saitsu H, Mizuguchi T, *et al.* Alu-related 5q35 microdeletions in Sotos
436 syndrome. *Clin. Genet.* 74(4), 384–391 (2008).
- 437 158. Malan V, De Blois MC, Prieur M, *et al.* Sotos syndrome caused by a paracentric
438 inversion disrupting the NSD1 gene. *Clin. Genet.* 73(1), 89–91 (2008).
- 439 159. Saugier-veber P, Bonnet C, Afenjar A, *et al.* Heterogeneity of NSD1 alterations in 116
440 patients with Sotos syndrome. *Hum. Mutat.* 28(11), 1098–1107 (2007).
- 441 160. Tei S, Tsuneishi S, Matsuo M. The first Japanese familial Sotos syndrome with a novel
442 mutation of the NSD1 gene. *Kobe J. Med. Sci.* 52(1-2), 1–8 (2006).
- 443 161. Kanemoto N, Kanemoto K, Nishimura G, *et al.* Nevo syndrome with an NSD1 deletion:
444 a variant of Sotos syndrome? *Am. J. Med. Genet. A.* 140(1), 70–73 (2006).
- 445 162. Douglas J, Tatton-Brown K, Coleman K, *et al.* Partial NSD1 deletions cause 5% of Sotos
446 syndrome and are readily identifiable by multiplex ligation dependent probe
447 amplification. *J. Med. Genet.* 42(9), e56 (2005).
- 448 163. Faravelli F. NSD1 mutations in Sotos syndrome. *Am. J. Med. Genet. C Semin. Med.*
449 *Genet.* 137C(1), 24–31 (2005).
- 450 164. Tatton-Brown K, Douglas J, Coleman K, *et al.* Genotype-phenotype associations in
451 Sotos syndrome: an analysis of 266 individuals with NSD1 aberrations. *Am. J. Hum.*
452 *Genet.* 77(2), 193–204 (2005).
- 453 165. Cecconi M, Forzano F, Milani D, *et al.* Mutation analysis of the NSD1 gene in a group of
454 59 patients with congenital overgrowth. *Am. J. Med. Genet. A.* 134(3), 247–253
455 (2005).
- 456 166. Melchior L, Schwartz M, Duno M. dHPLC screening of the NSD1 gene identifies nine
457 novel mutations--summary of the first 100 Sotos syndrome mutations. *Ann. Hum.*
458 *Genet.* 69(Pt 2), 222–226 (2005).
- 459 167. Kamimura J, Endo Y, Kurotaki N, *et al.* Identification of eight novel NSD1 mutations in
460 Sotos syndrome. *J. Med. Genet.* 40(11), e126 (2003).
- 461 168. Türkmen S, Gillissen-Kaesbach G, Meinecke P, *et al.* Mutations in NSD1 are
462 responsible for Sotos syndrome, but are not a frequent finding in other overgrowth
463 phenotypes. *Eur. J. Hum. Genet. EJHG.* 11(11), 858–865 (2003).
- 464 169. Rio M, Clech L, Amiel J, *et al.* Spectrum of NSD1 mutations in Sotos and Weaver
465 syndromes. *J. Med. Genet.* 40(6), 436–440 (2003).
- 466 170. Nagai T, Matsumoto N, Kurotaki N, *et al.* Sotos syndrome and haploinsufficiency of
467 NSD1: clinical features of intragenic mutations and submicroscopic deletions. *J. Med.*
468 *Genet.* 40(4), 285–289 (2003).

- 469 171. Höglund P, Kurotaki N, Kytölä S, Miyake N, Somer M, Matsumoto N. Familial Sotos
470 syndrome is caused by a novel 1 bp deletion of the NSD1 gene. *J. Med. Genet.* 40(1),
471 51–54 (2003).
- 472 172. Douglas J, Hanks S, Temple IK, *et al.* NSD1 mutations are the major cause of Sotos
473 syndrome and occur in some cases of Weaver syndrome but are rare in other
474 overgrowth phenotypes. *Am. J. Hum. Genet.* 72(1), 132–143 (2003).
- 475 173. Kurotaki N, Imaizumi K, Harada N, *et al.* Haploinsufficiency of NSD1 causes Sotos
476 syndrome. *Nat. Genet.* 30(4), 365–366 (2002).
- 477 174. Li J, Yin C, Okamoto H, *et al.* Identification of a novel proliferation-related protein,
478 WHSC1 4a, in human gliomas. *Neuro-Oncol.* 10(1), 45–51 (2008).
- 479 175. Kleefstra T, Brunner HG, Amiel J, *et al.* Loss-of-function mutations in euchromatin
480 histone methyl transferase 1 (EHMT1) cause the 9q34 subtelomeric deletion
481 syndrome. *Am. J. Hum. Genet.* 79(2), 370–377 (2006).
- 482 176. Jaffe JD, Wang Y, Chan HM, *et al.* Global chromatin profiling reveals NSD2 mutations in
483 pediatric acute lymphoblastic leukemia. *Nat. Genet.* 45(11), 1386–1391 (2013).
- 484 177. Huang Z, Wu H, Chuai S, *et al.* NSD2 is recruited through its PHD domain to oncogenic
485 gene loci to drive multiple myeloma. *Cancer Res.* 73(20), 6277–6288 (2013).
- 486 178. Keats JJ, Maxwell CA, Taylor BJ, *et al.* Overexpression of transcripts originating from
487 the MMSET locus characterizes all t(4;14)(p16;q32)-positive multiple myeloma
488 patients. *Blood.* 105(10), 4060–4069 (2005).
- 489 179. Kassambara A, Klein B, Moreaux J. MMSET is overexpressed in cancers: link with
490 tumor aggressiveness. *Biochem. Biophys. Res. Commun.* 379(4), 840–845 (2009).
- 491 180. Nimura K, Ura K, Shiratori H, *et al.* A histone H3 lysine 36 trimethyltransferase links
492 Nkx2-5 to Wolf-Hirschhorn syndrome. *Nature.* 460(7252), 287–291 (2009).
- 493 181. Rosati R, La Starza R, Veronese A, *et al.* NUP98 is fused to the NSD3 gene in acute
494 myeloid leukemia associated with t(8;11)(p11.2;p15). *Blood.* 99(10), 3857–3860
495 (2002).
- 496 182. Kleefstra T, van Zelst-Stams WA, Nillesen WM, *et al.* Further clinical and molecular
497 delineation of the 9q subtelomeric deletion syndrome supports a major contribution
498 of EHMT1 haploinsufficiency to the core phenotype. *J. Med. Genet.* 46(9), 598–606
499 (2009).
- 500 183. Kleefstra T, Smidt M, Banning MJG, *et al.* Disruption of the gene Euchromatin Histone
501 Methyl Transferase1 (Eu-HMTase1) is associated with the 9q34 subtelomeric deletion
502 syndrome. *J. Med. Genet.* 42(4), 299–306 (2005).
- 503 184. Rump A, Hildebrand L, Tzschach A, Ullmann R, Schrock E, Mitter D. A mosaic maternal
504 splice donor mutation in the EHMT1 gene leads to aberrant transcripts and to
505 Kleefstra syndrome in the offspring. *Eur. J. Hum. Genet. EJHG.* 21(8), 887–890 (2013).

- 506 185. Nillesen WM, Yntema HG, Moscarda M, *et al.* Characterization of a novel transcript of
507 the EHMT1 gene reveals important diagnostic implications for Kleeftstra syndrome.
508 *Hum. Mutat.* 32(7), 853–859 (2011).
- 509 186. Cebrian A, Pharoah PD, Ahmed S, *et al.* Genetic variants in epigenetic genes and
510 breast cancer risk. *Carcinogenesis.* 27(8), 1661–1669 (2006).
- 511 187. Northcott PA, Nakahara Y, Wu X, *et al.* Multiple recurrent genetic events converge on
512 control of histone lysine methylation in medulloblastoma. *Nat. Genet.* 41(4), 465–472
513 (2009).
- 514 188. Ryu H, Lee J, Hagerty SW, *et al.* ESET/SETDB1 gene expression and histone H3 (K9)
515 trimethylation in Huntington’s disease. *Proc. Natl. Acad. Sci. U. S. A.* 103(50), 19176–
516 19181 (2006).
- 517 189. Zhang Y, Leaves NI, Anderson GG, *et al.* Positional cloning of a quantitative trait locus
518 on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat.*
519 *Genet.* 34(2), 181–186 (2003).
- 520 190. Sakamoto LHT, Andrade RV de, Felipe MSS, Motoyama AB, Pittella Silva F. SMYD2 is
521 highly expressed in pediatric acute lymphoblastic leukemia and constitutes a bad
522 prognostic factor. *Leuk. Res.* 38(4), 496–502 (2014).
- 523 191. Komatsu S, Imoto I, Tsuda H, *et al.* Overexpression of SMYD2 relates to tumor cell
524 proliferation and malignant outcome of esophageal squamous cell carcinoma.
525 *Carcinogenesis.* 30(7), 1139–1146 (2009).
- 526 192. Skawran B, Steinemann D, Weigmann A, *et al.* Gene expression profiling in
527 hepatocellular carcinoma: upregulation of genes in amplified chromosome regions.
528 *Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc.* 21(5), 505–516 (2008).
- 529 193. Hamamoto R, Silva FP, Tsuge M, *et al.* Enhanced SMYD3 expression is essential for the
530 growth of breast cancer cells. *Cancer Sci.* 97(2), 113–118 (2006).
- 531 194. Frank B, Hemminki K, Wappenschmidt B, *et al.* Variable number of tandem repeats
532 polymorphism in the SMYD3 promoter region and the risk of familial breast cancer.
533 *Int. J. Cancer J. Int. Cancer.* 118(11), 2917–2918 (2006).
- 534 195. Gaedcke J, Grade M, Jung K, *et al.* Mutated KRAS results in overexpression of DUSP4, a
535 MAP-kinase phosphatase, and SMYD3, a histone methyltransferase, in rectal
536 carcinomas. *Genes. Chromosomes Cancer.* 49(11), 1024–1034 (2010).
- 537 196. Hamamoto R, Furukawa Y, Morita M, *et al.* SMYD3 encodes a histone
538 methyltransferase involved in the proliferation of cancer cells. *Nat. Cell Biol.* 6(8),
539 731–740 (2004).
- 540 197. Hu L, Zhu YT, Qi C, Zhu Y-J. Identification of Smyd4 as a potential tumor suppressor
541 gene involved in breast cancer development. *Cancer Res.* 69(9), 4067–4072 (2009).

- 542 198. Boi M, Rinaldi A, Kwee I, *et al.* PRDM1/BLIMP1 is commonly inactivated in anaplastic
543 large T-cell lymphoma. *Blood*. 122(15), 2683–2693 (2013).
- 544 199. Best T, Li D, Skol AD, *et al.* Variants at 6q21 implicate PRDM1 in the etiology of
545 therapy-induced second malignancies after Hodgkin’s lymphoma. *Nat. Med.* 17(8),
546 941–943 (2011).
- 547 200. Song Y, Cao Z, Li L, Zhang H-T, Zhang X. Blimp-1 protein and Hans classification on
548 prognosis of diffuse large B-cell lymphoma and their interrelation. *Chin. J. Cancer*.
549 29(9), 781–786 (2010).
- 550 201. Nie K, Zhang T, Allawi H, *et al.* Epigenetic down-regulation of the tumor suppressor
551 gene PRDM1/Blimp-1 in diffuse large B cell lymphomas: a potential role of the
552 microRNA let-7. *Am. J. Pathol.* 177(3), 1470–1479 (2010).
- 553 202. Courts C, Montesinos-Rongen M, Brunn A, *et al.* Recurrent inactivation of the PRDM1
554 gene in primary central nervous system lymphoma. *J. Neuropathol. Exp. Neurol.* 67(7),
555 720–727 (2008).
- 556 203. Tam W, Gomez M, Nie K. Significance of PRDM1beta expression as a prognostic
557 marker in diffuse large B-cell lymphomas. *Blood*. 111(4), 2488–2489; author reply
558 2489–2490 (2008).
- 559 204. Tate G, Hirayama-Ohashi Y, Kishimoto K, Mitsuya T. Novel BLIMP1/PRDM1 gene
560 mutations in B-cell lymphoma. *Cancer Genet. Cytogenet.* 172(2), 151–153 (2007).
- 561 205. Garcia J-F, Roncador G, García J-F, *et al.* PRDM1/BLIMP-1 expression in multiple B and
562 T-cell lymphoma. *Haematologica*. 91(4), 467–474 (2006).
- 563 206. Tam W, Gomez M, Chadburn A, Lee JW, Chan WC, Knowles DM. Mutational analysis of
564 PRDM1 indicates a tumor-suppressor role in diffuse large B-cell lymphomas. *Blood*.
565 107(10), 4090–4100 (2006).
- 566 207. Pasqualucci L, Compagno M, Houldsworth J, *et al.* Inactivation of the PRDM1/BLIMP1
567 gene in diffuse large B cell lymphoma. *J. Exp. Med.* 203(2), 311–317 (2006).
- 568 208. Luo J, Niu X, Liu H, Zhang M, Chen M, Deng S. Up-regulation of transcription factor
569 Blimp1 in systemic lupus erythematosus. *Mol. Immunol.* 56(4), 574–582 (2013).
- 570 209. Zhou X, Lu X, Lv J, *et al.* Genetic association of PRDM1-ATG5 intergenic region and
571 autophagy with systemic lupus erythematosus in a Chinese population. *Ann. Rheum.*
572 *Dis.* 70(7), 1330–1337 (2011).
- 573 210. Gateva V, Sandling JK, Hom G, *et al.* A large-scale replication study identifies TNIP1,
574 PRDM1, JAZF1, UHRF1BP1 and IL10 as risk loci for systemic lupus erythematosus. *Nat.*
575 *Genet.* 41(11), 1228–1233 (2009).
- 576 211. Ellinghaus D, Zhang H, Zeissig S, *et al.* Association between variants of PRDM1 and
577 NDP52 and Crohn’s disease, based on exome sequencing and functional studies.
578 *Gastroenterology*. 145(2), 339–347 (2013).

- 579 212. Cleynen I, González JR, Figueroa C, *et al.* Genetic factors conferring an increased
580 susceptibility to develop Crohn's disease also influence disease phenotype: results
581 from the IBDchip European Project. *Gut*. 62(11), 1556–1565 (2013).
- 582 213. Fransen K, Mitrovic M, van Diemen CC, *et al.* Limited evidence for parent-of-origin
583 effects in inflammatory bowel disease associated loci. *PLoS One*. 7(9), e45287 (2012).
- 584 214. Abbondanza C, De Rosa C, D'Arcangelo A, *et al.* Identification of a functional estrogen-
585 responsive enhancer element in the promoter 2 of PRDM2 gene in breast cancer cell
586 lines. *J. Cell. Physiol.* 227(3), 964–975 (2012).
- 587 215. Du Y, Carling T, Fang W, Piao Z, Sheu JC, Huang S. Hypermethylation in human cancers
588 of the RIZ1 tumor suppressor gene, a member of a histone/protein methyltransferase
589 superfamily. *Cancer Res.* 61(22), 8094–8099 (2001).
- 590 216. Sakurada K, Furukawa T, Kato Y, Kayama T, Huang S, Horii A. RIZ, the retinoblastoma
591 protein interacting zinc finger gene, is mutated in genetically unstable cancers of the
592 pancreas, stomach, and colorectum. *Genes. Chromosomes Cancer.* 30(2), 207–211
593 (2001).
- 594 217. Chadwick RB, Jiang GL, Bennington GA, *et al.* Candidate tumor suppressor RIZ is
595 frequently involved in colorectal carcinogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 97(6),
596 2662–2667 (2000).
- 597 218. Piao Z, Fang W, Malkhosyan S, *et al.* Frequent frameshift mutations of RIZ in sporadic
598 gastrointestinal and endometrial carcinomas with microsatellite instability. *Cancer*
599 *Res.* 60(17), 4701–4704 (2000).
- 600 219. Pan K-F, Lu Y-Y, Liu W-G, Zhang L, You W-C. Detection of frameshift mutations of RIZ in
601 gastric cancers with microsatellite instability. *World J. Gastroenterol. WJG.* 10(18),
602 2719–2722 (2004).
- 603 220. Oshimo Y, Oue N, Mitani Y, *et al.* Frequent epigenetic inactivation of RIZ1 by promoter
604 hypermethylation in human gastric carcinoma. *Int. J. Cancer J. Int. Cancer.* 110(2),
605 212–218 (2004).
- 606 221. Tokumaru Y, Nomoto S, Jerónimo C, *et al.* Biallelic inactivation of the RIZ1 gene in
607 human gastric cancer. *Oncogene.* 22(44), 6954–6958 (2003).
- 608 222. Nishida N, Kudo M, Nagasaka T, Ikai I, Goel A. Characteristic patterns of altered DNA
609 methylation predict emergence of human hepatocellular carcinoma. *Hepatol. Baltim.*
610 *Md.* 56(3), 994–1003 (2012).
- 611 223. Zhang C, Li H, Wang Y, *et al.* Epigenetic inactivation of the tumor suppressor gene RIZ1
612 in hepatocellular carcinoma involves both DNA methylation and histone
613 modifications. *J. Hepatol.* 53(5), 889–895 (2010).

- 614 224. Piao GH, Piao WH, He Y, Zhang HH, Wang GQ, Piao Z. Hyper-methylation of RIZ1
615 tumor suppressor gene is involved in the early tumorigenesis of hepatocellular
616 carcinoma. *Histol. Histopathol.* 23(10), 1171–1175 (2008).
- 617 225. Fang W, Piao Z, Buyse IM, *et al.* Preferential loss of a polymorphic RIZ allele in human
618 hepatocellular carcinoma. *Br. J. Cancer.* 84(6), 743–747 (2001).
- 619 226. Fang W, Piao Z, Simon D, Sheu JC, Huang S. Mapping of a minimal deleted region in
620 human hepatocellular carcinoma to 1p36.13-p36.23 and mutational analysis of the RIZ
621 (PRDM2) gene localized to the region. *Genes. Chromosomes Cancer.* 28(3), 269–275
622 (2000).
- 623 227. Tan S-X, Hu R-C, Liu J-J, Tan Y-L, Liu W-E. Methylation of PRDM2, PRDM5 and PRDM16
624 genes in lung cancer cells. *Int. J. Clin. Exp. Pathol.* 7(5), 2305–2311 (2014).
- 625 228. Geli J, Kiss N, Kogner P, Larsson C. Suppression of RIZ in biologically unfavourable
626 neuroblastomas. *Int. J. Oncol.* 37(5), 1323–1330 (2010).
- 627 229. Hoebeeck J, Michels E, Pattyn F, *et al.* Aberrant methylation of candidate tumor
628 suppressor genes in neuroblastoma. *Cancer Lett.* 273(2), 336–346 (2009).
- 629 230. Deng Q, Huang S. PRDM5 is silenced in human cancers and has growth suppressive
630 activities. *Oncogene.* 23(28), 4903–4910 (2004).
- 631 231. Watanabe Y, Toyota M, Kondo Y, *et al.* PRDM5 identified as a target of epigenetic
632 silencing in colorectal and gastric cancer. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer
633 Res.* 13(16), 4786–4794 (2007).
- 634 232. Turnbull J, Girard J-M, Lohi H, *et al.* Early-onset Lafora body disease. *Brain J. Neurol.*
635 135(Pt 9), 2684–2698 (2012).
- 636 233. He X-J, Ruan J, Du W-D, *et al.* PRDM9 gene polymorphism may not be associated with
637 defective spermatogenesis in the Chinese Han population. *Syst. Biol. Reprod. Med.*
638 59(1), 38–41 (2013).
- 639 234. Irie S, Tsujimura A, Miyagawa Y, *et al.* Single-nucleotide polymorphisms of the PRDM9
640 (MEISETZ) gene in patients with nonobstructive azoospermia. *J. Androl.* 30(4), 426–
641 431 (2009).
- 642 235. Miyamoto T, Koh E, Sakugawa N, *et al.* Two single nucleotide polymorphisms in
643 PRDM9 (MEISETZ) gene may be a genetic risk factor for Japanese patients with
644 azoospermia by meiotic arrest. *J. Assist. Reprod. Genet.* 25(11-12), 553–557 (2008).
- 645 236. Reid AG, Nacheva EP. A potential role for PRDM12 in the pathogenesis of chronic
646 myeloid leukaemia with derivative chromosome 9 deletion. *Leukemia.* 18(1), 178–180
647 (2004).
- 648 237. Nishikawa N, Toyota M, Suzuki H, *et al.* Gene amplification and overexpression of
649 PRDM14 in breast cancers. *Cancer Res.* 67(20), 9649–9657 (2007).

- 650 238. Schulte JH, Lim S, Schramm A, *et al.* Lysine-specific demethylase 1 is strongly
651 expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer*
652 *Res.* 69(5), 2065–2071 (2009).
- 653 239. Bennani-Baiti IM, Machado I, Llombart-Bosch A, Kovar H. Lysine-specific demethylase
654 1 (LSD1/KDM1A/AOF2/BHC110) is expressed and is an epigenetic drug target in
655 chondrosarcoma, Ewing’s sarcoma, osteosarcoma, and rhabdomyosarcoma. *Hum.*
656 *Pathol.* 43(8), 1300–1307 (2012).
- 657 240. Harris WJ, Huang X, Lynch JT, *et al.* The histone demethylase KDM1A sustains the
658 oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer Cell.* 21(4), 473–487
659 (2012).
- 660 241. Binda C, Valente S, Romanenghi M, *et al.* Biochemical, structural, and biological
661 evaluation of tranlycypromine derivatives as inhibitors of histone demethylases LSD1
662 and LSD2. *J. Am. Chem. Soc.* 132(19), 6827–6833 (2010).
- 663 242. Li Y, Deng C, Hu X, *et al.* Dynamic interaction between TAL1 oncoprotein and LSD1
664 regulates TAL1 function in hematopoiesis and leukemogenesis. *Oncogene.* 31(48),
665 5007–5018 (2012).
- 666 243. Fiskus W, Sharma S, Shah B, *et al.* Highly effective combination of LSD1 (KDM1A)
667 antagonist and pan-histone deacetylase inhibitor against human AML cells. *Leukemia.*
668 (2014).
- 669 244. Sankar S, Theisen ER, Bearss J, *et al.* Reversible LSD1 inhibition interferes with global
670 EWS/ETS transcriptional activity and impedes Ewing sarcoma tumor growth. *Clin.*
671 *Cancer Res. Off. J. Am. Assoc. Cancer Res.* (2014).
- 672 245. Miura S, Maesawa C, Shibazaki M, *et al.* Immunohistochemistry for histone h3 lysine 9
673 methyltransferase and demethylase proteins in human melanomas. *Am. J.*
674 *Dermatopathol.* 36(3), 211–216 (2014).
- 675 246. Ding D, Liu X, Guo S-W. Overexpression of lysine-specific demethylase 1 in ovarian
676 endometriomas and its inhibition reduces cellular proliferation, cell cycle progression,
677 and invasiveness. *Fertil. Steril.* 101(3), 740–749 (2014).
- 678 247. Konovalov S, Garcia-Bassets I. Analysis of the levels of lysine-specific demethylase 1
679 (LSD1) mRNA in human ovarian tumors and the effects of chemical LSD1 inhibitors in
680 ovarian cancer cell lines. *J. Ovarian Res.* 6(1), 75 (2013).
- 681 248. Kong L, Zhang G, Wang X, Zhou J, Hou S, Cui W. Immunohistochemical expression of
682 RBP2 and LSD1 in papillary thyroid carcinoma. *Romanian J. Morphol. Embryol. Rev.*
683 *Roum. Morphol. Embryol.* 54(3), 499–503 (2013).
- 684 249. Huang Z, Li S, Song W, *et al.* Lysine-specific demethylase 1 (LSD1/KDM1A) contributes
685 to colorectal tumorigenesis via activation of the Wnt/ β -catenin pathway by down-
686 regulating Dickkopf-1 (DKK1) [corrected]. *PLoS One.* 8(7), e70077 (2013).

- 687 250. Ding J, Zhang Z-M, Xia Y, *et al.* LSD1-mediated epigenetic modification contributes to
688 proliferation and metastasis of colon cancer. *Br. J. Cancer.* 109(4), 994–1003 (2013).
- 689 251. Yu Y, Wang B, Zhang K, *et al.* High expression of lysine-specific demethylase 1
690 correlates with poor prognosis of patients with esophageal squamous cell carcinoma.
691 *Biochem. Biophys. Res. Commun.* 437(2), 192–198 (2013).
- 692 252. Liu J, Liu F-Y, Tong Z-Q, *et al.* Lysine-specific demethylase 1 in breast cancer cells
693 contributes to the production of endogenous formaldehyde in the metastatic bone
694 cancer pain model of rats. *PLoS One.* 8(3), e58957 (2013).
- 695 253. Kashyap V, Ahmad S, Nilsson EM, *et al.* The lysine specific demethylase-1
696 (LSD1/KDM1A) regulates VEGF-A expression in prostate cancer. *Mol. Oncol.* 7(3), 555–
697 566 (2013).
- 698 254. Jie D, Zhongmin Z, Guoqing L, *et al.* Positive expression of LSD1 and negative
699 expression of E-cadherin correlate with metastasis and poor prognosis of colon
700 cancer. *Dig. Dis. Sci.* 58(6), 1581–1589 (2013).
- 701 255. Zhao Z-K, Yu H-F, Wang D-R, *et al.* Overexpression of lysine specific demethylase 1
702 predicts worse prognosis in primary hepatocellular carcinoma patients. *World J.*
703 *Gastroenterol. WJG.* 18(45), 6651–6656 (2012).
- 704 256. Serce N, Gnatzy A, Steiner S, Lorenzen H, Kirfel J, Buettner R. Elevated expression of
705 LSD1 (Lysine-specific demethylase 1) during tumour progression from pre-invasive to
706 invasive ductal carcinoma of the breast. *BMC Clin. Pathol.* 12, 13 (2012).
- 707 257. Lv T, Yuan D, Miao X, *et al.* Over-expression of LSD1 promotes proliferation, migration
708 and invasion in non-small cell lung cancer. *PLoS One.* 7(4), e35065 (2012).
- 709 258. Kauffman EC, Robinson BD, Downes MJ, *et al.* Role of androgen receptor and
710 associated lysine-demethylase coregulators, LSD1 and JMJD2A, in localized and
711 advanced human bladder cancer. *Mol. Carcinog.* 50(12), 931–944 (2011).
- 712 259. Hayami S, Kelly JD, Cho H-S, *et al.* Overexpression of LSD1 contributes to human
713 carcinogenesis through chromatin regulation in various cancers. *Int. J. Cancer J. Int.*
714 *Cancer.* 128(3), 574–586 (2011).
- 715 260. Suikki HE, Kujala PM, Tammela TLJ, van Weerden WM, Vessella RL, Visakorpi T.
716 Genetic alterations and changes in expression of histone demethylases in prostate
717 cancer. *The Prostate.* 70(8), 889–898 (2010).
- 718 261. Lim S, Janzer A, Becker A, *et al.* Lysine-specific demethylase 1 (LSD1) is highly
719 expressed in ER-negative breast cancers and a biomarker predicting aggressive
720 biology. *Carcinogenesis.* 31(3), 512–520 (2010).
- 721 262. Magerl C, Ellinger J, Braunschweig T, *et al.* H3K4 dimethylation in hepatocellular
722 carcinoma is rare compared with other hepatobiliary and gastrointestinal carcinomas

- 723 and correlates with expression of the methylase Ash2 and the demethylase LSD1.
724 *Hum. Pathol.* 41(2), 181–189 (2010).
- 725 263. Zhu Q, Liu C, Ge Z, *et al.* Lysine-specific demethylase 1 (LSD1) Is required for the
726 transcriptional repression of the telomerase reverse transcriptase (hTERT) gene. *PLoS*
727 *One.* 3(1), e1446 (2008).
- 728 264. Krug AW, Tille E, Sun B, *et al.* Lysine-specific demethylase-1 modifies the age effect on
729 blood pressure sensitivity to dietary salt intake. *Age Dordr. Neth.* 35(5), 1809–1820
730 (2013).
- 731 265. Pojoga LH, Williams JS, Yao TM, *et al.* Histone demethylase LSD1 deficiency during
732 high-salt diet is associated with enhanced vascular contraction, altered NO-cGMP
733 relaxation pathway, and hypertension. *Am. J. Physiol. Heart Circ. Physiol.* 301(5),
734 H1862–1871 (2011).
- 735 266. Hsia DA, Tepper CG, Pochampalli MR, *et al.* KDM8, a H3K36me2 histone demethylase
736 that acts in the cyclin A1 coding region to regulate cancer cell proliferation. *Proc. Natl.*
737 *Acad. Sci. U. S. A.* 107(21), 9671–9676 (2010).
- 738 267. He J, Nguyen AT, Zhang Y. KDM2b/JHDM1b, an H3K36me2-specific demethylase, is
739 required for initiation and maintenance of acute myeloid leukemia. *Blood.* 117(14),
740 3869–3880 (2011).
- 741 268. Qiu J, Shi G, Jia Y, *et al.* The X-linked mental retardation gene PHF8 is a histone
742 demethylase involved in neuronal differentiation. *Cell Res.* 20(8), 908–918 (2010).
- 743 269. Kleine-Kohlbrecher D, Christensen J, Vandamme J, *et al.* A functional link between the
744 histone demethylase PHF8 and the transcription factor ZNF711 in X-linked mental
745 retardation. *Mol. Cell.* 38(2), 165–178 (2010).
- 746 270. Loenarz C, Ge W, Coleman ML, *et al.* PHF8, a gene associated with cleft lip/palate and
747 mental retardation, encodes for an Nepsilon-dimethyl lysine demethylase. *Hum. Mol.*
748 *Genet.* 19(2), 217–222 (2010).
- 749 271. Abidi FE, Miano MG, Murray JC, Schwartz CE. A novel mutation in the PHF8 gene is
750 associated with X-linked mental retardation with cleft lip/cleft palate. *Clin. Genet.*
751 72(1), 19–22 (2007).
- 752 272. Laumonier F, Holbert S, Ronce N, *et al.* Mutations in PHF8 are associated with X
753 linked mental retardation and cleft lip/cleft palate. *J. Med. Genet.* 42(10), 780–786
754 (2005).
- 755 273. Parrish JK, Sechler M, Winn RA, Jedlicka P. The histone demethylase KDM3A is a
756 microRNA-22-regulated tumor promoter in Ewing Sarcoma. *Oncogene.* (2013).
- 757 274. Guo X, Lu J, Wang Y, Gui Y, Duan X, Cai Z. Ascorbate antagonizes nickel ion to regulate
758 JMJD1A expression in kidney cancer cells. *Acta Biochim. Biophys. Sin.* 44(4), 330–338
759 (2012).

- 760 275. Anderton JA, Bose S, Vockerodt M, *et al.* The H3K27me3 demethylase, KDM6B, is
761 induced by Epstein-Barr virus and over-expressed in Hodgkin's Lymphoma. *Oncogene*
762 [Internet]. (2011). Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21242977>.
- 763 276. Shen Y, Guo X, Wang Y, *et al.* Expression and significance of histone H3K27
764 demethylases in renal cell carcinoma. *BMC Cancer*. 12, 470 (2012).
- 765 277. Banka S, Lederer D, Benoit V, *et al.* Novel KDM6A (UTX) mutations and a clinical and
766 molecular review of the X-linked Kabuki syndrome (KS2). *Clin. Genet.* (2014).
- 767 278. Miyake N, Koshimizu E, Okamoto N, *et al.* MLL2 and KDM6A mutations in patients
768 with Kabuki syndrome. *Am. J. Med. Genet. A*. 161(9), 2234–2243 (2013).
- 769 279. Miyake N, Mizuno S, Okamoto N, *et al.* KDM6A point mutations cause Kabuki
770 syndrome. *Hum. Mutat.* 34(1), 108–110 (2013).
- 771 280. Lederer D, Grisart B, Digilio MC, *et al.* Deletion of KDM6A, a histone demethylase
772 interacting with MLL2, in three patients with Kabuki syndrome. *Am. J. Hum. Genet.*
773 90(1), 119–124 (2012).
- 774 281. Gossage L, Murtaza M, Slatter AF, *et al.* Clinical and pathological impact of VHL,
775 PBRM1, BAP1, SETD2, KDM6A, and JARID1c in clear cell renal cell carcinoma. *Genes*.
776 *Chromosomes Cancer*. 53(1), 38–51 (2014).
- 777 282. Paolicchi E, Crea F, Farrar WL, Green JE, Danesi R. Histone lysine demethylases in
778 breast cancer. *Crit. Rev. Oncol. Hematol.* 86(2), 97–103 (2013).
- 779 283. Dubuc AM, Remke M, Korshunov A, *et al.* Aberrant patterns of H3K4 and H3K27
780 histone lysine methylation occur across subgroups in medulloblastoma. *Acta*
781 *Neuropathol. (Berl.)*. 125(3), 373–384 (2013).
- 782 284. Liu J, Lee W, Jiang Z, *et al.* Genome and transcriptome sequencing of lung cancers
783 reveal diverse mutational and splicing events. *Genome Res*. 22(12), 2315–2327 (2012).
- 784 285. Jones DTW, Jäger N, Kool M, *et al.* Dissecting the genomic complexity underlying
785 medulloblastoma. *Nature*. 488(7409), 100–105 (2012).
- 786 286. Murati A, Brecqueville M, Devillier R, Mozziconacci M-J, Gelsi-Boyer V, Birnbaum D.
787 Myeloid malignancies: mutations, models and management. *BMC Cancer*. 12, 304
788 (2012).
- 789 287. Robinson G, Parker M, Kranenburg TA, *et al.* Novel mutations target distinct
790 subgroups of medulloblastoma. *Nature*. 488(7409), 43–48 (2012).
- 791 288. McDevitt MA. Clinical applications of epigenetic markers and epigenetic profiling in
792 myeloid malignancies. *Semin. Oncol.* 39(1), 109–122 (2012).
- 793 289. Muramatsu H, Makishima H, Maciejewski JP. Chronic myelomonocytic leukemia and
794 atypical chronic myeloid leukemia: novel pathogenetic lesions. *Semin. Oncol.* 39(1),
795 67–73 (2012).

- 796 290. Jankowska AM, Makishima H, Tiu RV, *et al.* Mutational spectrum analysis of chronic
797 myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX,
798 EZH2, and DNMT3A. *Blood*. 118(14), 3932–3941 (2011).
- 799 291. Gui Y, Guo G, Huang Y, *et al.* Frequent mutations of chromatin remodeling genes in
800 transitional cell carcinoma of the bladder. *Nat. Genet.* 43(9), 875–878 (2011).
- 801 292. Wartman LD, Larson DE, Xiang Z, *et al.* Sequencing a mouse acute promyelocytic
802 leukemia genome reveals genetic events relevant for disease progression. *J. Clin.*
803 *Invest.* 121(4), 1445–1455 (2011).
- 804 293. Van Haaften G, Dalgliesh GL, Davies H, *et al.* Somatic mutations of the histone H3K27
805 demethylase, UTX, in human cancer. *Nat. Genet.* 41(5), 521–523 (2009).
- 806 294. Li L-L, Xue A-M, Li B-X, *et al.* JMJD2A contributes to breast cancer progression through
807 transcriptional repression of the tumor suppressor ARHI. *Breast Cancer Res. BCR.*
808 16(3), R56 (2014).
- 809 295. Wang H-L, Liu M-M, Ma X, *et al.* Expression and effects of JMJD2A histone
810 demethylase in endometrial carcinoma. *Asian Pac. J. Cancer Prev. APJCP.* 15(7), 3051–
811 3056 (2014).
- 812 296. Hu C-E, Liu Y-C, Zhang H-D, Huang G-J. JMJD2A predicts prognosis and regulates cell
813 growth in human gastric cancer. *Biochem. Biophys. Res. Commun.* 449(1), 1–7 (2014).
- 814 297. Li B-X, Li J, Luo C-L, *et al.* Expression of JMJD2A in infiltrating duct carcinoma was
815 markedly higher than fibroadenoma, and associated with expression of ARHI, p53 and
816 ER in infiltrating duct carcinoma. *Indian J. Exp. Biol.* 51(3), 208–217 (2013).
- 817 298. Berry WL, Janknecht R. KDM4/JMJD2 histone demethylases: epigenetic regulators in
818 cancer cells. *Cancer Res.* 73(10), 2936–2942 (2013).
- 819 299. Kogure M, Takawa M, Cho H-S, *et al.* Deregulation of the histone demethylase JMJD2A
820 is involved in human carcinogenesis through regulation of the G(1)/S transition.
821 *Cancer Lett.* 336(1), 76–84 (2013).
- 822 300. Berry WL, Shin S, Lightfoot SA, Janknecht R. Oncogenic features of the JMJD2A histone
823 demethylase in breast cancer. *Int. J. Oncol.* 41(5), 1701–1706 (2012).
- 824 301. Hong Q, Yu S, Yang Y, Liu G, Shao Z. A polymorphism in JMJD2C alters the cleavage by
825 caspase-3 and the prognosis of human breast cancer. *Oncotarget.* (2014).
- 826 302. Luo W, Chang R, Zhong J, Pandey A, Semenza GL. Histone demethylase JMJD2C is a
827 coactivator for hypoxia-inducible factor 1 that is required for breast cancer
828 progression. *Proc. Natl. Acad. Sci. U. S. A.* 109(49), E3367–3376 (2012).
- 829 303. Vinatzer U, Gollinger M, Müllauer L, Raderer M, Chott A, Streubel B. Mucosa-
830 associated lymphoid tissue lymphoma: novel translocations including rearrangements
831 of ODZ2, JMJD2C, and CNN3. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* 14(20),
832 6426–6431 (2008).

- 833 304. Rui L, Emre NCT, Kruhlak MJ, *et al.* Cooperative epigenetic modulation by cancer
834 amplicon genes. *Cancer Cell.* 18(6), 590–605 (2010).
- 835 305. Kim T-D, Fuchs JR, Schwartz E, *et al.* Pro-growth role of the JMJD2C histone
836 demethylase in HCT-116 colon cancer cells and identification of curcuminoids as
837 JMJD2 inhibitors. *Am. J. Transl. Res.* 6(3), 236–247 (2014).
- 838 306. Li W, Zhao L, Zang W, *et al.* Histone demethylase JMJD2B is required for tumor cell
839 proliferation and survival and is overexpressed in gastric cancer. *Biochem. Biophys.*
840 *Res. Commun.* 416(3-4), 372–378 (2011).
- 841 307. Toyokawa G, Cho H-S, Iwai Y, *et al.* The histone demethylase JMJD2B plays an essential
842 role in human carcinogenesis through positive regulation of cyclin-dependent kinase
843 6. *Cancer Prev. Res. Phila. Pa.* 4(12), 2051–2061 (2011).
- 844 308. Pryor JG, Brown-Kipphut BA, Iqbal A, Scott GA. Microarray comparative genomic
845 hybridization detection of copy number changes in desmoplastic melanoma and
846 malignant peripheral nerve sheath tumor. *Am. J. Dermatopathol.* 33(8), 780–785
847 (2011).
- 848 309. Shi L, Sun L, Li Q, *et al.* Histone demethylase JMJD2B coordinates H3K4/H3K9
849 methylation and promotes hormonally responsive breast carcinogenesis. *Proc. Natl.*
850 *Acad. Sci. U. S. A.* 108(18), 7541–7546 (2011).
- 851 310. Kawazu M, Saso K, Tong KI, *et al.* Histone demethylase JMJD2B functions as a co-factor
852 of estrogen receptor in breast cancer proliferation and mammary gland development.
853 *PLoS One.* 6(3), e17830 (2011).
- 854 311. Kuźbicki L, Lange D, Strączyńska-Niemiec A, Chwirot BW. JARID1B expression in human
855 melanoma and benign melanocytic skin lesions. *Melanoma Res.* 23(1), 8–12 (2013).
- 856 312. Radberger P, Radberger A, Bykov VJN, Seregard S, Economou MA. JARID1B protein
857 expression and prognostic implications in uveal melanoma. *Invest. Ophthalmol. Vis.*
858 *Sci.* 53(8), 4442–4449 (2012).
- 859 313. Held M, Bosenberg M. A role for the JARID1B stem cell marker for continuous
860 melanoma growth. *Pigment Cell Melanoma Res.* 23(4), 481–483 (2010).
- 861 314. Roesch A, Fukunaga-Kalabis M, Schmidt EC, *et al.* A temporarily distinct subpopulation
862 of slow-cycling melanoma cells is required for continuous tumor growth. *Cell.* 141(4),
863 583–594 (2010).
- 864 315. Roesch A, Mueller AM, Stempffl T, Moehle C, Landthaler M, Vogt T. RBP2-H1/JARID1B
865 is a transcriptional regulator with a tumor suppressive potential in melanoma cells.
866 *Int. J. Cancer J. Int. Cancer.* 122(5), 1047–1057 (2008).
- 867 316. Xiang Y, Zhu Z, Han G, *et al.* JARID1B is a histone H3 lysine 4 demethylase up-regulated
868 in prostate cancer. *Proc. Natl. Acad. Sci. U. S. A.* 104(49), 19226–19231 (2007).

- 869 317. Mitra D, Das PM, Huynh FC, Jones FE. Jumonji/ARID1 B (JARID1B) protein promotes
870 breast tumor cell cycle progression through epigenetic repression of microRNA let-7e.
871 *J. Biol. Chem.* 286(47), 40531–40535 (2011).
- 872 318. Catchpole S, Spencer-Dene B, Hall D, *et al.* PLU-1/JARID1B/KDM5B is required for
873 embryonic survival and contributes to cell proliferation in the mammary gland and in
874 ER+ breast cancer cells. *Int. J. Oncol.* 38(5), 1267–1277 (2011).
- 875 319. Izawa A, Kobayashi D, Nasu S, *et al.* Relevance of c-erbB2, PLU-1 and survivin mRNA
876 expression to diagnostic assessment of breast cancer. *Anticancer Res.* 22(5), 2965–
877 2969 (2002).
- 878 320. Madsen B, Tarsounas M, Burchell JM, Hall D, Poulsom R, Taylor-Papadimitriou J. PLU-
879 1, a transcriptional repressor and putative testis-cancer antigen, has a specific
880 expression and localisation pattern during meiosis. *Chromosoma.* 112(3), 124–132
881 (2003).
- 882 321. Yamane K, Tateishi K, Klose RJ, *et al.* PLU-1 is an H3K4 demethylase involved in
883 transcriptional repression and breast cancer cell proliferation. *Mol. Cell.* 25(6), 801–
884 812 (2007).
- 885 322. Barrett A, Santangelo S, Tan K, *et al.* Breast cancer associated transcriptional repressor
886 PLU-1/JARID1B interacts directly with histone deacetylases. *Int. J. Cancer J. Int. Cancer.*
887 121(2), 265–275 (2007).
- 888 323. Jensen LR, Amende M, Gurok U, *et al.* Mutations in the JARID1C gene, which is
889 involved in transcriptional regulation and chromatin remodeling, cause X-linked
890 mental retardation. *Am. J. Hum. Genet.* 76(2), 227–236 (2005).
- 891 324. Tzschach A, Lenzner S, Moser B, *et al.* Novel JARID1C/SMCX mutations in patients with
892 X-linked mental retardation. *Hum. Mutat.* 27(4), 389 (2006).
- 893 325. Santos C, Rodriguez-Revenge L, Madrigal I, Badenas C, Pineda M, Milà M. A novel
894 mutation in JARID1C gene associated with mental retardation. *Eur. J. Hum. Genet.*
895 *EJHG.* 14(5), 583–586 (2006).
- 896 326. Tahiliani M, Mei P, Fang R, *et al.* The histone H3K4 demethylase SMCX links REST
897 target genes to X-linked mental retardation. *Nature.* 447(7144), 601–605 (2007).
- 898 327. Iwase S, Lan F, Bayliss P, *et al.* The X-linked mental retardation gene SMCX/JARID1C
899 defines a family of histone H3 lysine 4 demethylases. *Cell.* 128(6), 1077–1088 (2007).
- 900 328. Abidi FE, Holloway L, Moore CA, *et al.* Mutations in JARID1C are associated with X-
901 linked mental retardation, short stature and hyperreflexia. *J. Med. Genet.* 45(12), 787–
902 793 (2008).
- 903 329. Abidi F, Holloway L, Moore CA, *et al.* Novel human pathological mutations. Gene
904 symbol: JARID1C. Disease: mental retardation, X-linked. *Hum. Genet.* 125(3), 345
905 (2009).

- 906 330. Rujirabanjerd S, Nelson J, Tarpey PS, *et al.* Identification and characterization of two
907 novel JARID1C mutations: suggestion of an emerging genotype-phenotype correlation.
908 *Eur. J. Hum. Genet. EJHG.* 18(3), 330–335 (2010).
- 909 331. Jensen LR, Bartenschlager H, Rujirabanjerd S, *et al.* A distinctive gene expression
910 fingerprint in mentally retarded male patients reflects disease-causing defects in the
911 histone demethylase KDM5C. *PathoGenetics.* 3(1), 2 (2010).
- 912 332. Santos-Rebouças CB, Fintelman-Rodrigues N, Jensen LR, *et al.* A novel nonsense
913 mutation in KDM5C/JARID1C gene causing intellectual disability, short stature and
914 speech delay. *Neurosci. Lett.* 498(1), 67–71 (2011).
- 915 333. Ounap K, Puusepp-Benazzouz H, Peters M, *et al.* A novel c.2T > C mutation of the
916 KDM5C/JARID1C gene in one large family with X-linked intellectual disability. *Eur. J.*
917 *Med. Genet.* 55(3), 178–184 (2012).
- 918 334. Hou J, Wu J, Dombkowski A, *et al.* Genomic amplification and a role in drug-resistance
919 for the KDM5A histone demethylase in breast cancer. *Am. J. Transl. Res.* 4(3), 247–256
920 (2012).
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