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H3K27 Methylation: A Focal Point of Epigenetic Deregulation in Cancer

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Abstract

Epigenetics, the modification of chromatin without changing the DNA sequence itself, determines whether a gene is expressed, and how much of a gene is expressed. Methylation of lysine 27 on histone 3 (H3K27me), a modification usually associated with gene repression, has established roles in regulating the expression of genes involved in lineage commitment and differentiation. Not surprisingly, alterations in the homeostasis of this critical mark have emerged as a recurrent theme in the pathogenesis of many cancers. Perturbations in the distribution or levels of H3K27me occur due to deregulation at all levels of the process, either by mutation in the histone itself, or changes in the activity of the writers, erasers or readers of this mark. Additionally, as no single histone mark alone determines the overall transcriptional readiness of a chromatin region, deregulation of other chromatin marks can also have dramatic consequences. Finally, the significance of mutations altering H3K27me is highlighted by the poor clinical outcome of patients whose tumors harbor such lesions. Current therapeutic approaches targeting aberrant H3K27 methylation remain to be proven useful in the clinic. Understanding the biological consequences and gene expression pathways affected by aberrant H3K27 methylation may lead to identification of new therapeutic targets and strategies.

Keywords

Epigenetics; H3K27 methylation; EZH2; PRC2; Cancer

1. Introduction

The transcriptional programs that determine cell fate are stimulus- and cell-type specific. Epigenetic regulation of genes allows different cells to integrate environmental signals with the genetic code to precisely control gene expression and shape cell identity. Epigenetic modifications, namely DNA methylation at CpG sites, and covalent modifications of the N-terminal tails and the core structure of core histones, are critical regulators of chromatin function and help determine accessibility of DNA to the transcription machinery (Kouzarides, 2007). Given the critical role of such modifications in regulating gene expression, it is not surprising that over the past decade, aberrant epigenetic regulation and alteration of histone modifications have emerged as a prominent and recurrent theme in malignancy (Berdasco and Esteller, 2013).

Epigenetic information is deposited by “writer” proteins, such as histone methyl lysine and arginine transferases, removed by “eraser” proteins, such as histone deacetylases and demethylases, and decoded by “reader” proteins adapted to bind to chromatin marks using specific structures such as the chromo, bromo, and PHD domains (Falkenberg and Johnstone, 2014). The antagonistic activities of two broad classes of protein complexes, trithorax (TrxG) and Polycomb (PcG), are responsible for writing the histone methylation marks that maintain or suppress gene expression. TrxG is associated with activation of gene expression characterized by methylation of lysine 4 of histone H3 (H3K4me), whereas PcG correlated with suppression of gene expression and trimethylation of lysine 27 of histone H3 (H3K27me³) (Schuettengruber et al., 2011).

In mammals, there are two distinct PcG complexes, PRC1 and PRC2 (Fig. 1). PRC2 is the primary writer of di- and tri- methylation of H3K27 which, at certain sites, leads to the recruitment of PRC1. PRC1 ubiquitinates H2A at lysine 119 (H2AK119ub¹), which can then be followed by DNA methyltransferase (DNMT) binding and consequent DNA methylation. Alternatively, H2AK119ub¹ by a variant PRC1 complex can recruit PRC2 *de novo*, indicating that either PRC1 or PRC2 can initiate a repressive chromatin domain, with recruitment of the other to help maintain or propagate it (Blackledge et al., 2014). PRC2 is composed of four core components, enhancer of zeste homologue 2 (EZH2), suppressor of zeste 12 (SUZ12), and two WD40 domain proteins, EED and RBBP4 (Cao et al., 2002; Muller et al., 2002). The conserved suppressor of variegation, enhancer of zeste, trithorax (SET) domain is the catalytic subunit of EZH2 and has methyltransferase activity not only towards H3K27, but also weakly towards lysine 26 of histone H1 (Kuzmichev et al., 2002). Early purifications of the PRC2 complex demonstrated that the EZH2 enzyme is active only when associated with EED and SUZ12, and has weak histone methyltransferase activity on its own *in vitro* (Cao and Zhang, 2004; Czermin et al., 2002; Muller et al., 2002). Thus, the non-catalytic subunits have essential roles in regulating the activity and integrity of the complex (Pasini et al., 2004). EZH2 can also interact with histone deacetylases (HDACs) through EED (van der Vlag and Otte, 1999; Zhao et al., 2010) and with noncoding RNA (Jeon and Lee, 2011; van der Vlag and Otte, 1999), thereby providing functional links between the cellular gene repression systems. Other accessory factors including AEBP2 (Cao and Zhang, 2004; Kim et al., 2009), JARID2 (Li et al., 2010; Peng et al., 2009) and three Polycomb-like proteins (PCLs: PHF1, MTF2, and PHF19) (Boulay et al., 2011; Li et

al., 2011; Nekrasov et al., 2007; Walker et al., 2010; Zhang et al., 2011b) have been implicated in the recruitment of PRC2 to its target genes and modulation of its activity. EZH1, a close homolog of EZH2, contains a SET domain, forms an alternative PRC2 complex with SUZ12 and EED, and also catalyzes H3K27 methylation. However, EZH1 and EZH2 exhibit different expression patterns, and the EZH1-PRC2 and EZH2-PRC2 complexes have different methyltransferase and chromatin binding activities (Margueron et al., 2008).

Demethylation of H3K27 is performed by the histone demethylases, UTX/KMD6A and JMJD3/KDM6B, which contain a JmjC (Jumonji) catalytic domain that uses α -ketoglutarate as a co-factor to oxidize and remove the methyl group (Agger et al., 2007; Hong et al., 2007; Lee et al., 2007). UTX interacts with mixed-lineage-leukemia (MLL)-containing complexes (Cho et al., 2007; Issaeva et al., 2007; Lee et al., 2007), which are responsible for the activating histone H3 lysine 4 (H3K4) mark, as well as CBP which is a histone acetyl transferase (Tie et al., 2012). These results suggest a coordinated mechanism for transcriptional activation, in which repressive H3K27 methyl marks are removed and activating marks are placed.

The amount and distribution of a specific histone modification can be pathologically altered by aberrant expression or function of the writers or erasers, or by mutations of the histone that prevents the residue from being modified. In addition, alterations in the “readers” can mis-transduce the signal, thereby altering the functional outcome of the mark. For example, in leukemia, chromosomal translocations involving the Plant Homeodomain (PHD) fingers of “reader” proteins with the transcriptional activator NUP98, results in inappropriate interpretation of the H3K4 tri-methylation mark (H3K4me³) at genes critical for differentiation (Wang et al., 2009). Finally, histone modifications are intricately coordinated, and alterations of one histone mark can affect the levels and distribution of other modifications. A good example of this *trans*-regulation is the stimulation of SET1-dependent H3K4me^{2/3} by histone H2B (H2B) ubiquitination (Kim et al., 2013b).

Given the wealth of evidence of the importance of H3K27me in the pathogenesis of cancer, in this review we will focus on mechanisms that affect the levels and distribution of this mark in human malignancies.

2. Histone H3 Mutations

The human histone H3 family of proteins consists of seven members, the two “canonical” H3, H3.1 and H3.2, and five variants, H3.3, CENP-A, H3t (Witt et al., 1996), H3.X and H3.Y (Wiedemann et al., 2010). Histone proteins are decorated by a variety of protein posttranslational modifications (PTMs), which are critical to dynamic modulation of chromatin structure and function and contribute to the cellular gene expression program. As with canonical H3, histone tails of the variants are still subject to PTMs.

Recurrent, cancer-driving mutations are found in the genes encoding histones. Specifically, sequencing studies in pediatric and young adult high-grade gliomas have identified somatic missense mutations of histone H3 that results in a lysine 27 to methionine change

(H3K27M) (Schwartzentruber et al., 2012; Sturm et al., 2012; Wu et al., 2012a). The majority of K27M mutations have been found in the gene encoding H3.3, *H3F3A*, with a few occurrences in genes that encode canonical H3, *HIST1H3B* and *HIST1H3C* (Buczkowicz et al., 2014; Fontebasso et al., 2014; Khuong-Quang et al., 2012; Schwartzentruber et al., 2012; Sturm et al., 2012; Wu et al., 2012a). The K27M mutations in H3 are predictors of poor survival (Khuong-Quang et al., 2012; Sturm et al., 2012).

Histone H3.3 deposition is the result of the replacement of histone H3 (normally incorporated into chromatin during S phase) with the variant, which is incorporated outside S phase at particular loci (Ahmad and Henikoff, 2002). H3.3 is enriched at pericentromeric and telomeric regions (Szenker et al., 2011), and importantly, at transcriptionally “active” or “poised” regions of the genome (Goldberg et al., 2010). H3.3’s pronounced patterns of genomic enrichment are mediated by at least two independent histone chaperones, with HIRA mainly facilitating deposition at gene loci (Ray-Gallet et al., 2002) and ATRX/DAXX responsible for repeat regions (Lewis et al., 2010). The poised regions into which histone H3.3 is placed are enriched for lysine 4 trimethylation of H3 (H3K4me³), or for both lysine 27 trimethylation (H3K27me³) and H3K4me³ (Delbarre et al., 2010). Thus the deposition of mutant histones could perturb the delicate balance of histone modifications in these crucial regions.

In vitro evidence suggests that the mutations act in a dominant negative manner by directly inhibiting PRC2 methyltransferase activity (Bender et al., 2013; Lewis et al., 2013), and “trap” or “poison” the PRC2 complex. In doing so, the mutant histones prevent the deposition of methyl marks on other PRC2 target regions, and thus can affect the entirety of the cellular transcriptional program. In support of this, chromatin immunoprecipitation sequencing (ChIP-Seq) analysis of cultured glioma cells with the H3.3K27M mutation measured fewer H3K27me³ peaks compared to controls (Bender et al., 2013; Chan et al., 2013), but interestingly, the lower abundance was not uniform over the genome. Instead, there was a genomic redistribution of H3K27me³ marks, with increases and decreases in gene expression corresponding to decreases and increases in local H3K27me³ levels, respectively. An innovative model of diffuse intrinsic pontine glioma (DIPG), was created by differentiating human embryonic stem (ES) cells into neural progenitor cells and then transducing them with a viral vector to force expression of H3.3K27M (Funato et al., 2014). This study by Funato and colleagues demonstrated that expression of H3.3K27M alone is insufficient to confer features of neoplastic cells, but it does synergize with other common DIPG gene alterations, in this case p53 loss and PDGFRA activation, to cause oncogenic transformation (Funato et al., 2014). The same study showed that H3.3K27M-induced mitogenic stimulation was restricted to neural progenitors, with no effect seen in undifferentiated ES cells or astrocytes, suggesting that the histone mutation is oncogenic only within the appropriate developmental window. An overlapping analysis of gene expression patterns and chromatin modifications showed that H3.3K27M expression promoted de-differentiation to a more primitive, stem-like state (Funato et al., 2014). Additionally, studies have demonstrated co-dependency between DNA methylation and histone H3K27me³ patterns (Brinkman et al., 2012; Statham et al., 2012), and the overall reductions in DNA methylation seen in patient samples with H3.3K27M (Bender et al.,

2013; Sturm et al., 2012), may work in concert with the mutated histone to help stabilize the tumor phenotype.

Mutations of other histone residues may also indirectly affect H3K27me³. A significant proportion of chondroblastoma and giant cell bone tumors have a mutation of lysine 36 histone H3 to methionine (K36M) (Behjati et al., 2013). Similar to the K27M mutants, K36M mutants display global reductions in H3K36 methylation. The H3K36me^{2/3} and H3K27me^{2/3} marks are mutually exclusive (Yuan et al., 2011; Zheng et al., 2012), and thus, the reduction of H3K36me levels may relieve remove a damper on PRC2 action, resulting in expansion of the H3K27me³ mark and aberrant repression of many loci. However, further research is needed to determine the nature of genes deregulated in such tumors.

Finally, altering the epigenetic landscape may not be the only mechanism by which histone H3 mutants affect gene expression programs. Mutation of the N-terminal tail could alter recognition of H3.3 by chromatin remodeling enzymes or chaperones, such as DAXX (Lewis et al., 2010), that may disrupt normal incorporation of H3.3 in pericentromeric regions. This in turn could affect chromosomal segregation, as was observed when H3.3 levels were knocked-down (Bush et al., 2013; Lin et al., 2013). Additionally, these mutations could affect H3.3 turnover kinetics or induce conformational changes that disrupt normal chromatin architecture. Thus, histone H3 mutants might disrupt the cytogenetic integrity of a cell, changes which might facilitate carcinogenesis.

3. Alterations in H3K27me “Writers”

3.1 EZH2 overexpression

Accumulating evidence suggests that *EZH2* acts as an oncogene and its aberrant overexpression has been documented in both solid tumors and hematological malignancies. Amplification of *EZH2* (Bracken et al., 2003; Saramaki et al., 2006) and alterations in microRNA levels have been proposed as the cause of *EZH2* deregulation (Friedman et al., 2009; Kong et al., 2012; Varambally et al., 2008).

Overexpression of *EZH2* in chronic lymphocytic leukemia (CLL) patients correlates with important indicators of poor prognosis, including high white blood cell counts, ZAP-70 expression and chromosomal abnormalities (Rabello Ddo et al., 2015). Overexpression was also reported in patients with high-risk myelodysplastic syndrome (MDS), MDS-derived acute myeloid leukemia (AML), and AML, particularly in patients with complex karyotypes and correlates with levels of DNA methylation and poor prognostic scoring (Grubach et al., 2008; Xu et al., 2011). Overexpression of *EZH2* itself is insufficient to cause leukemia, but does prevent hematopoietic stem cell (HSC) exhaustion (Kamminga et al., 2006). When *EZH2* was knocked out in the MLL-AF9 mouse model of AML, growth of leukemic cells was compromised *in vitro* and leukemia progression slowed *in vivo* (Tanaka et al., 2012). In this model, *EZH2* represses genes relevant to differentiation, apoptosis and stem cell function, such as *EGR1*, and reduces leukemia initiating cell (LIC) frequency. However, the results of this model, where the leukemia induced is produced via perturbation of another histone methyltransferase, MLL1, complicates interpretation of the results and makes it difficult to delineate a role for *EZH2* alone.

EZH2 is aberrantly overexpressed in a majority of natural killer/T-cell lymphoma (NKTL), as gene expression profiling (GEP) of formalin-fixed, paraffin-embedded (FFPE) tissues showed overexpression of EZH2 compared to normal NK cells (Ng et al., 2011). The overexpression is due to MYC suppression of microRNAs that normally target and inhibit EZH2 expression.

In solid tumors, high levels of EZH2 are clinically associated with aggressive biology, metastasis and poor clinical outcome (Bachmann et al., 2006; Kleer et al., 2003; Sasaki et al., 2008; Varambally et al., 2002). Similarly, in model cell lines, overexpression of EZH2 promotes anchorage-independent growth and cell invasion and correlates with cancer progression (Kleer et al., 2003; Xu et al., 2012).

Traditional thinking dictates that overexpressed EZH2 exerts its oncogenic activity via its SET domain, and presumably, its ability to mark histones for gene repression. Indeed, EZH2 overexpression has been linked to increased H3K27me³ levels and repression of tumor suppressor genes (Kim and Yu, 2012; Wang et al., 2012). In line with these findings, GEP of human melanoma patients identified a new set of EZH2 target genes that when expressed at low levels, correlated poorly with survival. Importantly, these target genes displayed tumor-suppressive functions affecting either melanoma growth or metastatic spread (Zingg et al., 2015).

However, evidence suggests that EZH2 has potential functions other than that of a transcriptional repressor, although potential mechanisms remain incompletely characterized. In NKTL, EZH2 behaves unconventionally in that its promotion of growth is independent of its SET domain (i.e. methyltransferase activity), and it serves as a transcriptional activator, by binding to and positively regulating the *CCND1* promoter (Yan et al., 2013). In prostate cancer cell lines (Xu et al., 2012), phosphorylated EZH2 did not associate with the PRC2 complex, but bound the androgen receptor, and was present at actively transcribed genes. This activity did require the SET domain of EZH2, suggesting that methylation of non-histone targets may also be relevant for the oncogenic action of EZH2. In support of the idea that non-histone targets are important mediators of EZH2 action, in glioblastoma, phosphorylated EZH2 methylates STAT3, increasing the activation of this oncogenic transcription factor (Kim et al., 2013a) (Fig. 2).

3.2 EZH2 mutations

Just as overexpression of EZH2 has pathological consequences, mutations in EZH2, both activating and inactivating, are found in a variety of tumors.

Non-Hodgkins Lymphoma (NHL) is a heterogeneous disease with frequent mutations in histone-modifying enzymes. In 2010, next generation sequencing identified heterozygous somatic missense mutations in 7% of Follicular Lymphoma (FL) and in up to 22% of Diffuse Large B-Cell Lymphoma (DLBCL) cases (Morin et al., 2010). Notably, the DLBCL patients that were positive for *EZH2* mutations, were of the germinal center B-cell like (GCB), and not the activated B-cell like (ABC) molecular subtype. The mutations are found within the SET domain of EZH2 at tyrosine 641 (Y64N, F, S or H) (Morin et al., 2010), alanine 677 (A677G) (McCabe et al., 2012), and alanine 687 (A687V) (Majer et al., 2012).

The Y641 mutant can be incorporated into the PRC2 complex, and it was initially reported to be a loss-of-function (LOF) mutation (Morin et al., 2010). Subsequent work however demonstrated a gain-of-function (GOF) for the mutant, whereby the mutant EZH2 has minimal activity for H3K27me⁰ and H3K27me¹, but has enhanced catalytic efficiency for the H3K27me² to H3K27me³ conversion. The fact that all the Y641 mutations are heterozygous implies that the malignant phenotype of disease requires the coordinated activities of the wild-type (WT) EZH2, to monomethylate H3K27, and the mutant EZH2, for increased conversion of H3K27 to the trimethylated form (Sneeringer et al., 2010; Yap et al., 2011). EZH2 A687V works similarly to the Y641 mutations (Majer et al., 2012). In contrast, EZH2 A677G has equal affinity for all three methylation substrates (H3K27me⁰, H3K27me¹, and H3K27me²) (Majer et al., 2012; McCabe et al., 2012; Wu et al., 2012b).

In lymphoma, the end result of all documented mutations is elevated global H3K27 trimethylation, constitutive repression of genes required for B-cell differentiation and an expansion of B cells at the germinal center stage that can ultimately lead to malignancy (Beguelin et al., 2013) (Fig. 2). The recurrent mutations in EZH2 make it an attractive therapeutic target in B-cell malignancies. Indeed, small molecule inhibitors of EZH2 function (EZH2i) have been developed that show potent inhibition of DLBCL-cell line proliferation *in vitro*. The clinical targeting of EZH2 in DLBCL and other malignancies is discussed in more detail in Section 7.

Paradoxically, evidence in leukemia suggests that it is the loss of EZH2 that contributes to tumor development. In T-cell acute lymphoblastic leukemia (T-ALL), myeloproliferative disorders and myeloid malignancies, a range of missense, nonsense and frameshift mutations of *EZH2* occur (Ernst et al., 2010; Nikoloski et al., 2010; Ntziachristos et al., 2012). These lesions can be heterozygous or homozygous, are found throughout the gene body, and generally are predicted to ablate HMT activity via truncation of the SET domain. Loss of EZH2 potentiates oncogenic NOTCH1 and RUNX1 signaling in T-ALL and myelodysplastic syndromes (MDS), respectively (Ntziachristos et al., 2012; Sashida et al., 2014). Loss of EZH2 is an indicator of poor prognosis in MDS (Ernst et al., 2010; Nikoloski et al., 2010), but the same association with *de novo* AML cannot be drawn, as EZH2 mutations remain comparatively rare in this setting (Wang et al., 2013). While MDS may often convert to AML, this does not to be the case for MDS associated with EZH2 loss, suggesting that such patients succumb to progressive pancytopenia rather than leukemic progression.

In contrast to the wealth of data describing EZH2 mutations in a hematopoietic setting, the role of EZH2 mutations in solid malignancies remains relatively uncharacterized. EZH2 Y641 mutations have recently been identified in roughly 2% of human melanomas (Hodis et al., 2012). Ectopic expression of EZH2 GOF mutations in a melanoma cell line resulted in increased H3K27me³ and dramatic changes in 3D culture morphology *in vitro* and larger tumor size *in vivo* (Barsotti et al., 2015). Sequencing of head and neck squamous cell carcinoma patient's tumors revealed the presence inactivating mutations in EZH2 (Stransky et al., 2011), although many other studies suggest that EZH2 acts as an oncogene in HNSCC cell lines (Gannon et al., 2013).

The fact that loss- and gain-of function EZH2 mutations occur in cancer implies binary, tissue-specific roles for the protein as both oncogene and tumor suppressor and highlights the importance of the balance of this histone mark for cell homeostasis.

3.3 Mutations in Polycomb-group associated proteins

Efficient H3K27 methylation requires the cooperation of several PRC2 core components in addition to EZH2, specifically SUZ12 and EED. Knockout of either of these genes in ES cells results in severe global reduction of H3K27me³ (Pasini et al., 2007). SUZ12 and EED are found mutated in several cancers, like nerve sheath tumors (Lee et al., 2014), myeloproliferative neoplasms (MPN) (Brecqueville et al., 2011) and early T-cell T-ALL (Zhang et al., 2012). In some of them decreased H3K27me³ was observed. The PRC2 complex recognizes the product of its own catalysis, H3K27me^{2/3}, through its EED subunit, leading to a stimulation of its methyltransferase activity (Xu et al., 2010). Therefore, as EED serves as a reader for H3K27me^{2/3}, we consider it in the following section (Section 4).

JARID2, a more recently identified PRC2-associated protein, is important for recruiting both PRC1 and PRC2 to promoters (Pasini et al., 2010a). However, its role in directly regulating H3K27 methylation remains uncertain. JARID2 is not required for maintaining global H3K27 methylation, although it does participate in maintaining this mark at selected promoters. In T-cell acute lymphoblastic leukemia (T-ALL) and MDS, rare missense mutations in JARID2 were found (Score et al., 2012; Simon et al., 2012), but were not predicted to be inactivating, and therefore the relevance of these mutations in H3K27me³ patterning is unclear.

Mimicking the loss of EZH2 in myelodysplastic syndromes (MDS), MPN and myeloid malignancies, recurrent somatic mutations and deletions of *ASXL1*, which encodes another PRC2-associated factor (Abdel-Wahab et al., 2012), result in loss of PRC2-mediated H3K27me³. As a result, many genes are aberrantly activated, including the *HOXA* cluster, contributing to myeloid transformation and inducement of an MDS-like disease in mice (Abdel-Wahab et al., 2012; Inoue et al., 2013).

4. Alterations in H3K27me “Readers”

The chromodomain of “reader” proteins recognizes methylation marks on histones. With the help of WD40 domains that recruit effector molecules, chromodomains determine the propagation and maintenance of a silent chromatin conformation. Aberrations in chromodomain proteins are commonly found in cancer, and lead to misinterpretation of the chromatin state (Fig. 3).

The chromodomain protein CDYL binds H3K27me³ and recruits EZH2, bridging the PRC2 complex to chromatin. CDYL's interaction with H3K27me³ enhances PRC2 methyltransferase activity and thus, creates a positive feedback loop that helps to maintain chromatin in a silent state (Zhang et al., 2011a). CDYL loss of heterozygosity is found in cervical cancer and is associated with poor prognosis. The decreased expression in CDYL was shown to de-repress the proto-oncogene *NTRK3* and to lead to oncogenic transformation *in vitro* (Mulligan et al., 2008). Likewise, the chromodomain of CDYL, and

its homologue CDYL2, exhibit missense and nonsense mutations that could impact their ability to recognize methylation marks on chromatin (Cbioportal for Cancer Genomics, 2016).

CBX (Chromobox Homolog) proteins are responsible for the recruitment of the PRC1 complex at specific loci by recognizing H3K27me marks. PRC1 will further create a silent chromatin conformation by methylating H3K9. In many cancer types, loss of CBX7 expression is associated with invasiveness and epithelial to mesenchymal transition (EMT) (Federico et al., 2009; Forzati et al., 2012; Karamitopoulou et al., 2010; Pallante et al., 2014). However, CBX7 was also shown to act as an oncogene. Overexpression of CBX7 in hematopoietic stem progenitor cells enhances self-renewal and induces leukemia, an effect that requires a functional chromodomain (Klauke et al., 2013).

Although EED does not possess a chromodomain, some residues in its WD40 domain form a pocket interacting with H3K27me (Margueron et al., 2009). One rare mutation in the WD40 motif was shown to inhibit its interaction with H3K27me³, and global H3K27me³ is severely impaired in the cells overexpressing this mutant (Ueda et al., 2012). The most frequent mutations in EED seem to disrupt the formation of PRC2 and abrogate the H3K27 methyltransferase activity of PRC2 (Khan et al., 2013; Score et al., 2012; Denisenko et al., 1998).

Methylation of H3K27 can also inhibit binding of effector molecules. AF10 and AF17 recognize unmodified H3K27 through a PHD finger-Zn knuckle-PHD finger (PZP) module and this is required for DOT1L mediated H3K79 methylation and gene expression (Chen et al., 2015). Of note, *AF10* and *AF17* are involved in recurrent chromosomal translocations with MLL in leukemia, making these tumors addicted to DOT1L (Chen et al., 2015; Deshpande et al., 2014). PZP-mediated interaction between AF10 and H3K27 plays a critical role in regulating both the expression of DOT1L-target genes and the proliferation of DOT1L addicted leukemic cells. However, the binding to H3K27 by AF10-containing oncofusions has not been investigated.

Aberrations in readers of H3K27me³ can therefore contribute to outcomes as dramatic as the ones caused by deregulation of chromatin writers and erasers. However, more work is needed to better characterize the mechanisms of action of these mutations.

5. Alterations in H3K27 “Erasers”

The H3K27me³ mark may be removed by the UTX (KDM6A) and JMJD3 (KDM6B) proteins, both containing the jumonji domains and using an oxidation based mechanism of removing the methyl residues. In both hematological malignancies and solid tumors, disruption of H3K27 demethylase activity via mutation of *UTX* is far more common in cancer patients than disruption due to *JMJD3* mutations, (Gui et al., 2011; Statham et al., 2012; Van der Meulen et al., 2015; van Haaften et al., 2009). However, a subset of glioblastoma multiforme (GBM) patients does have somatic mutations of *JMJD3*, or down regulation of its mRNA expression secondary to DNA hypermethylation (Ene et al., 2012; Monti et al., 2012). Furthermore, cases of follicular lymphoma that have transformed to

large cell lymphoma may suffer from inactivating mutations of JMJD3, which are mutually exclusive from KMT2D/MLL2 mutations (Carlotti et al., 2015).

Several recent whole-genome sequencing studies of bladder cancer patients revealed that *UTX* was inactivated in about 25% cases (Guo et al., 2013; Nickerson et al., 2014; Waddell et al., 2015). Exome sequencing also revealed a significant frequency of *UTX* mutations in pancreatic tumors and gene expression profiling revealed that the mutations were enriched in a subtype in which p53 mutations were also enriched, and that subtype had a poorer prognosis (Bailey et al., 2016). *UTX* is located on the X-chromosome and the mutations are homozygous in females and hemizygous in males (van Haaften et al., 2009). However, in males, loss of *UTX* was accompanied by loss of *UTX*'s paralogue located on the Y chromosome, *UTY* (van Haaften et al., 2009).

As with EZH2, *UTX* behaves paradoxically, and both tumor suppressor and oncogenic effects are seen with alterations in *UTX*. In some models, loss of *UTX* enhances the proliferation of cancer cells (Ho et al., 2013; Van der Meulen et al., 2015; van Haaften et al., 2009) and in others, *UTX* overexpression promotes proliferation (Kim et al., 2014). The effect is not tissue specific, as in breast cancer *UTX* expression is associated with invasion and clinically, high levels of *UTX* are associated with poor prognosis in patients (Kim et al., 2014). However, *UTX* expression also plays an important role in breast tumor suppression by silencing transcription factors important in the epithelial-mesenchymal transition (EMT) (Choi et al., 2015). In these cases, the confusion concerning *UTX*'s role may be attributed to its partner at the time (KMT2D versus LSD1/HDAC1) or whether the activity is H3K27me-demethylase dependent or independent.

Finally, the mutual exclusivity of *UTX* mutations with other genes might provide clues as to *UTX*'s place in essential biological pathways in different tissues. For example, inactivating mutations of *UTX* and the H3K4 methyltransferase, KMT2D (Nickerson et al., 2014) in bladder cancer are mutually exclusive. Expression of some *UTX*-modulated genes are also regulated by the H3K4 methyltransferase KMT2D, whose C-terminal region interacts with *UTX* (Kim et al., 2014). The mutual exclusivity seen with these two proteins suggests a functional redundancy and that they cooperatively regulate gene expression programs. In multiple myeloma (MM), *UTX* null samples are all negative for the MMSET-activating t(4;14) translocation (van Haaften et al., 2009). Together, these findings indicate that balances in H3K27/H3K4 and H3K27/H3K36 methylation are critical for cell homeostasis.

6. Cross Talk with Other Chromatin Regulators

The discovery that chromatin modifiers were heavily mutated in all cancers paved the way for studies attempting to understand the interplay between the epigenetic modifiers and histone modifications affected by these mutations. Many histone-modifying enzymes involved in the pathogenesis of cancer indirectly impact on H3K27me level genome wide and at specific locus.

6.1 MMSET overexpression

In multiple myeloma (MM) harboring the t(4;14) translocation, the H3K36 specific HMT, MMSET/NSD2 is fused to the immunoglobulin heavy chain locus, leading to MMSET overexpression (Keats et al., 2005). This genetic aberration is associated with poor prognosis (Keats et al., 2003), and the increase in MMSET expression was shown to drive cell proliferation, clonogenicity, and invasion of MM cells (Brito et al., 2009; Ezponda et al., 2013; Kuo et al., 2011; Lauring et al., 2008; Martinez-Garcia et al., 2011). Overexpression of MMSET causes a genome-wide increase in H3K36me², concomitant with a global reduction in H3K27me³ (Fig. 4A) (Ezponda et al., 2013). However, some specific loci were shielded from the effects of the overexpressed MMSET and exhibited high H3K27me³ levels with increased EZH2 binding, leading to repression of transcription of such loci (Popovic et al., 2012). Thus, MMSET overexpression simultaneously leads to global decreases, but local increases in H3K27me³, suggesting that a few genes are mediating the oncogenic effect. Consistent with this hypothesis, cells expressing high levels of MMSET are more sensitive to molecules inhibiting EZH2 (Popovic et al., 2012), and this correlates with activation of the specific-loci repressed genes. Therefore, H3K27me³ mediated repression of some genes is relevant to the molecular pathogenesis of this form of malignancy.

A similar interplay between H3K36me and H3K27me was observed in other malignancies. In pediatric B-ALL and mantle cell lymphoma, a recurrent mutation within the catalytic SET domain of MMSET mimics the effects of MMSET overexpression on cell growth and was similarly able to induce a global increase in H3K36me² concomitantly with a genome-wide decrease in H3K27me³ (Bea et al., 2013; Jaffe et al., 2013; Oyer et al., 2014). Furthermore, the t(5;11) translocation found in AML, leading to fusion of the MMSET homologue *NSD1*, and *NUP98* was shown to cause local increases in H3K36me² simultaneously with loss of EZH2 and H3K27me³ at the *HOXA* locus. This effect was associated with the transforming properties of NUP98-NSD1 and required NSD1 HMT activity (Wang et al., 2007).

EZH2 and MMSET expression is tightly correlated in cancers and EZH2 function was suggested to be required for MMSET activity. In fact, EZH2 regulates MMSET expression by attenuating the expression microRNAs, such as miR-203, miR-26a and miR-31 and indirectly cause increase H3K36me². Furthermore, EZH2 neoplastic properties were shown to require MMSET expression (Asangani et al., 2013; Ezponda et al., 2013).

6.2 SWI/SNF chromatin remodeling complex inactivation

The SWI/SNF chromatin-remodeling complex antagonize the PRC2 complex activity at its target genes (Kia et al., 2008). SWI/SNF is frequently inactivated in cancer, most notably in rhabdoid tumors, where almost all cases present with a mutation in one of the SWI/SNF complex subunits. It was hypothesized that nucleosome density, which is regulated by SWI/SNF, affects PRC2 activity, and deposition of methylation on H3K27 (Yuan et al., 2012). For instance, loss of SNF5, also found in T-cell lymphoma, decreases polycomb protein displacement at specific loci leading to an increase in EZH2 and H3K27me³ (Fig. 4B) (Wilson et al., 2010). This causes the repression of genes critical for differentiation and

tumor suppression such as *HOXB1* (Wilson et al., 2010) and *CDKN2A* (Kia et al., 2008). *ARID1A* encodes a component of the SWI/SNF chromatin-remodeling complex that is also frequently mutated in many tumors (Lawrence et al., 2014), including ovarian clear cell carcinoma (OCCC). Inhibition of EZH2 reduced overall H3K27me³ levels in both wild-type and *ARID1A*-mutated OCCC cells, while selectively suppressing proliferation of *ARID1A*-mutated cell lines, suggesting that H3K27me³ at specific loci drives tumorigenesis. Indeed, EZH2 inhibitor (EZH2i) selectivity towards ARID1A mutated tumors was associated with increased PIK3IP1 expression and decreased AKT activation (Bitler et al., 2015). Overall, these studies suggest that SWI/SNF inactivated tumors depend on PRC2-mediated H3K27me³ at specific genes. However, non-SET activity may be also important, as one study demonstrated that SWI/SNF-mutated cancer cells were dependent on both catalytic and non-catalytic activity of EZH2 and this was abolished by the presence of mutations in RAS pathway (Kim et al., 2015).

6.3 Histone acetylation

Lysine K27 on H3 can also be targeted by acetylation, and this modification is mutually exclusive with methylation (Tie et al., 2009) (Pasini et al., 2010b). The acetyltransferases CBP/p300 catalyze this reaction and consequently, antagonize the methylation and repression of transcription by PRC2 (Pasini et al., 2010b). Histone deacetylases (HDACs) catalyze the reverse reaction. HDAC1 and HDAC3 interact with PRC2 complex (van der Vlag and Otte, 1999) and promote H3K27me by removing acetylation on H3K27, thus making lysine 27 on H3 free for subsequent methylation by EZH2. Indeed, ablation or inhibition of PRC2 promotes H3K27ac genome wide and also at specific loci such as proximal promoter and enhancers (Pasini et al., 2010b; Xu et al., 2015).

CBP/p300 are inactivated in about 30% cases of DLBCL (Pasqualucci et al., 2011) and in 18% of cases of acute lymphoblastic leukemia (ALL), raising the possibility that PRC2-regulated genes are maintained in a repressed state in these cancers. However, no studies have examined the effect of histone acetyltransferase (HAT) inactivation on the regulation of PRC2 target genes. HDAC expression is increased in various cancers (Adams et al., 2010; Choi et al., 2001; Marquard et al., 2008; Wada et al., 2009; Zhang et al., 2005), and inhibitors of HDAC activity have been shown to decrease global H3K27me³ (Fiskus et al., 2006). HDAC inhibitors also markedly attenuated EZH2-mediated invasion of cancer cell lines (Cao et al., 2008). Moreover, a correlation was established between HDAC1 and H3K27me³ occupancy genome-wide and the correlation was more significant at promoter regions (Song et al., 2015). Overall, these data show that HDAC/HAT activity is determinant in establishing H3K27me³ marks throughout the genome.

6.4 WT1 mutations

Wilms' tumor 1 (WT1) is a transcription factor harboring inactivating mutations within its DNA-binding zinc finger domain in about 10% of AML cases. WT1 interacts with the enzyme mediating DNA demethylation, TET2, to regulate its target genes (Wang et al., 2015). Consequently, the loss of *WT1* was associated with global increase in DNA methylation (Rampal et al., 2014), and hypermethylated genes were shown to strongly overlap with genes targeted by PRC2/H3K27me, leading to aberrant repression of H3K27me

marked gene in WT1 mutant tumors (Sinha et al., 2015). Moreover, inhibition of EZH2 is able to induce a better myeloid differentiation response in WT1 mutant than in WT1 wild-type primary acute promyelocytic leukemia.

6.5 MLL oncofusions

The H3K4-specific HMT, mixed-lineage leukemia (MLL1/KMT2A), is frequently rearranged with a large number of varied genes in AML and this is associated with poor prognosis (Behm et al., 1996). All fusion oncoproteins involving MLL have lost HMT activity, but still recruit coactivators of transcription, epigenetic modifiers, including DOT1L, and together aberrantly induce transcription of oncogenes like *MYC*, *MEIS1* and *HOX*, and also *EZH2*. Even though the genome-wide pattern of H3K27me in MLL-rearranged leukemias has not yet been investigated, these tumors have been shown to depend on PRC2 activity to promote growth and self-renewal of leukemia (Neff et al., 2012; Shi et al., 2013). Furthermore, they exhibit sensitivity towards inhibition of both EZH1 and EZH2 enzymes, and this is associated with decreased H3K27me³ at enhancers and promoters of specific genes related to development and differentiation (Xu et al., 2015). Inhibition of PRC2 only minimally affects the expression of well-established direct target genes of MLL-AF9, which also harbor minimal levels of H3K27 methylation at their promoters (Shi et al., 2013). This is consistent with the fact that methylation of H3K27 inhibits recruitment of the MLL-fusion interacting partner, AF10 (Chen et al., 2015). Together these results suggest that MLL-fusions and PRC2 target distinct site in the genome and act complementary in promoting tumorigenesis.

6.6 H2A monoubiquitination

H3K27me³ can direct the recruitment of the PRC1 complex to chromatin. PRC1 possesses E3 ubiquitin ligase activity towards H2AK119. Monoubiquitinated H2AK119 (H2AK119ub¹) can in turn recruit PRC2 complex to maintain chromatin in a repressed conformation. H2AK119ub¹ increases the affinity of the PRC2 complex to nucleosomes, and is sufficient to drive H3K27me³ deposition, although the mechanism by which this occurs is still undefined (Blackledge et al., 2014; Kalb et al., 2014). Inversely, UTX-mediated demethylation of H3K27 reduces the recruitment of the canonical PRC1 complex and consequently, will decrease H2A monoubiquitination (Lee et al., 2007).

In cancer, perturbations in this relationship can favor the increases in H3K27me³ (Figure 4C). The ubiquitin ligase TRIM37 catalyzes H2AK119ub¹. This gene is amplified in about 40% of breast cancers and this is associated with decreased survival of estrogen-receptor positive patients. TRIM37-targeted gene promoters were shown to recruit PRC2, and exhibited high levels of H3K27me³ and transcriptional silencing. Furthermore, knock-down of *TRIM37* reversed these events (Bhatnagar et al., 2014). The deubiquitinating (DUB) enzyme targeting H2AK119ub¹, BRCA1 associated protein-1 (BAP-1), is a tumor suppressor inactivated in a variety of malignancies (Dey et al., 2012; Harbour et al., 2010; Pena-Llopis et al., 2012; Testa et al., 2011). BAP-1 loss was shown to transform cells in an EZH2-dependent manner, leading to a global increase in H3K27me³ (LaFave et al., 2015). Interestingly, BAP-1 mutations co-occur with UTX mutations in bladder cancer, which suggest that these factors may have complementary functions in driving tumorigenesis

(Nickerson et al., 2014). These data suggest that deregulation of enzymes mediating H2AK119ub¹ in cancer may lead to outcomes similar to EZH2 overexpression.

Conversely, the deregulation of enzymes regulating H2AK119ub¹ levels can tip the scale against H3K27 methylation (Fig. 4D). The PRC2 component, ASXL1, activates BAP-1 by increasing its affinity for ubiquitin (Sahtoe et al., 2016). *ASXL1* mutations found in AML aberrantly enhance the DUB activity of BAP-1 and thus, deplete the levels of H3K27me³ (Balasubramani et al., 2015). Such tumors mimic the effect of EZH2 inactivating mutations.

7. Targeting Deregulated H3K27me

The convergence of many genetic aberrations in the deregulation of H3K27me in cancer has led to the development of inhibitors of the PRC2 complex catalytic core, EZH2. The first one identified, Deazaneplanocin A (DZNep)(Glazer et al., 1986), was shown to reactivate PRC2 target genes, lead to degradation of EZH2 and demonstrated antitumor activity (Deb et al., 2014; Tan et al., 2007). DZNep inhibits SAH-hydrolase, a cofactor needed for the activity of many HMTs, therefore it has a poor specificity toward EZH2. Hence, highly selective molecules directly targeting EZH2 were developed. These inhibitors directly compete for interaction with the methyl-group donor *S*-adenosyl methionine (SAM). They decrease levels of H3K27me^{2/3}, and reactivate transcription of PRC2/H3K27me³ repressed genes involved in cell cycle regulation and differentiation (Beguelin et al., 2013; Kim et al., 2013b; Knutson et al., 2014; McCabe et al., 2012). Some studies have shown that these inhibitors have selective antitumor effects toward lymphoma cells harboring activating mutations in EZH2 (Beguelin et al., 2013). On the other hand, in GCB type DLBCL, these inhibitors target equally WT and mutated EZH2 cells (Beguelin et al., 2013; McCabe et al., 2012). Elevated levels of H3K27me³ and EZH2 expression do not correlate with the GOF mutations in EZH2 (Zhou et al., 2015), and cell sensitivity to EZH2 inhibitors does not always correlate with globally high levels of H3K27me³. For instance, the overexpression of MMSET sensitizes MM cells to EZH2 inhibitors, even though genome-wide H3K27me³ levels decrease. It appears that cancer cells showing increased H3K27me at tumor suppressing genes are more sensitive to EZH2i, as in the case of rhabdoid tumors lacking the SNF5 protein (Knutson et al., 2013).

Furthermore, loss of UTX in MM and T-ALL has been associated with increases in H3K27me³ at specific loci, and correlates with increased sensitivity to EZH2i (Ezponda et al., 2014; Van der Meulen et al., 2015). Many cancers are negative for UTX (Ibragimova et al., 2013; Nickerson et al., 2014), and therefore may benefit from therapy targeting EZH2.

Phase 1/2 clinical trials have been initiated using the EZH2 inhibitor tazemetostat (Epizyme), in patients with advanced solid tumors or with relapsed or refractory B-cell lymphoma. Tazemetostat demonstrates a favorable safety profile and tolerability. The overall response rate in B-cell lymphoma has reached 60%, where almost all responders were wild-type for EZH2 (Nickerson et al., 2014; Ribrag et al., 2015) suggesting that EZH2 hyperactivity can be mimicked by other alterations. Interestingly, clinical activity was observed in patients with *INI1*-negative and *SMARCA4*-negative tumors, both leading to an

inactive SWI/SNF complex, giving hope for patient with such tumors, which represent 20% of all cancers.

The status of chromatin regulators deregulated in cancer that cross-talk with PRC2 should be taken into consideration while designing future clinical trials using drugs targeting H3K27 methylation (Fig. 5). It is yet to be proven whether the status of commonly deregulated chromatin enzymes could serve as biomarkers of response to EZH2i, such as *TRIM37* amplification in breast cancer, MMSET overexpression, the presence of MLL oncofusions, and *WT1* inactivated tumors.

With any anti-tumor agent, there is the possibility that resistance will occur, and some studies have focused on modeling this phenomenon. In an *in vitro* model of acquired resistance to EZH2i, secondary mutations were identified in wild-type and mutant EZH2 alleles (Gibaja et al., 2016). The presence of Ras-pathway mutations, along with mutations in SWI/SNF correlated with resistance to EZH2 inhibition (Baude et al., 2014; De Raedt et al., 2011). These studies underlie the need to develop other EZH2 or PRC2 inhibitors, and to design combination strategies.

EZH2 oncogenic activity has been attributed to non-enzymatic functions (Kim et al., 2015), and therefore inhibitors of its catalytic site may not fully suppress its tumor promoting activity. Since the interaction of EED with EZH2 is essential for activity of the PRC2 complex (Cao et al., 2002; Chamberlain et al., 2008; Denisenko et al., 1998; Han et al., 2007), a small molecule was developed to target the alpha-helical domain of EZH2 that binds EED and thus, disrupts this interaction (Kim et al., 2013b). This molecule, known as stabilized alpha-helix of EZH2 (SAH-EZH2) is specific to both EZH1 and 2, and leads to global dose dependent decreases in H3K27me³. SAH-EZH2 also leads to degradation of EZH2, eliminating both enzymatic and structural functions of EZH2. Notably, SAH-EZH2 is effective in some SWI/SNF-mutant cancers cell lines that rely on non-enzymatic functions of EZH2 and are resistant to catalytic EZH2 inhibitors. Moreover, an antiproliferative effect and induction of monocytic differentiation was observed upon treatment in leukemia cells harboring the AF9-MLL translocation or EZH2 activating mutations. SAH-EZH2 exhibits mechanistic differences with the first generation of catalytic inhibitors developed and therefore, the two synergize together to repress leukemic cells growth.

Approaches in epigenetic therapy have also recently focused on the development of molecules that block the “readers” of methyl mark, for instance the methyl mark on histone H4 lysine 20 (H4K20), which is involved in the DNA damage response, DNA replication and mitotic condensation (James et al., 2013; Ma et al., 2014). Likewise, compounds targeting H3K27me “readers” may be developed to treat cancers showing a gain of function of these factors. Recently, an inhibitor of CBX7 was identified and demonstrated interaction with key residues in the methyl-lysine binding pocket of CBX7 chromodomain (Ren et al., 2015). This molecule disrupts the interaction of CBX7 with H3K27me³, and efficiently decreases its occupancy on *INK4A/ARF* locus in prostate cancer cells. However, no antitumor effect has been reported yet, and this drug exhibit poor specificity because it also targets several other CBX proteins.

Conversely, the PRC2 complex is inactivated in myeloid malignancies, for example T-ALL, and in peripheral nerve sheath tumors, where PRC2 inactivation correlates with loss of H3K27me³ and activation of specific pathways. Elevated expression of the oncogenic transcription factor TAL-1 defines a major sub-group of T-ALL that has a specific expression signature (Ferrando et al., 2002). TAL-1 interacts with UTX to activate its target genes in these tumors (Benyoucef et al., 2016). An inhibitor of Jumonji H3K27 demethylase (Kruidenier et al., 2012) demonstrated anti-tumor activity in such tumors, which correlated with global increase in H3K27me and repression of the gene expression program specific to TAL-1 positive tumors (Ntziachristos et al., 2014). Jumonji H3K27 demethylase/UTX inhibitors may benefit patients presenting PRC2 inactivation.

In the same way, the H3K27M mutation in pediatric gliomas leads to decrease in H3K27me and these tumors demonstrate better susceptibility to inhibitors of the Jmjd3 demethylase (Hashizume et al., 2014). Developing an epigenetic targeting strategy for tumors bearing K27M-H3.3 mutations seems complex, as these aberrations lead to both global decreases and local increases in H3K27me^{2/3}. Funato *et al.*, who developed the pediatric glioma model discussed in Section 2, performed a chemical screen on their model using a commercially available small-molecule library of compounds that target epigenetic regulators (Funato et al., 2014). Their top hit was the menin inhibitor, MI-2. Menin is a component of MLL complexes, where it serves as a transcriptional cofactor. The Menin gene (*MEN1*) acts as a tumor-suppressor in endocrine cancers (Matkar et al., 2013) but conversely, is highly oncogenic in MLL rearranged leukemias (Yokoyama et al., 2005). Silencing of *MEN1* decreased proliferation specifically in H3.3K27M mutant cell lines and similarly, MI-2 also showed anti-neoplastic effects on cells derived from a patient sample.

Other therapeutic approaches may require the identification of biologically relevant transcription factors that normally compete with the PRC2 complex (such as NOTCH1 in T-ALL), or genes where H3K27me deregulation contributes to oncogenic transformation. The PRC2 complex has shown opposing functions in cancer, therefore an appropriate use of EZH2/UTX inhibitors will need a careful consideration of the biological context, and certainly a better understanding of epigenetics regulation in different cancers. A more detailed mapping of these altered epigenomes is critical to ascertain which cancer subtypes could benefit from these drugs.

8. Conclusions and Future Perspectives

Alterations in levels and distribution of H3K27 methylation is a hallmark of transformation in many cancers and has been demonstrated to be a suitable target for anti-neoplastic therapy both *in vitro* and *in vivo*. Various epigenetic pathways work in concert to alter H3K27me³ in cancer, thereby providing numerous approaches for targeting. The genes most affected by deregulated H3K27me are cancer-subtype specific drivers of oncogenesis. Identification of the other transcription factors and epigenetic regulators also present at these affected genes may provide crucial insights for the design of new combination strategies, and for the identification of biomarkers of response to those agents already being tested in the clinic, such as EZH2i. Many questions about how perturbations in H3K27me patterning leads to tumorigenesis remain, mostly due to conflicting reports of differences in the roles of the

writers and readers of H3K27 methylation in different tissues, and even in different developmental stages. This highlights the need for more basic *in vitro* studies, mouse models and careful validation of the drugs targeting this pathway in order to gain a more complete picture of the importance of this epigenetic mark.

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Fig. 1.

PRC2 is composed of the core subunits, EZH2, SUZ12, RBBP4 and EED, and is associated with accessory factors, JARID2, AEBP2 and the Polycomb-like proteins (PCLs). PRC2 catalyzes the methylation of histone H3 lysine 27 (H3K27me). PRC1 recognizes, or “reads,” the tri-methylation of the H3K27 residue (H3K27me³), a repressive mark, and in turn adds an ubiquitin to histone H2A lysine 119 (H2AK119ub¹). This causes the recruitment of DNA methyltransferases, which will add methyl groups to DNA at CpG islands, and in this way, the repression of chromatin is maintained and propagated.

Fig. 2. EZH2 is overexpressed and mutated in several cancers. In natural killer/T-cell lymphoma (NKTL), prostate cancer and glioblastoma, overexpression of EZH2 causes it to have activities beyond H3K27 methylation (left panel). In diffuse large B-cell lymphoma (DLBCL), common mutations in EZH2 increases the enzyme's efficiency, resulting in an increase in H3K27me³ levels. These mutations are heterozygous and work with the wild-type EZH2 (right panel).

H3K27me³ reader mutations

Fig. 3.

Mutations in readers of H3K27me³ impede recruitment of both PRC2 and PRC1 complexes, which prevents the maintenance and/or propagation of a silent chromatin state.

Fig. 4.

A. Overexpression of MMSET induces global increases in H3K36me², which prevents its recognition by PRC2. Therefore, PRC2 accumulates at specific loci, where it binds chromatin and represses transcription. **B.** Through modulation of nucleosome density, the chromatin remodeling complex SWI/SNF prevents methylation of H3K27 by PRC2. The many mutations in SWI/SNF subunits inactivate its activity and lead to increased levels of H3K27me³. **C.** H2A ubiquitination increases PRC2 affinity for nucleosomes, which leads to increased H3K27me³. The inactivation of the DUB enzyme, BAP-1, and overexpression of the ubiquitinase TRIM37 favors global increases in H3K27me³ and silencing of chromatin. **D.** On the other hand, mutated ASXL1 enhances the DUB activity of BAP-1, leading to decreased levels of H3K27me³ with gene activation.

Potential markers for response to inhibitors of H3K27 methylation

Potential markers for response to inhibitors of H3K27 demethylation

Fig. 5.

Many factors deregulated in cancer converge to increase or decrease H3K27me³ in cancer, and they may represent new therapeutic targets, or biomarkers of response to existing agents.