CellPress
REVIEWS

Review

The Unexpected Noncatalytic Roles of Histone Modifiers in Development and Disease

Yann Aubert,[1,2,5,6] Shaun Egolf,[1,2,5,6] and Brian C. Capell[1,2,3,4,5,6,*,@]

Epigenetic regulation is critical for the precise control of cellular fate and developmental programs. Disruption of epigenetic information is increasingly appreciated as a potential driving mechanism in both developmental disorders as well as ubiquitous diseases such as cancer. Consistent with this, mutations in histone modifying enzymes are amongst the most frequent events in all of human cancer. While early studies have focused on the canonical enzymatic functions involved in catalyzing modifications to histones, more recent studies have uncovered a new layer of critical nonenzymatic roles in transcriptional regulation for these proteins. Here, we provide an overview of these surprising, yet exciting, noncanonical, noncatalytic roles, and highlight how these revelations may have important implications for understanding disease and the future of epigenome-targeting therapies.

Highlights

An abundance of recent findings suggest that, surprisingly, numerous epigenetic regulators also possess an array of critical noncatalytic roles in addition to their canonical histone modifying enzymatic activities.

These functions range from the recruitment of other epigenetic modifiers and critical transcription factors to unexpected cytoplasmic functions and roles in three-dimensional genome organization, together offering extensive new insights into epigenome function and gene regulation.

These discoveries reveal how the aberrant function of these epigenetic modifiers leads to profoundly altered transcriptional landscapes, ultimately disrupting developmental programs or driving carcinogenesis.

Collectively, these mechanistic insights are providing exciting rationale for the testing of novel epigenome-targeting therapies in order to harness the tremendous potential of epigenetic therapy for human disease.

Histone Modifiers in the Establishment and Maintenance of Cellular Fate and Identity

Dynamic **epigenetic** (see Glossary) regulation is dictated by the intricate organization of each cell's genome into chromatin. The fundamental unit of chromatin is the **nucleosome**, and the compaction of chromatin has a profound influence on gene expression levels, with more open regions (euchromatin) tending to be transcriptionally active, while more compacted regions (heterochromatin) tend to be transcriptionally repressed [1]. Histone post-translational modifications are able to orchestrate these processes through their direct regulation of chromatin organization, as at least 60 different covalent chemical modifications on the histone amino (N) and carboxy (C) terminal tails and within the globular domains have been described [2]. These modifications are deposited (written), removed (erased), and interpreted (read) by numerous well-characterized enzymes with specific catalytic functions, highlighting the promise for harnessing the ability of small molecules to affect epigenetic function and, therefore, ultimately treat disease (Box 1) [3].

Through these essential roles in gene regulation, epigenetic processes are critical for proper organismal development, cellular differentiation, and the reprogramming of cell fate. Transcription factors and chromatin modifiers interact to establish the epigenomic landscape, which both enables and stabilizes cell type-specific gene expression, while simultaneously performing stable repression of alternative cell fates [4]. Consequently, chromatin profiles alone in differentiated cells are often sufficient for identifying cell type, particularly at the key regulatory nodes of gene **enhancers** [5,6]. In fact, epigenome profiling of the chromatin landscape in tumors of unknown cell of origin can provide a more accurate determination of the tissue of origin than sequencing of the tumor itself [7]. Through chromatin organization, cell type-specific genes will be turned on, while genes leading towards alternative cell fates will be repressed, forming an epigenetic barrier that prevents cells from returning to the stem cell state. Beyond the period of embryonic development, epigenetic mechanisms continue to coordinate the balance between adult stem cell renewal and differentiation [8]. This epigenetic balance is essential, as impaired self-renewal can

[1]Penn Epigenetics Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[2]Department of Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[3]Department of Genetics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[4]Abramson Cancer Center, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA

Box 1. Targeting the Epigenome

The pervasive epigenetic dysregulation observed in cancer, combined with the directly targetable nature of epigenetic enzymes, have made epigenome-targeting therapies an exceptionally promising avenue of discovery. In cancers where overexpression or gain-of-function mutations have been observed, inhibitors have been developed to directly target various histone modifiers such as EZH2, DOT1L, and KDM1A (LSD1) [3]. These compounds have been advanced into human clinical trials [74]. In contrast, strategies to treat loss-of-function or expression in cancer have focused on synthetic lethality approaches [75]. Importantly, these inhibitors have demonstrated efficacy as adjunctive therapies in overcoming drug resistance in a variety of contexts, as well as enhancing the efficacy of immunotherapies [76]. In the majority of cases, these inhibitors have been designed to target the catalytic functions of these enzymes. While some inhibitors have been developed to directly interrupt the ability of a histone modifier to interact with a nonhistone target (i.e., inhibitors of the interaction of KMT2A and its cofactor, menin), even in these cases, the goal was to disrupt the enzymatic capabilities of the histone modifier in question. Therefore, given the recent developments in this field highlighted in this review, it is very likely that new inhibitors will emerge in the coming years to directly target noncatalytic functions of the histone modifiers. Indeed, given the emergence of new technologies for drug development such as CRISPR/Cas9-based therapeutics for epigenome editing [77], as well as targeted protein degradation systems such as proteolysis-targeting chimeras (PROTACs) [78,79] will undoubtedly lead to new approaches to epigenetic therapy.

[5]Laboratory website: https://www.med.upenn.edu/capelllab/.
[6]These authors contributed equally to this work.

*Correspondence: capellb@pennmedicine.upenn.edu (B.C. Capell).
@Twitter: @briancapell (B.C. Capell).

lead to stem cell exhaustion and aging, while excessive self-renewal may promote cancer. Consistent with this, a loss of key chromatin regulatory factors that promote cell differentiation can push cells towards a more stem cell-like phenotype, and this is a recurrent theme across human cancers [9,10].

To coordinate the establishment and maintenance of these cellular trajectories, epigenetic 'writers' and 'erasers', such as lysine methyltransferases (KMTs) and demethylases (KDMs), respectively, provide highly nuanced transcriptional regulation. For example, some of the most well-studied methylated lysines on histone H3 are associated with transcriptional activation (e.g., H3K4me3, H3K36me3, H3K79me3), transcriptional repression (e.g., H3K27me3), or DNA repeat and centromeric silencing (e.g., H3K9me3) [1]. Collectively, this establishes an impressively complex system for the fine-tuning of gene regulation. For instance, embryonic stem cells exist in a **bivalent state**, where they are marked by both the activating histone modification, H3K4me3, as well as the repressive modification, H3K27me3 [4]. From this state the genes are considered to be 'poised' and primed either for activation or for repression, depending upon their ultimate cell fate.

The majority of our understanding of these epigenetic enzymes stems from their canonical enzymatic roles in catalyzing histone modifications. Intriguingly, there has been a recent abundance of discoveries uncovering critical noncatalytic, nonenzymatic roles for these KMTs and KDMs in transcriptional regulation and cellular physiology. While these exciting findings have vastly expanded the potential of these chromatin regulators to influence cellular physiology and disease, they have also highlighted potential caveats that need to be considered in the development of epigenome-targeting therapies. Here, we highlight these findings and consider both their immediate implications as well as new questions that they raise. Given limitations of space, we focus here on the KMTs and KDMs, where the majority of these observations have been made. Readers are referred to the following reviews for recent updates regarding the other major epigenetic mechanisms of histone acetylation and DNA methylation [11–14].

All En-COMPASS-ing Regulation at Enhancers and Promoters

Methylation on histone 3 lysine 4 (H3K4) marks actively transcribed genes and their enhancers and promoters during cellular development, differentiation, and homeostasis [15]. From flies to humans, these modifications are highly conserved and catalyzed by family members of a macromolecular epigenetic machine known as **COMPASS** (complex of proteins associated with Set 1) [15] (Box 2). Investigations into COMPASS enzymes and how they may play a role in disease have provided significant rationale for uncovering the basic biological roles of H3K4

Box 2. COMPASS and H3K4 Methylation in Development and Disease

The mono-, di-, or trimethylated states of H3K4 (H3K4me1, 2, 3) have distinct localization patterns genome-wide. H3K4me1 primarily marks enhancers, whereas H3K4me2 is present at both promotors and enhancers, and H3K4me3 is enriched at gene promoters [80]. In yeast, all H3K4 methylation is mediated by the enzymatic activity of SET1 when in association with COMPASS. These functions are delegated across three separate SET-like enzymes in *Drosophila*, named Trx, Trr, and dSet1. Humans form six distinct COMPASS-like complexes containing either the H3K4 histone methyltransferase KMT2A (MLL1), KMT2B (MLL2), KMT2C (MLL3), KMT2D (MLL4), KMT2F (SETD1A), or KMT2G (SETD1B). Sequence homology and functional studies suggest conservation between KMT2A/B and Trx, KMT2C/D and Trr, and KMT2F/G and dSet1. Importantly, each set of KMT2 enzymes and its homolog play nonredundant, essential roles, including functional diversity in H3K4 methylation [15]. Supporting the critical nature of these enzymes, all of them have been shown to play critical roles during mammalian development. Indeed, individual genetic knockouts of each of these proteins leads to either embryonic or perinatal lethality in mice [17,18,81–83]. In humans, hemizygous mutations in *KMT2A* (*MLL1*) are associated with the developmental disorder Wiedemann-Steiner syndrome [84], while heterozygous loss-of-function or missense mutations in *KMT2D* or its binding partner *UTX* (*KDM6A*) are causative in the developmental disorder **Kabuki syndrome** [85,86]. Beyond development, countless studies have identified a significant link between KMT2 family members and cancer [87]. Rearrangement of *KMT2A*, commonly referred to as *MLL1*, occurs in approximately 10% of all leukemias and correlates with poor prognosis in both children and adults [88,89]. Cancer genome sequencing has identified mutations in *KMT2C* and *KMT2D* across numerous solid and liquid tumors [87]. These mutations are believed to be of functional importance during carcinogenesis, as *KMT2D* is tumor suppressive in diffuse large B cell lymphoma and pancreatic cancer [90–92], while promoting tumor proliferation in breast and prostate cancer [93–95]. While other KMT2 family members are not frequently mutated across cancer, SETD1A (KMT2F) is required for MLL-AF9 leukemia cell growth and survival and is also associated with breast cancer metastasis [43,96].

methyltransferases and how their mutations may drive disease. While many have hypothesized that these enzymes may primarily act through catalyzing H3K4 methylation to create a permissive chromatin landscape and subsequently activate gene expression, recent surprising evidence demonstrates that KMT2 proteins also possess essential noncatalytic roles (Table 1, Key Table). Many of these findings present compelling data to suggest that these noncatalytic roles may, in fact, better explain the biological importance of KMT2 proteins than their H3K4 methylation activity. For example, KMT2C and KMT2D play partially overlapping, but nonredundant functions at gene enhancers, despite having conserved enzymatic subunits. Fundamental studies have established the role of these enzymes in catalyzing H3K4 monomethylation (H3K4me1) at enhancer regions in order to prime enhancers for transcriptional activation (Box 2), and coordinate downstream gene expression changes during cell differentiation [16–20]. Following H3K4me1 enhancer priming, the addition of H3K27ac at these regions is associated with transcriptional activation [21]. Intriguingly, in addition to the well-known catalytic function of KMT2D, recent studies have now demonstrated its requirement for the recruitment of the H3K27 histone acetyltransferase p300 to enhancers [19,22]. This work suggested that the KMT2D protein, rather than KMT2D-dependent H3K4me1, was required for p300 recruitment during enhancer activation [19]. Functionally, KMT2D depletion reduced levels of H3K27ac via reduced p300 recruitment, disrupted normal gene expression programs, and, in turn, impaired both adipogenesis and differentiation of mouse embryonic stem cells (mESCs) [19,22]. Indeed, at 5537 amino acids, the particularly large size of KMT2D may serve several functional purposes in allowing it to serve as a scaffold for other proteins, such as p300, thereby allowing it to participate in various nonenzymatic roles (Figure 1).

To further separate the catalytic versus noncatalytic functions of KMT2C and KMT2D, a recent study employed KMT2C and KMT2D proteins containing point mutations in the catalytic domain, rendering them incapable of histone methylation. This selective catalytic inactivation of KMT2C/D displayed unexpectedly minor effects on transcription from enhancers or promotors in mESCs [23]. In contrast, complete deletion of KMT2C/D led to extensive alterations in enhancer Pol II occupancy, enhancer RNA (eRNA) synthesis, and gene expression [23]. Moreover, catalytically dead KMT2C/D were able to partially maintain H3K27ac levels, albeit incompletely, suggesting

Glossary

BAF (SWI/SNF) chromatin remodeling complex: BRG1/BRM associated factor (BAF) is one of the four mammalian ATP-dependent chromatin-remodeling complexes. In humans, this 2-MDa protein complex contains up to 15 subunits, including BAF250a/b, BRG1/BRM, BAF155/170, BAF60, BAF53, BAF47, and BAF45. Mechanistically, the BAF chromatin remodeling complex contributes to chromatin remodeling by hydrolyzing ATP, which generates energy for nucleosomal unwrapping, mobilization, ejection, or histone dimer exchange, ultimately leading to the relaxation of the condensed chromatin fiber and facilitating transcription factor access to chromatin.
Bivalent state: bivalent gene promoters are characterized by the coexistence of activating H3K4me3 and repressive H3K27me3 modifications. Depending on the developmental and cellular context, those promoters can either get activated through demethylation and subsequent acetylation of H3K27 or silenced through H3K4 demethylation to regulate cellular fate trajectories.
Cohesin: eukaryotic ring-shaped multiprotein complex composed of the core subunits SMC1, SMC3, RAD21, and STAG1/SA1 or STAG2/SA2, which collectively contribute to the regulation of gene expression by mediating topological *cis*-interactions between a gene and its regulatory elements.
COMPASS: COMPASS stands for complex of protein associated with Set1. Multiprotein complex conserved from yeast to flies and humans, composed in humans of the core subunits Ash2, Rbbp5, Dpy30, Wdr5, and of a complex-specific Set1-like subunits harboring H3K4 methyltransferase activity.
Enhancer: *cis*-regulatory DNA sequence located either downstream or upstream of a given gene, bound by specific transcription factors, that contribute to increase a gene's transcription level. Enhancers contribute to the spatio-temporal regulation of a gene's expression pattern to achieve specific developmental or cell type-specific gene expression patterns. Active enhancers are characterized by high levels of H3K4me1 and H3K27ac histone modifications, whereas 'poised'

that both enzymatically dependent and independent functions of KMT2D are required for H3K27ac deposition [23]. Supporting these findings, it was recently demonstrated that while deletion of the *KMT2C/D Drosophila* homolog *trr* is embryonic lethal, catalytically deficient Trr produces normal offspring with no overt phenotype or disrupted gene expression programs under normal homeostasis [24]. However, in the setting of environmental stress or altered **cohesin** levels, *Drosophila* embryos did display some subtle abnormalities, suggesting that H3K4me1 was potentially more important for transcriptional regulation under these under environmental stress conditions and, potentially, in a cohesin-dependent manner [24].

Indeed, while these above studies highlight the multiple, essential noncatalytic roles of KMT2C and KMT2D, other recent reports have supported a model whereby KMT2C/D-dependent H3K4me1 plays important roles in enhancer activation. For example, there seems to be unique epigenetic crosstalk between KMT2D and p300, by which p300 stimulates KMT2D-mediated H3K4me1 at enhancers to boost gene transcription [25]. Combined with the role of KMT2D in p300 recruitment [19], these findings establish a potential epigenetic feedforward loop between these enzymes, by which interplay between H3K4me1 and H3K27ac may fine-tune enhancer activity [25]. In addition, another recent study demonstrated KMT2C/D-dependent H3K4me1 was required for establishing enhancer–promoter looping and cohesin occupancy at KMT2C/D-dependent enhancers [26], as well as recruitment of the **BAF (SWI/SNF) chromatin remodeling complex** [27] (Figure 1). Collectively, these data provide compelling evidence that KMT2C and KMT2D have critical noncatalytic roles important for their gene regulatory functions, but also roles that are clearly optimized by their catalysis of H3K4me1.

KMT2C and KMT2D also interact with UTX (KDM6A), an important mediator of gene enhancer regulation and transcriptional activation [28]. UTX is an X-linked gene that belongs to a family of histone demethylases responsible for demethylation of H3K27me3, a histone modification canonically associated with transcriptional repression [29–31]. Given the interactions with KMT2C and KMT2D, it was hypothesized that UTX-mediated removal of repressive H3K27me3 at inactive enhancers might combine with KMT2C/D-mediated deposition of activating H3K4me1 to mediate enhancer activation. However, many reports have broadened the scope of the potential roles of UTX, showing that its dominant role in many contexts occurs primarily through demethylase-independent activities. Indeed, several early studies demonstrated that UTX is a critical regulator of mammalian development, independent of its catalytic function [32–34]. Unexpectedly, these *in vivo* studies were able to show that UTX deficiency could in part be compensated for in males by the Y-linked paralog of UTX, known as UTY, which carries very minimal histone demethylase activity [32,33,35]. Similar observations were made in *Caenorhabditis elegans* [36]. These somewhat surprising discoveries are likely explained by recent mechanistic studies, which showed that depletion of UTX does not alter H3K27me3 levels at UTX enhancer sites, but instead reduces H3K4me1, H3K27ac, and gene transcription [25]. Significantly, this study went on to show that UTX complexes with KMT2D in order to serve as a critical mediator of p300 recruitment to enhancers through direct protein–protein interactions (Figure 2) [25].

These expansive roles for UTX have particular relevance in cancer, as *UTX* is highly mutated across numerous human cancers [37]. For example, UTX suppresses myeloid leukemogenesis and does so through noncatalytic mechanisms. Specifically during leukemogenesis, UTX loss results in broad enhancer dysregulation that mediates both lost tumor-suppressive GATA signaling and upregulation of oncogenic ETS activity, despite very minimal changes in H3K27me3 genome-wide [38]. Similarly, *UTX* mutations are enriched in the aggressive squamous-like subtype of pancreatic cancer, where a loss of UTX drives extensive enhancer rewiring and activation

enhancers are characterized by H3K4me1.

Epigenetic: mechanisms by which gene expression and phenotypes are influenced independent of any changes to the underlying DNA sequence. Fundamental to epigenetic regulation is the intricate organization of each cell's genome into chromatin, which is controlled by three main reversible processes: direct methylation of the DNA strand itself, chromatin remodeling, and post-translational modifications of histone tails (which we focus on in this review).

Kabuki syndrome: pediatric congenital disorder of genetic origin associated with mild to severe developmental delay and intellectual disability, as well as distinctive facial features Between 55 and 80% of cases of Kabuki syndrome are caused by mutations in *KMT2D/MLL4*, while between 4% and 6% of cases are caused by mutations in *UTX/KDM6A*.

Mitogen-activated protein kinase (MAPK) pathway: cell signaling transduction module characterized by the binding of an extracellular ligand to a transmembrane receptor tyrosine kinase, which results in a cascade of phosphorylation events involving three mitogen-activated protein kinases (MAPKKK, MAPKK, ERK/MAPK), ultimately leading to the nuclear translocation of ERK/MAPK and the subsequent activation of target gene expression through phosphorylation of specific transcription factors. The MAPK pathway is frequently dysregulated and thought to serve as a driver in numerous human cancers.

Nucleosome: the fundamental unit of chromatin, made up of 147 base pairs of DNA, wrapped around an octamer of histone proteins (two copies of each of the four core histone proteins: H2A, H2B, H3, and H4).

Ubiquitination: covalent modification of proteins by ubiquitin and ubiquitin-like proteins, resulting in the proteasomal degradation of the ubiquitinated protein.

Key Table

Table 1. Noncanonical, Noncatalytic Functions of Histone Modifiers

Histone modifier	Catalytic function	Representative noncatalytic role(s)	Mutations in disease
KDM6A (UTX)	H3K27me3 demethylation	• Complexes with KMT2D in order to serve as a critical mediator of p300 recruitment to enhancers through direct protein–protein interactions [25] • Senses cellular oxygen levels [40] • Promotes tumor-suppressive GATA signaling and suppresses oncogenic ETS activity in acute myeloid leukemia [38] • Suppresses oncogenic activation of p63 and Myc in pancreatic cancer [39]	• Kabuki syndrome [85,86] • Mutated in numerous cancers, particularly epithelial ranging from bladder and esophageal to skin, lung, and head and neck [37]
KDM6B (JMJD3)	H3K27me3 demethylation	• Bridges interaction between T-box transcription factor T-Bet and SWI/SNF-chromatin remodeling complex subunit [61] • Contributes to the regulation of inflammatory gene expression in macrophages in response to lipopolysaccharide [62] • Functions as a negative regulator of somatic cell reprogramming and cancer stem cells by targeting PHF20 for proteasomal degradation and reducing Oct4 levels [63,64] • Activates genes in the MAPK pathway in a H3K27 demethylase-independent manner in multiple myeloma [66]	• Mutated in numerous cancers, including small cell lung cancer, melanoma, and prostate
KMT2A (MLL1)	H3K4me2/3 methylation	• Required for murine viability and the maintenance of hematopoietic stem and progenitor cells [46,47] • Binds MOF to promote H4K16ac at target genes [47]	• Wiedemann-Steiner syndrome [84] • Rearranged in leukemia and mutated in numerous cancers such as melanoma, colon, bladder, uterine, breast, and lung [87–89]
KMT2C (MLL3)	H3K4me1 methylation	• Required for Pol II occupancy and eRNA synthesis at enhancers [23] • Involved in long-range enhancer interactions via cohesin recruitment [26] • Interacts with the tumor suppressor BAP1 to prevent cancerous proliferation [42]	• Kleefstra syndrome • Mutated in numerous cancers, particularly epithelial cancers ranging from breast, bladder, and esophageal to skin, lung, and head and neck [87]
KMT2D (MLL4)	H3K4me1 methylation	• Scaffolds p300 and UTX to mediate H3K27ac at gene enhancers and proper target gene expression [19,22] • Required for Pol II occupancy and eRNA synthesis at enhancers [23] • Involved in long-range enhancer interactions via cohesin recruitment [26] • Interacts with RECQL5 and promotes genome stability [41]	• Kabuki syndrome [85,86] • Mutated in numerous cancers, particularly diffuse large B cell lymphoma, medulloblastoma, and epithelial cancers ranging from bladder and esophageal to skin, lung, and head and neck [87,90–95]
KMT2F (SETD1A)	H3K4me3 methylation	• Scaffolds Cyclin K to promote cell survival in acute myeloid leukemia [43]	• Mutated in several cancers such as melanoma, bladder, colon, breast, prostate, uterine, pancreas [43,87,96]
KMT2G (SETD1B)	H3K4me3 methylation	• Binds BOD1 to regulate tumor metabolism in triple negative breast cancer [45]	• Mutated in numerous cancers: uterine, colon, prostate, breast [87]
KMT6A (EZH2)	H3K27me3 methylation	• Methylation of nonhistone targets [50–54] • Directly activates expression of the androgen receptor gene [55] • Binds to and enhances stability of p53 mRNA, as well as promoting translation of both wild type and gain-of-function mutant p53 [56] • Promotes cancer cell proliferation in cancers harboring mutations in components of the SWI/SNF chromatin remodeling complex [57] • Promotes proliferation and viability of neuroblastoma [58]	• Overexpression in numerous human cancers [49]
KMT6B (EZH1)	H3K27me3 methylation	• Cytosolic form of EZH1 sequesters a component of the PRC1 repressive complex, EED [59]	• Overexpression in numerous human cancers [49]

(continued on next page)

Table 1. (continued)

Histone modifier	Catalytic function	Representative noncatalytic role(s)	Mutations in disease
RING1B (*RNF2*)	H2AK119ub1 ubiquitination	• H2AK119ub1-independent transcriptional repression in murine and fly development [68,69] • Transcriptional activator through its association with the protein AUTS2 [70] • Activation of key epidermal development genes [71,72] • Promotes expression of oncogenes and oncogenic transcriptional programs through RING1B-mediated super-enhancers [73]	• Amplified in several cancers, including breast, pancreas, prostate, and bladder

of oncogenes such as *TP63* and *MYC*, again with only minor changes in H3K27me3 enrichment [39]. And most recently, UTX was recently shown to be able to sense cellular oxygen levels, which then directly impacts its histone demethylase activity and downstream cellular functions such as differentiation, which collectively have significant potential implications for carcinogenesis (Figure 2) [40].

The above work defining these interrelationships between KMT2D, UTX, and p300 nicely illustrates the regulatory importance of protein–protein interactions for proper transcription and enhancer regulation (Figure 1). This provides a broad model through which to better understand the complex functions that other binding partners of histone modifiers may play during development and disease. For example, KMT2D was recently identified to bind RECQL5, a transcriptional elongation factor [41]. Interestingly, loss of KMT2D led to genome instability and transcriptional stress without inducing changes in H3K4 methylation at affected genes [41]. Whether these effects were secondary to a potential role for KMT2D in recruiting or maintaining RECQL5 levels during transcriptional elongation was not established. In addition, KMT2C was shown to bind to the tumor suppressor BAP1 through its PHD domain in cancer cells, and disruption of this interaction via *KMT2C* mutations led to impaired KMT2C and UTX recruitment to enhancers and increased cancer cell proliferation [42].

Finally, beyond KMT2C and KMT2D, recent work has also revealed crucial noncatalytic roles of other COMPASS family members, typically better known for their roles in catalyzing H3K4me3 at gene promoters. For example, the protein KMT2F, or SETD1A, is required for cell survival in acute myeloid leukemia (AML), though its catalytic domain is completely dispensable for this function. Rather, a newly characterized functional location on SETD1A (FLOS) domain of SETD1A, which acts as a Cyclin K binding site, is required for the prosurvival effects in AML [43]. Similarly, noncatalytic functions of SETD1A also mediate basic, nontumorigenic cell functions, as a complete loss of SETD1A in ESCs disrupts proliferation and self-renewal, while catalytically deficient SETD1A mutants are capable of mediating these processes [44]. Interestingly, the nonenzymatic functions of COMPASS family members may extend beyond the nucleus, as a recent study in triple negative breast cancer revealed that KMT2G (SETD1B) is primarily cytoplasmic in these cells where it associates with BOD1 to regulate tumor metabolism. Loss of the catalytic-specific SETD1B form is expendable for the cell survival advantage afforded by the full-length protein in this system [45]. Finally, KMT2A (MLL1) has several acknowledged noncatalytic functions. This is unsurprising given that while complete *Kmt2a* deletion is embryonic lethal, mice lacking only the catalytic domain of Kmt2a survive into adulthood [46]. The catalytic domain of KMT2A is also unnecessary for maintaining hematopoietic stem and progenitor cells and the initiation of leukemogenesis, despite the requirement for wild type KMT2A during these processes [47]. Through association with the histone acetyltransferase MOF, KMT2A promotes

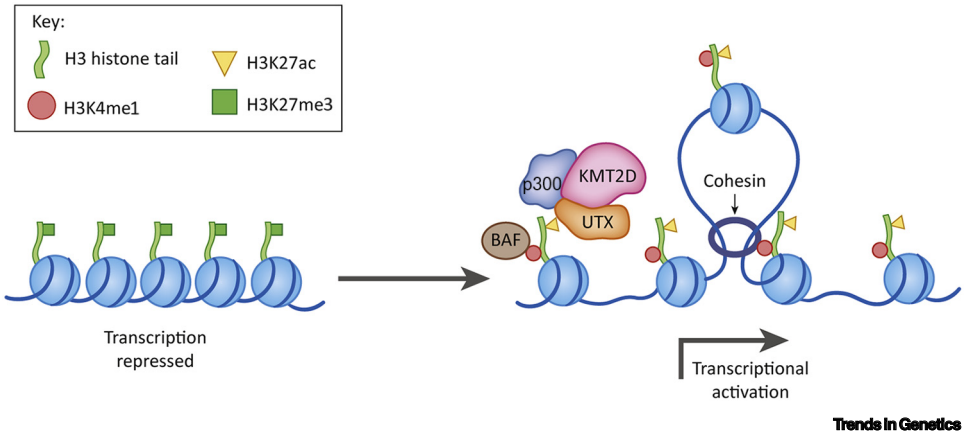

Figure 1. KMT2D Activates Enhancer Transcription through Catalytic and Noncatalytic Means. KMT2D canonically catalyzes H3K4me1 to prime enhancers for activation. In addition, recent studies have established noncatalytic means through which it also can activate transcription. First, KMT2D is required for the recruitment of the histone acetyltransferase, p300, to catalyze H3K27ac and activate genes. The KMT2D-interacting protein, UTX, contributes to this recruitment, and in a feedforward loop, p300 then enhances KMT2D-catalyzed H3K4me1 deposition. Then, KMT2D-mediated H3K4me1 has been shown to be critical for the proper recruitment of both cohesin (to coordinate long-range chromatin interactions) and the BAF complex (to coordinate chromatin remodeling).

histone H4 lysine 16 acetylation at its target genes, which is necessary for proper gene expression and recruitment of transcriptional initiation and elongation machinery [47].

Polycomb Complexes: Marking the Balance between Development, Differentiation, and Cancer

Opposing the canonical transcriptional activating abilities of the COMPASS complex is the polycomb repressive complex [28]. This dynamic repression system is marked typically by trimethylation of histone H3K27, which is mediated by polycomb repressive complex 2 (PRC2). The core PRC2 is composed of the SET-domain containing methyltransferase enhancer of zeste 2 (EZH2/KMT6A) or its closely related homolog EZH1 (KMT6B) and also comprises the core subunits suppressor of zeste 12 (SUZ12) and embryonic ectoderm development (EED), which are required for the methyltransferase activity of the complex [48]. H3K27me3 is commonly associated with the repression of genes involved in cellular functions ranging from cell cycle regulation, differentiation, and the suppression of carcinogenesis. As a consequence, aberrant expression of PRC2 subunits has been associated with an array of diseases and malignancies through abnormal H3K27me3 deposition. Consistent with this, EZH2 overexpression in particular has been shown to be a common theme across numerous human cancers [49]. These collective findings have driven the development of EZH2 inhibitors, which have entered clinical trials for the treatment of various cancers (Box 1) [49].

Similar to the COMPASS complex, an increasing number of studies have also highlighted noncanonical and noncatalytic functions of EZH2 (Figure 3). Although EZH2 primarily acts on H3 to methylate H3K27me3, recent studies also have reported nonhistone targets of EZH2. For example, EZH2 has been shown to bind to and methylate STAT3, which enhances its activity in glioblastoma [50]. Similarly, EZH2 methylation of Jarid2 serves as a feedforward loop to promote further PRC2 activity [51]. In contrast, EZH2 methylation of GATA4 represses its transcriptional activation [52]. Alternatively, EZH2-mediated methylation of the transcription factors RORα and PLZF results in their targeting for proteasomal degradation [53,54].

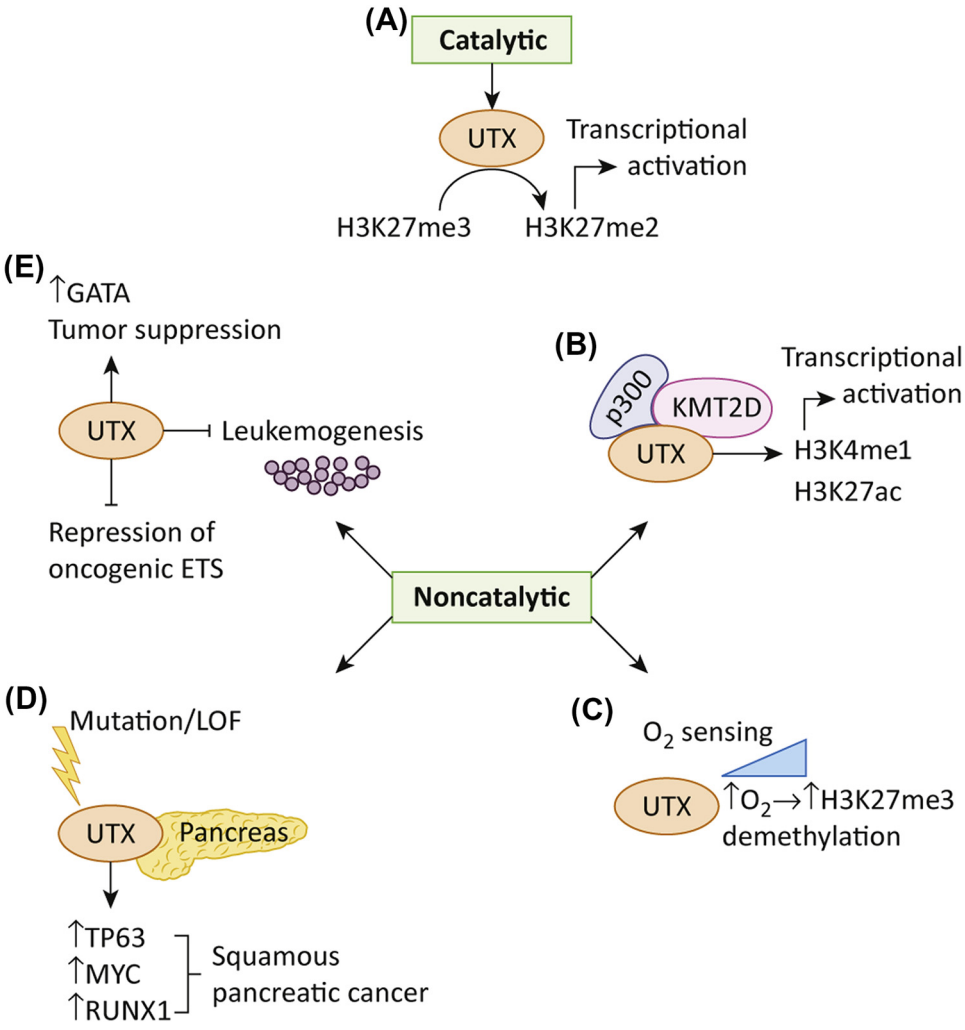

Figure 2. UTX Displays Both Catalytic and Noncatalytic Mechanisms in Development and Cancer. The canonical role of UTX in histone demethylation involves the demethylation of H3K27me3 at enhancers to promote transcription (A). Recently, however, UTX has been shown to also be important for the maintenance of H3K4me1 and H3K27ac, likely through its ability to contribute to the recruitment of p300 to acetylate H3K27, which in turn may then enhance KMT2D-mediated H3K4me1 deposition (B). UTX also possesses the ability to sense cellular oxygen levels and this influences its demethylase activity, with functional consequences observed during cellular differentiation and implications for carcinogenesis (C). In some cancers, loss-of-function (LOF) of UTX leads to broad enhancer dysregulation, despite minimal impacts on H3K27me3 levels. For example, a loss of UTX drives the aberrant activation of p63, MYC, and RUNX1 to drive the squamous-like subtype of pancreatic cancer (D), as well as a loss of tumor suppressive GATA signaling and gains in oncogenic ETS signaling in myeloid leukemogenesis (E).

Beyond its enzymatic functions, several cancer studies have identified noncatalytic roles for EZH2 in driving disease. Despite its canonical roles in transcriptional repression, paradoxically, recent reports suggest that EZH2 may play roles in promoting both transcription and translation. For instance, in prostate cancer, EZH2 binds to and directly activates expression of the androgen receptor (AR) gene, independent of the rest of the PRC2 complex and its methyltransferase function [55]. Also, in prostate cancer, EZH2 protein was shown to bind directly to p53 mRNA at an internal ribosomal entry site, both enhancing p53 mRNA stability and promoting translation of both wild type and gain-of-function mutant p53 [56]. In cancers harboring mutations in

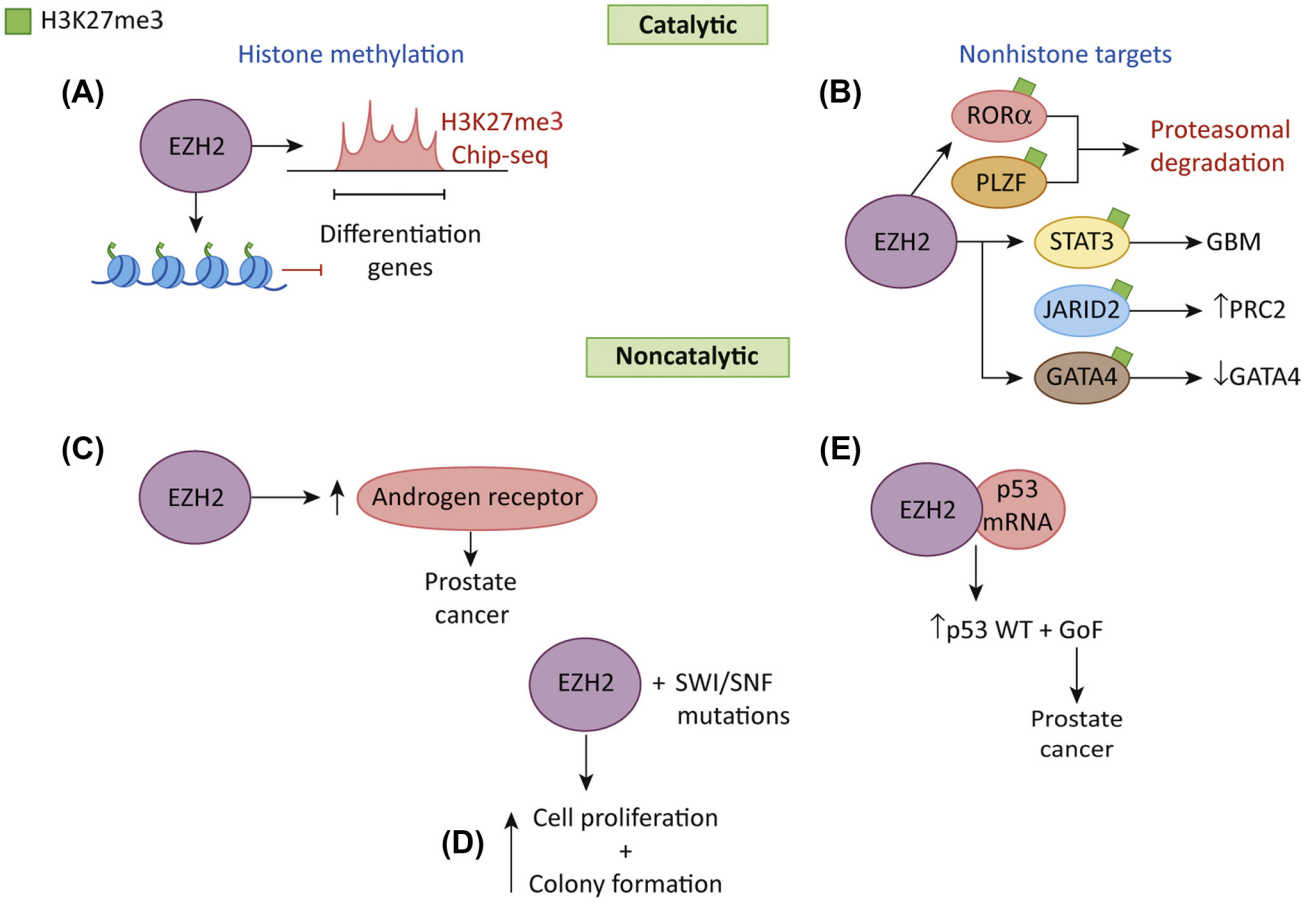

Figure 3. EZH2 Acts through Both Methylation-Dependent and -Independent Mechanisms. EZH2 canonically catalyzes H3K27me3 to repress genes, with key roles in gene repression during cellular differentiation (A). Beyond histone methylation however, EZH2 has now been shown to affect numerous other cellular processes through noncanonical methylation of nonhistone targets (B). These include RORα and PZLF to target them for proteasomal degradation, STAT3 to promote proliferation in glioblastoma (GBM), JARID2 to increase activity of PRC2, and GATA4 to reduce its transcription. Numerous other noncatalytic roles have been identified in cancer, where EZH2 is frequently elevated. EZH2 has been shown to increase levels of the androgen receptor to drive prostate carcinogenesis (C). Cancers carrying mutations in members of the SWI/SNF chromatin remodeling complex have been shown to be dependent on nonenzymatic functions of EZH2 in order to proliferate and form colonies (D). In addition, EZH2 has been demonstrated to bind to the mRNA of both wild type (WT) and mutant gain-of-function (GoF) p53, enhancing both the mRNA stability and increasing translation of the protein in prostate cancer.

components of the SWI/SNF chromatin remodeling complex, genetic knockdown of EZH2 reduced cell proliferation and prevented colony formation. Reintroduction of catalytically inactive EZH2 offered almost complete rescue of proliferation, suggesting that these cancers were primarily dependent on the nonenzymatic activity of EZH2 in stabilizing the PRC2 complex [57]. In neuroblastoma, high EZH2 expression levels correlate with poor prognosis and reduced overall survival, however pharmacological inhibition of EZH2 enzymatic activity had virtually no effect on cellular phenotypes, despite a complete loss of H3K27me3. In contrast, genetic knockdown of the complete EZH2 protein by small hairpin RNAs resulted in decreased global H3K27me3 levels, induction of cell cycle arrest, and apoptosis [58]. Reintroduction of a catalytically dead version of EZH2 reduced apoptosis and restored proliferation, highlighting an important role for EZH2 in neuroblastoma viability independent of its histone methyltransferase enzymatic activity and providing significant rationale for using EZH2 inhibitors that would target the entire protein and not just the catalytic function [58].

Finally, a cytosolic isoform of EZH1 lacking a functional histone methyltransferase SET domain (EZH1β) has been identified in postmitotic muscle cells. EZH1β regulates the cytosolic-nuclear localization of the PRC2 subunit EED, thereby preventing nuclear assembly of a functional PRC2–EZH1 complex in absence of atrophic oxidative stress. Under stress, EZH1β is targeted for proteasomal degradation, allowing EED to move to the nucleus and assemble a functional PRC2–EZH1 complex on chromatin to repress target genes in muscle [59]. Overall, these results shed light on noncanonical and nonenzymatic functions of PRC2 methyltransferase subunits EZH1 and EZH2, suggest additional roles for those proteins in transcriptional regulation beyond their histone methyltransferase activity, and provide a basis for the discovery of new roles for EZH1/EZH2 in development and cancer.

In combination with the H3K27 demethylase UTX (KDM6A) discussed above, PRC2-mediated H3K27 methyltransferase activity is counterbalanced by the Jumonji-C domain-containing JMJD3 (KDM6B) catalyzing removal of H3K27me3 [60]. Like UTX, JMJD3 contributes to a variety of tissue-specific and developmental processes through catalytic-dependent and -independent functions. During T cell development, JMJD3, independent of its demethylase function, was shown to be required for mediating interactions between a key T cell transcription factor (T-bet) and Brg1, a member of the SWI/SNF chromatin remodeling complex [61]. Further work exploring functions of JMJD3 in transcription has demonstrated that it contributes to the regulation of inflammatory gene expression in macrophages in response to lipopolysaccharide. However, this role was deemed to be independent of its H3K27 demethylase activity, as no changes in H3K27me3 enrichment were observed in response to the loss of JMJD3 binding [62]. In addition, JMJD3 has been demonstrated to act as a negative regulator of somatic cell reprogramming through both histone demethylase-dependent and -independent mechanisms. For example, JMJD3 was found to target a major reprogramming factor, Plant Homeodomain finger protein 20 (PHF20), for **ubiquitination** by bringing it together with the ubiquitin-ligase Trim26 [63]. This JMJD3-triggered ubiquitination of PHF20 was also observed to result in reduced Oct4 expression and the suppression of stem cell-like characteristics of breast cancer cells [64]. Further evidence has also revealed critical noncatalytic roles for JMJD3 in cancer. Like EZH2, JMJD3 also has been shown to have some nonhistone targets and can demethylate the retinoblastoma (RB) protein to help promote the tumor suppressive mechanism of cellular senescence [65]. In multiple myeloma, JMJD3 was found to be upregulated, and while JMJD3 knockdown promoted apoptosis and reduced cell proliferation, JMJD3 overexpression enhanced cell growth. Mechanistically, this study found that JMJD3 activates genes in the **mitogen-activated protein kinase (MAPK) pathway** in a H3K27 demethylase-independent manner, since JMJD3 knockdown resulted in no changes in H3K27me3 deposition at MAPK genes, while expression of a catalytically dead JMJD3 was able to enhance multiple myeloma cell growth and induce expression of MAPK genes to the same extent that wild type JMJD3 overexpression did [66].

Besides PRC2-mediated H3K27 trimethylation, other polycomb repressive complex regulatory mechanisms arise from canonical and noncanonical PRC1 complexes. An essential function of PRC1 is the monoubiquitination of histone 2A (H2A) on serine 119 (H2AK119ub1) by the E3 ubiquitin ligase activity of its core RING-PCGF heterodimer components. PRC1-mediated H2AK119ub1 contributes to the repression of cell fate-determining genes in cooperation with PRC2-mediated H3K27me3 deposition [67]. Similar to PRC2, recent findings have highlighted surprising noncanonical, noncatalytic functions of PRC1. For example, homozygous deletion of the core PRC1 component *Ring1b* in mESCs produced more drastic transcriptional changes than mutation of the *Ring1b* catalytic site alone, supporting the idea that Ring1b-mediated gene repression is enhanced by, but not primarily dependent on, its catalytic activity. Moreover, while *Ring1b* whole body deletion was embryonic lethal at E10.5, catalytically inactive Ring1b

mouse embryos completed gastrulation and developed to E15.5, suggesting that the activity of Ring1b is dispensable for early mouse development [68]. A similar phenomenon was observed in *Drosophila*, where a total loss of Sce, the *Drosophila* RING1B ortholog, resulted in total loss of H2AK118ub1, decreased H3K27me3 bulk deposition, and embryos with extensive abnormalities. In contrast, embryos harboring a catalytically inactive form of Sce were identical to wild type embryos [69]. Significantly, in each of these two above examples, loss of H2A monoubiquitination activity reduced H3K27me3 deposition at RING1B binding sites, highlighting the interplay between PRC1-mediated H2A ubiquitination and PRC2-mediated H3K27me3 deposition and supporting a model whereby PRC1-mediated H2A ubiquitination contributes to regulate PRC2 binding at Polycomb target genes.

In contrast to the canonical roles of PRC1 in gene repression, a noncanonical PRC1 complex was identified as a transcriptional activator through its association with the protein AUTS2. Specifically, AUTS2 recruits the kinase CK2 to phosphorylate RING1B at serine 168 and prevent RING1B-mediated H2A ubiquitination activity. Then this PRC1–AUTS2 complex recruits p300 to promote gene activation through increased H3K27ac [70]. Other more recent findings investigating skin epidermal development support these findings. Here, *in vivo* studies demonstrated that PRC1 function is essential for proper skin development, though its catalytic activity is dispensable. Furthermore, these studies presented further evidence for a role for PRC1 in gene activation [71,72]. Finally, in multiple cancer cell lines, RING1B was found to bind super-enhancers and to promote the expression of known oncogenes [73]. Overall, these findings shed light on the flexibility and context-dependence of PRC1-mediated gene regulation and demonstrate new, unexpected, and enzymatic-independent PRC1-mediated gene regulatory mechanisms.

Concluding Remarks

This collective body of recent studies highlights the multiple, diverse, and essential noncatalytic functions of several histone modifier families during proper cellular development, homeostasis, differentiation, and disease. While we were not able to address the potential noncatalytic roles of all histone modifiers here due to limitations of space, we believe the studies highlighted here are highly representative and serve as a great framework from which to consider all chromatin regulators. Importantly, as discussed above, these recent developments provide crucial new insight into defining the role of both histone methylation and modifier dysfunction in development and cancer. In addition, these discoveries raise exciting new research questions (see Outstanding Questions) and offer critical insights into potential therapeutic strategies. In the coming years, it is likely that the findings presented here will not only inspire new discoveries of noncatalytic roles for other epigenetic regulators, but will also be harnessed for a more nuanced approach in terms of targeting the epigenome for clinical benefit.

Outstanding Questions

What other broad noncatalytic mechanisms may be performed by histone modifiers? Protein scaffolding is the primary one that we highlight, but also intriguing evidence exists for the maintenance of genome stability (KMT2D) and sensing global cellular oxygen levels (UTX). Do other metabolite- or nutrient-sensitive mechanisms exist and potentially function in a catalytic-independent fashion?

Which protein domains mediate noncatalytic functions for particular histone modifiers?

Can these noncatalytic functions/domains be targeted to treat cancer? Will targeting noncatalytic activities need to be coupled with inhibition of the catalytic activity as well? Would dual targeting of both functions be synergistically advantageous?

Are these noncatalytic functions required for cellular homeostasis and, in line with this, are catalytic functions dependent on noncatalytic functions? [If you deleted the particular domain required for p300 recruitment on KMT2D would it be able to: (i) mediate H3K4 methylation and (ii) conduct H3K4methylation-dependent functions like BAF recruitment?]

Which of these noncatalytic roles for histone modifiers are cell type-specific and what are the implications for the treatment of disease?

Is the recruitment of ubiquitin-ligase to target interacting proteins for proteasomal degradation a specific feature of JMJD3, or does this constitutes a more generalizable mechanism that also extends to other H3K27 demethylases or to other histone modifiers?

Do other histone modifiers also exhibit cytosolic functions beyond EZH1β and KMT2G/SETD1B?

Acknowledgments

We apologize to those whose work could not be included in this review article, owing to space limitations. The B.C.C. laboratory is funded by grants from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) of the National Institutes of Health under Award Number K08AR070289, the Damon Runyon Cancer Foundation, the Dermatology Foundation, and the Penn Skin Biology and Diseases Resource-based Center, funded by NIAMS 1P30AR069589-01.

References

1. Allis, C.D. and Jenuwein, T. (2016) The molecular hallmarks of epigenetic control. *Nat. Rev. Genet.* 17, 487–500
2. Sidoli, S. and Garcia, B.A. (2017) Characterization of individual histone posttranslational modifications and their combinatorial patterns by mass spectrometry-based proteomics strategies. *Methods Mol. Biol.* 1528, 121–148
3. Shortt, J. *et al.* (2017) A chemical probe toolbox for dissecting the cancer epigenome. *Nat. Rev. Cancer* 17, 160–183
4. Atlasi, Y. and Stunnenberg, H.G. (2017) The interplay of epigenetic marks during stem cell differentiation and development. *Nat. Rev. Genet.* 18, 643–658
5. Sur, I. and Taipale, J. (2016) The role of enhancers in cancer. *Nat. Rev. Cancer* 16, 483–493

6. Long, H.K. *et al.* (2016) Ever-changing landscapes: transcriptional enhancers in development and evolution. *Cell* 167, 1170–1187
7. Polak, P. *et al.* (2015) Cell-of-origin chromatin organization shapes the mutational landscape of cancer. *Nature* 518, 360–364
8. Avgustinova, A. and Benitah, S.A. (2016) Epigenetic control of adult stem cell function. *Nat. Rev. Mol. Cell Biol.* 17, 643–658
9. Nguyen, L.V. *et al.* (2012) Cancer stem cells: an evolving concept. *Nat. Rev. Cancer* 12, 133–143
10. Beck, B. and Blanpain, C. (2013) Unravelling cancer stem cell potential. *Nat. Rev. Cancer* 13, 727–738
11. Edwards, J.R. *et al.* (2017) DNA methylation and DNA methyltransferases. *Epigenetics Chromatin* 10, 23
12. Narita, T. *et al.* (2019) Functions and mechanisms of non-histone protein acetylation. *Nat. Rev. Mol. Cell Biol.* 20, 156–174
13. Verdin, E. and Ott, M. (2015) 50 years of protein acetylation: from gene regulation to epigenetics, metabolism and beyond. *Nat. Rev. Mol. Cell Biol.* 16, 258–264
14. Lyko, F. (2018) The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. *Nat. Rev. Genet.* 19, 81–92
15. Shilatifard, A. (2012) The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annu. Rev. Biochem.* 81, 65–95
16. Hu, D. *et al.* (2013) The MLL3/MLL4 branches of the COMPASS family function as major histone H3K4 monomethylases at enhancers. *Mol. Cell. Biol.* 33, 4745–4754
17. Lee, J.E. *et al.* (2013) H3K4 mono- and di-methyltransferase MLL4 is required for enhancer activation during cell differentiation. *eLife* 2, e01503
18. Lee, J. *et al.* (2008) Targeted inactivation of MLL3 histone H3-Lys-4 methyltransferase activity in the mouse reveals vital roles for MLL3 in adipogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 105, 19229–19234
19. Wang, C. *et al.* (2016) Enhancer priming by H3K4 methyltransferase MLL4 controls cell fate transition. *Proc. Natl. Acad. Sci. U. S. A.* 113, 11871–11876
20. Lin-Shiao, E. *et al.* (2018) KMT2D regulates p63 target enhancers to coordinate epithelial homeostasis. *Genes Dev.* 32, 181–193
21. Creyghton, M.P. *et al.* (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. U. S. A.* 107, 21931–21936
22. Lai, B. *et al.* (2017) MLL3/MLL4 are required for CBP/p300 binding on enhancers and super-enhancer formation in brown adipogenesis. *Nucleic Acids Res.* 45, 6388–6403
23. Dorighi, K.M. *et al.* (2017) Mll3 and Mll4 facilitate enhancer RNA synthesis and transcription from promoters independently of H3K4 monomethylation. *Mol. Cell* 66, 568–576
24. Rickels, R. *et al.* (2017) Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability. *Nat. Genet.* 49, 1647–1653
25. Wang, S.P. *et al.* (2017) A UTX-MLL4-p300 transcriptional regulatory network coordinately shapes active enhancer landscapes for eliciting transcription. *Mol. Cell* 67, 308–321
26. Yan, J. *et al.* (2018) Histone H3 lysine 4 monomethylation modulates long-range chromatin interactions at enhancers. *Cell Res.* 28, 204–220
27. Local, A. *et al.* (2018) Identification of H3K4me1-associated proteins at mammalian enhancers. *Nat. Genet.* 50, 73–82
28. Piunti, A. and Shilatifard, A. (2016) Epigenetic balance of gene expression by Polycomb and COMPASS families. *Science* 352, aad9780
29. Agger, K. *et al.* (2007) UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* 449, 731–734
30. Lan, F. *et al.* (2007) A histone H3 lysine 27 demethylase regulates animal posterior development. *Nature* 449, 689–694
31. Hong, S. *et al.* (2007) Identification of JmjC domain-containing UTX and JMJD3 as histone H3 lysine 27 demethylases. *Proc. Natl. Acad. Sci. U. S. A.* 104, 18439–18444
32. Wang, C. *et al.* (2012) UTX regulates mesoderm differentiation of embryonic stem cells independent of H3K27 demethylase activity. *Proc. Natl. Acad. Sci. U. S. A.* 109, 15324–15329
33. Shpargel, K.B. *et al.* (2012) UTX and UTY demonstrate histone demethylase-independent function in mouse embryonic development. *PLoS Genet.* 8, e1002964
34. Yoo, K.H. *et al.* (2016) Histone demethylase KDM6A controls the mammary luminal lineage through enzyme-independent mechanisms. *Mol. Cell. Biol.* 36, 2108–2120
35. Walport, L.J. *et al.* (2014) Human UTY(KDM6C) is a male-specific N-methyl lysyl demethylase. *J. Biol. Chem.* 289, 18302–18313
36. Vandamme, J. *et al.* (2012) The *C. elegans* H3K27 demethylase UTX-1 is essential for normal development, independent of its enzymatic activity. *PLoS Genet.* 8, e1002647
37. Wang, L. and Shilatifard, A. (2019) UTX mutations in human cancer. *Cancer Cell* 35, 168–176
38. Gozdecka, M. *et al.* (2018) UTX-mediated enhancer and chromatin remodeling suppresses myeloid leukemogenesis through noncatalytic inverse regulation of ETS and GATA programs. *Nat. Genet.* 50, 883–894
39. Andricovich, J. *et al.* (2018) Loss of KDM6A activates super-enhancers to induce gender-specific squamous-like pancreatic cancer and confers sensitivity to BET inhibitors. *Cancer Cell* 33, 512–526
40. Chakraborty, A.A. *et al.* (2019) Histone demethylase KDM6A directly senses oxygen to control chromatin and cell fate. *Science* 363, 1217–1222
41. Kantidakis, T. *et al.* (2016) Mutation of cancer driver MLL2 results in transcription stress and genome instability. *Genes Dev.* 30, 408–420
42. Wang, L. *et al.* (2018) Resetting the epigenetic balance of Polycomb and COMPASS function at enhancers for cancer therapy. *Nat. Med.* 24, 758–769
43. Hoshii, T. *et al.* (2018) A non-catalytic function of SETD1A regulates Cyclin K and the DNA damage response. *Cell* 172, 1007–1021
44. Sze, C.C. *et al.* (2017) Histone H3K4 methylation-dependent and -independent functions of Set1A/COMPASS in embryonic stem cell self-renewal and differentiation. *Genes Dev.* 31, 1732–1737
45. Wang, L. *et al.* (2017) A cytoplasmic COMPASS is necessary for cell survival and triple-negative breast cancer pathogenesis by regulating metabolism. *Genes Dev.* 31, 2056–2066
46. Terranova, R. *et al.* (2006) Histone and DNA methylation defects at Hox genes in mice expressing a SET domain-truncated form of Mll. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6629–6634
47. Mishra, B.P. *et al.* (2014) The histone methyltransferase activity of MLL1 is dispensable for hematopoiesis and leukemogenesis. *Cell Rep.* 7, 1239–1247
48. Laugesen, A. *et al.* (2019) Molecular mechanisms directing PRC2 recruitment and H3K27 methylation. *Mol. Cell* 74, 8–18
49. Kim, K.H. and Roberts, C.W. (2016) Targeting EZH2 in cancer. *Nat. Med.* 22, 128–134
50. Kim, E. *et al.* (2013) Phosphorylation of EZH2 activates STAT3 signaling via STAT3 methylation and promotes tumorigenicity of glioblastoma stem-like cells. *Cancer Cell* 23, 839–852
51. Sanulli, S. *et al.* (2015) Jarid2 methylation via the PRC2 complex regulates H3K27me3 deposition during cell differentiation. *Mol. Cell* 57, 769–783
52. He, A. *et al.* (2012) PRC2 directly methylates GATA4 and represses its transcriptional activity. *Genes Dev.* 26, 37–42
53. Lee, J.M. *et al.* (2012) EZH2 generates a methyl degron that is recognized by the DCAF1/DDB1/CUL4 E3 ubiquitin ligase complex. *Mol. Cell* 48, 572–586
54. Vasanthakumar, A. *et al.* (2017) A non-canonical function of Ezh2 preserves immune homeostasis. *EMBO Rep.* 18, 619–631
55. Kim, J. *et al.* (2018) Polycomb- and methylation-independent roles of EZH2 as a transcription activator. *Cell Rep.* 25, 2808–2820
56. Zhao, Y. *et al.* (2019) EZH2 cooperates with gain-of-function p53 mutants to promote cancer growth and metastasis. *EMBO J.* 38, e99599
57. Kim, K.H. *et al.* (2015) SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat. Med.* 21, 1491–1496

58. Bate-Eya, L.T. *et al.* (2017) Enhancer of zeste homologue 2 plays an important role in neuroblastoma cell survival independent of its histone methyltransferase activity. *Eur. J. Cancer* 75, 63–72
59. Bodega, B. *et al.* (2017) A cytosolic Ezh1 isoform modulates a PRC2-Ezh1 epigenetic adaptive response in postmitotic cells. *Nat. Struct. Mol. Biol.* 24, 444–452
60. Arcipowski, K.M. *et al.* (2016) Histone demethylases in physiology and cancer: a tale of two enzymes, JMJD3 and UTX. *Curr. Opin. Genet. Dev.* 36, 59–67
61. Miller, S.A. *et al.* (2010) Jmjd3 and UTX play a demethylase-independent role in chromatin remodeling to regulate T-box family member-dependent gene expression. *Mol. Cell* 40, 594–605
62. De Santa, F. *et al.* (2009) Jmjd3 contributes to the control of gene expression in LPS-activated macrophages. *EMBO J.* 28, 3341–3352
63. Zhao, W. *et al.* (2013) Jmjd3 inhibits reprogramming by upregulating expression of INK4a/Arf and targeting PHF20 for ubiquitination. *Cell* 152, 1037–1050
64. Xun, J. *et al.* (2017) JMJD3 suppresses stem cell-like characteristics in breast cancer cells by downregulation of Oct4 independently of its demethylase activity. *Oncotarget* 8, 21918–21929
65. Zhao, L. *et al.* (2015) JMJD3 promotes SAHF formation in senescent WI38 cells by triggering an interplay between demethylation and phosphorylation of RB protein. *Cell Death Differ.* 22, 1630–1640
66. Ohguchi, H. *et al.* (2017) KDM6B modulates MAPK pathway mediating multiple myeloma cell growth and survival. *Leukemia* 31, 2661–2669
67. Marsh, D.J. and Dickson, K.A. (2019) Writing histone monoubiquitination in human malignancy-the role of RING finger E3 ubiquitin ligases. *Genes (Basel)* 10, E67
68. Illingworth, R.S. *et al.* (2015) The E3 ubiquitin ligase activity of RING1B is not essential for early mouse development. *Genes Dev.* 29, 1897–1902
69. Pengelly, A.R. *et al.* (2015) Transcriptional repression by PRC1 in the absence of H2A monoubiquitylation. *Genes Dev.* 29, 1487–1492
70. Gao, Z. *et al.* (2014) An AUTS2-Polycomb complex activates gene expression in the CNS. *Nature* 516, 349–354
71. Cohen, I. *et al.* (2018) PRC1 fine-tunes gene repression and activation to safeguard skin development and stem cell specification. *Cell Stem Cell* 22, 726–739
72. Cohen, I. *et al.* (2019) PRC1 preserves epidermal tissue integrity independently of PRC2. *Genes Dev.* 33, 55–60
73. Chan, H.L. *et al.* (2018) Polycomb complexes associate with enhancers and promote oncogenic transcriptional programs in cancer through multiple mechanisms. *Nat. Commun.* 9, 3377
74. Berdasco, M. and Esteller, M. (2019) Clinical epigenetics: seizing opportunities for translation. *Nat. Rev. Genet.* 20, 109–127
75. Pfister, S.X. and Ashworth, A. (2017) Marked for death: targeting epigenetic changes in cancer. *Nat. Rev. Drug Discov.* 16, 241–263
76. Sheng, W. *et al.* (2018) LSD1 ablation stimulates anti-tumor immunity and enables checkpoint blockade. *Cell* 174, 549–563
77. Xie, N. *et al.* (2018) Novel epigenetic techniques provided by the CRISPR/Cas9 system. *Stem Cells Int.* 2018, 7834175
78. Wang, P. and Zhou, J. (2018) Proteolysis targeting chimera (PROTAC): a paradigm-shifting approach in small molecule drug discovery. *Curr. Top. Med. Chem.* 18, 1354–1356
79. Roth, S. *et al.* (2019) Advances in targeted degradation of endogenous proteins. *Cell Mol. Life Sci.* 76, 2761–2777
80. Maunakea, A.K. *et al.* (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* 466, 253–257
81. Glaser, S. *et al.* (2006) Multiple epigenetic maintenance factors implicated by the loss of Mll2 in mouse development. *Development* 133, 1423–1432
82. Yu, B.D. *et al.* (1998) MLL, a mammalian trithorax-group gene, functions as a transcriptional maintenance factor in morphogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 95, 10632–10636
83. Bledau, A.S. *et al.* (2014) The H3K4 methyltransferase Setd1a is first required at the epiblast stage, whereas Setd1b becomes essential after gastrulation. *Development* 141, 1022–1035
84. Jones, W.D. *et al.* (2012) De novo mutations in MLL cause Wiedemann-Steiner syndrome. *Am. J. Hum. Genet.* 91, 358–364
85. Ng, S.B. *et al.* (2010) Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat. Genet.* 42, 790–793
86. Miyake, N. *et al.* (2013) MLL2 and KDM6A mutations in patients with Kabuki syndrome. *Am. J. Med. Genet. A* 161A, 2234–2243
87. Rao, R.C. and Dou, Y. (2015) Hijacked in cancer: the KMT2 (MLL) family of methyltransferases. *Nat. Rev. Cancer* 15, 334–346
88. Winters, A.C. and Bernt, K.M. (2017) MLL-rearranged leukemias-an update on science and clinical approaches. *Front. Pediatr.* 5, 4
89. Krivtsov, A.V. *et al.* (2017) Mixed-lineage leukemia fusions and chromatin in leukemia. *Cold Spring Harb. Perspect Med.* 7, a026658
90. Ortega-Molina, A. *et al.* (2015) The histone lysine methyltransferase KMT2D sustains a gene expression program that represses B cell lymphoma development. *Nat. Med.* 21, 1199–1208
91. Zhang, J. *et al.* (2015) Disruption of KMT2D perturbs germinal center B cell development and promotes lymphomagenesis. *Nat. Med.* 21, 1190–1198
92. Dawkins, J.B. *et al.* (2016) Reduced expression of histone methyltransferases KMT2C and KMT2D correlates with improved outcome in pancreatic ductal adenocarcinoma. *Cancer Res.* 76, 4861–4871
93. Lv, S. *et al.* (2018) Histone methyltransferase KMT2D sustains prostate carcinogenesis and metastasis via epigenetically activating LIFR and KLF4. *Oncogene* 37, 1354–1368
94. Toska, E. *et al.* (2017) PI3K pathway regulates ER-dependent transcription in breast cancer through the epigenetic regulator KMT2D. *Science* 355, 1324–1330
95. Kim, J.H. *et al.* (2014) UTX and MLL4 coordinately regulate transcriptional programs for cell proliferation and invasiveness in breast cancer cells. *Cancer Res.* 74, 1705–1717
96. Salz, T. *et al.* (2015) Histone methyltransferase hSETD1A is a novel regulator of metastasis in breast cancer. *Mol. Cancer Res.* 13, 461–469