J Biosci (2020)45:6
DOI: 10.1007/s12038-019-9969-0

Mini-Review

The role of histone modifications in leukemogenesis

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Histone modifications play a critical role in coordinating accurate gene expression. Aside from genetic mutations which cause altered DNA sequence, it has become increasingly clear that aberrant post-translational modifications of histone tails are also associated with leukemogenesis. The functional roles of specific histone marks has informed the basis of our understanding for underlying mechanisms of leukemia, while global analyses of interacting histone modifications has begun to distinguish subtypes of leukemia with prognostic and therapeutic implications. In this current era of personalized and precision medicine, it will be necessary to not only identify the specific genetic mutations present in a patient's leukemia but to also appreciate the dynamic chromatin states which are driven by histone modifications that can aid our diagnostic and therapeutic strategies for improved management of leukemia.

Keywords. Histone modification; leukemia; chromatin; methylation; acetylation

1. Introduction

Hematopoiesis is the process by which multipotent stem cells give rise to the distinct cells that compromise our circulatory and immune systems. Just as each set of tissues in the body must undergo growth and differentiation during embryonic development and fetal maturation, the hematopoietic system is finely choreographed to express the right set of genes at just the right time in precisely the right cell types. When this coordinated process works effectively, hematopoietic stem cells can provide lifelong population of all cellular components in our blood, but when this regulated process goes awry, dysfunctional hematopoiesis can cause fatigue, bleeding complications, susceptibility to infections, and life-threatening disease like acute leukemia. Disordered hematopoiesis can arise from direct mutations in the DNA sequence of individual genes or from chromosomal breaks resulting in oncogenic proteins. Alternatively, the disruption of regulated chromatin interactions including histone modifications can affect critical expression of hematopoietic genes leading to leukemia. Indeed, with the advent of genome-wide approaches to characterize hematologic malignancies, recurrent pathologic changes in chromatin regulators have been identified (Cancer Genome Atlas Research, Ley *et al.* 2013; Papaemmanuil *et al.* 2016). Given our increasing awareness of mutations in epigenetic modifiers which have been associated with leukemia and given the development of epigenetic therapies, this review will provide a brief overview on the role of histone modifications in leukemogenesis.

The term *epigenetics* was first coined by Conrad Waddington in the 1940s to describe the process by which genes are regulated through post-translational modifications to manifest an expressed, heritable phenotype (Slack 2002; Berger *et al.* 2009). These epigenetic changes include covalent modification of nucleotides

This article is part of the Topical Collection: Chromatin Biology and Epigenetics.

Published online: 20 January 2020

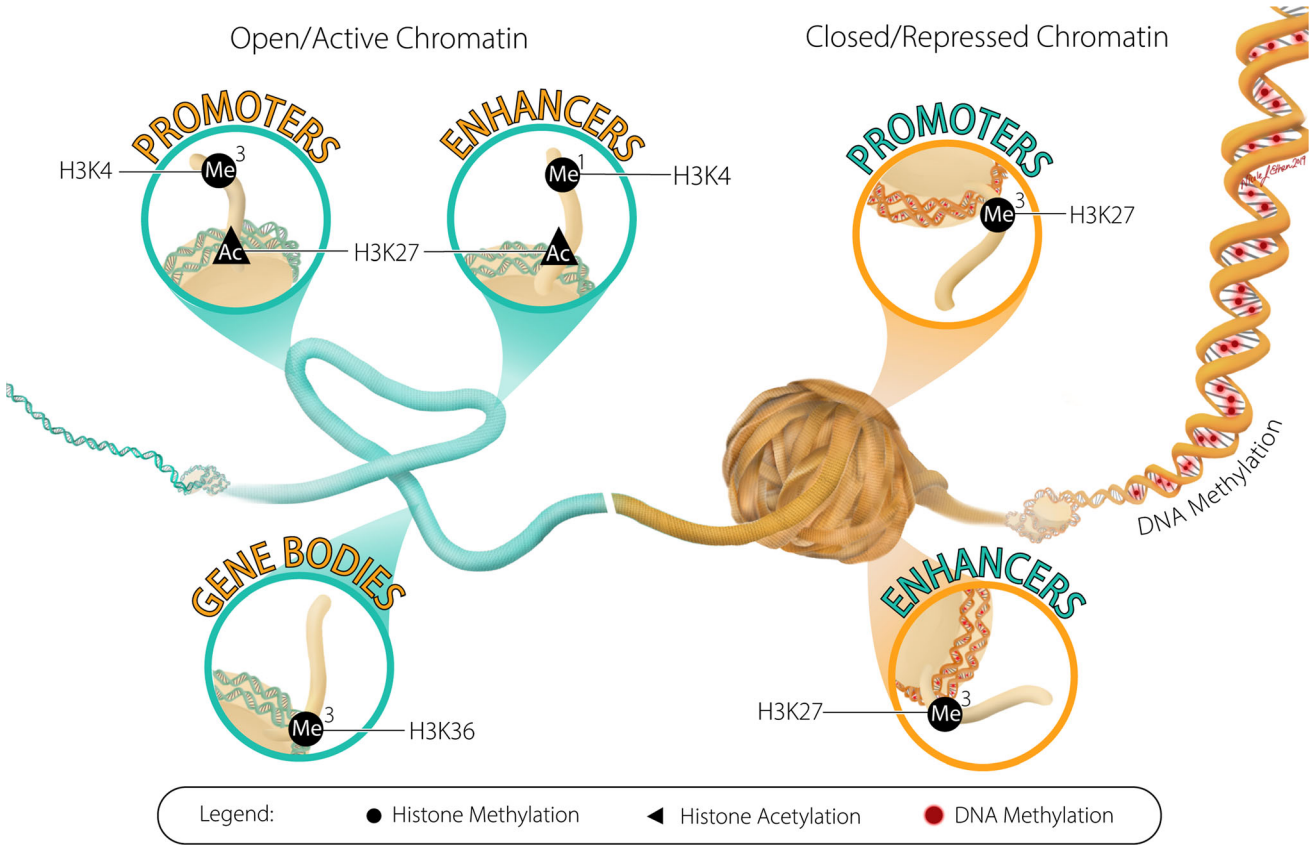

Figure 1. Schematic illustration of histone modifications in the context of open/active or closed/repressed chromatin states. Interacting histone marks located within gene structures at enhancers, promoters, and gene bodies form a coordinated epigenetic network that influences local chromatin structure and ultimately regulates gene transcription (illustration by Nicole Ethen).

and/or amino acids within chromatin. In recent decades, much research has been focused on identifying specific modifications which are typically placed on lysine or arginine residues of histone tails. Many of these histone marks have been associated with gene activation or repression and have been observed to be increased or decreased in preclinical models of leukemia suggesting pathogenic roles in leukemogenesis (figure 1).

2. Histone acetylation

Histone acetylation occurs at lysine residues on histone tails promoting an open chromatin conformation due to loss of the positive lysine charge which attracts the negatively charged phosphate backbone of DNA. Histone acetyltransferases (HATs) are the enzymes which catalyze acetylation of lysine residues. Two HATs have been directly implicated in leukemia – MOZ and CBP/P300 – through chromosomal translocations resulting in enhanced HAT activity, i.e. at H3K9 and H3K27(Sobulo *et al.* 1997; Santillan *et al.* 2006; Audia and Campbell 2016). Bromodomains (BRDs) which recognize and bind acetylated residues play important roles in hematopoiesis and have been implicated in leukemogenesis. In particular, CBP/P300 contain BRDs in addition to catalytic HAT activity which are critical for recognizing and maintaining open chromatin. The bromodomain and extraterminal domain (BET) family of proteins bind acetylated residues of which BRD4 has been extensively studied for its role in promoting transcriptional elongation of target genes in coordination with positive transcription elongation factor b (P-TEFb) (Chen *et al.* 2018). The ability of BRD4 to bind activated genes through its bromodomain and induce overexpression of oncogenes has made bromodomain targeting an active area of therapeutic development in recent years (Dawson *et al.* 2011; Cai *et al.* 2015; Roe *et al.* 2015).

3. Histone methylation

Aside from acetylation changes, lysine residues on histone tails can also be covalently modified through methylation by histone methyltransferases (HMTs).

More complex than a single acetylation mark, these residues can undergo mono-, di-, or trimethylation with altered implications based on the location of the methylation mark(s). The complex of proteins associated with Set1 (COMPASS) family is responsible for catalyzing methylation of H3K4 of which the KMT2A (or MLL1) protein has been directly implicated in aggressive forms of acute leukemia. In MLL-rearranged leukemia, this C-terminal SET domain with catalytic HMT activity is replaced by the C-terminus of an aberrant fusion partner (Popovic and Zeleznik-Le 2005; Shilatifard 2012). Despite the loss of the catalytic HMT activity, these MLL fusion proteins drive overexpression of target genes through recruitment of super elongation complex (SEC) components often as direct fusion partners resulting from the pathogenic chromosomal translocation (Popovic and Zeleznik-Le 2005; Shilatifard 2012). Overexpression of MLL-target genes is associated with increased H3K4me3 marks at gene promoters. Furthermore, MLL fusions have been associated with acquired methylation of H3K79 through recruitment of the methyltransferase DOT1L further resulting in sustained expression of MLL-target genes (Bernt *et al.* 2011; Nguyen *et al.* 2011).

The activating methylation marks placed by MLL/COMPASS on H3K4 are balanced by repressive methylation marks on H3K27 (Piunti and Shilatifard 2016). The Polycomb repressive complex 2 (PRC2) component EZH2 catalyzes methylation of H3K27 to promote gene repression. In the context of MLL-rearranged leukemia, the overactivation by MLL fusion proteins overcomes the repressive signals of the PRC2 complex (Piunti and Shilatifard 2016). While specific mutations in EZH2 are rarely found in acute leukemia, EZH2 mutations can be found in a subset of pre-leukemic myeloid malignancies, i.e. myelodysplastic syndrome and myeloproliferative neoplasms (Lund *et al.* 2014). The role of H3K27 methylation in hematologic malignancies was further observed in the context of T-cell acute lymphoid leukemia (T-ALL) where mutations in the H3K27 demethylase UTX or other PRC2 components, SUZ12 and EED, have contributed to altered levels of H3K27 methylation (Ntziachristos *et al.* 2012, 2014). It is hypothesized that inactivating mutations of UTX may lead to further repression of tumor suppressor genes contributing to leukemogenesis (Wang and Shilatifard 2019). Interestingly, though, another demethylase JMJD3 has been proposed to have an opposing role at these same H3K27 loci functioning as an oncogene in T-ALL, indicating that two distinct chromatin regulatory pathways may converge at similar H3K27 loci (Ntziachristos *et al.* 2014).

In contrast to H3K4 and H3K27 methylation marks which are typically placed on histone tails of non-coding regulatory regions of chromatin, methylation of H3K36 is associated with histones located *within* the gene bodies of actively transcribed genes (Bannister *et al.* 2005). The H3K36 methylation marks are placed by HMTs of the nuclear receptor-binding SET domain (NSD) family. Both AML patient samples and ALL cell lines have been associated with aberrations in NSD proteins – respective NUP98-NSD1 fusion proteins and activating NSD2 (E1099K) mutations – in which elevated levels of activating H3K36me3 marks have been observed (Cerveira *et al.* 2003; Jaffe *et al.* 2013).

4. Chromatin cross-talk: A complex network of interacting epigenetic modifications

Histone modifications are commonly found within the context of larger networks of chromatin interactions in which nearby modifications will influence the local chromatin environment to coordinate regulation of gene transcription. *Cis*-acting regulatory elements within non-coding regions of DNA, for example, make up enhancers and promoters with long- and short-range influence on gene expression each associated with distinct combinations of histone marks (Shlyueva *et al.* 2014; Rickels and Shilatifard 2018). Active promoters are marked with H3K4me3 and H3K27ac while active enhancers are associated with H3K4me1 and H3K27ac marks (Heintzman *et al.* 2007; Rickels and Shilatifard 2018). In contrast, poised enhancers exhibit bivalent chromatin marks of H3K4me1 and repressive H3K27me3 (Heintzman *et al.* 2007; Rickels and Shilatifard 2018). A schematic representation of interacting histone marks within the context of active or repressed chromatin is illustrated in figure 1.

Histone modifications form complex networks of interactions with other types of epigenetic changes including DNA methylation and hydroxymethylation which coordinate together to impact local gene expression (Hu and Shilatifard 2016). A more complete understanding of these epigenetic dynamics is being elucidated with the advent of next generation sequencing and genome-wide mapping of interacting histone modifications within the context of DNA methylation during normal hematopoiesis and pathologic leukemogenesis. A recent study by Yi *et al* used an integrated genome-wide approach to analyze global epigenetic changes to classify AML patient samples (Yi *et al.* 2019). By associating distinct ChIP-Seq histone methylation changes with RNA-Seq, DNaseI-Seq, and whole genome bisulfate sequencing, they were able to identify two

subtypes of AML phenotypes: one associated with active enhancers defined by strong H3K4me1 and H3K27ac signals with hypomethylated CpG regions correlating to open chromatin and active expression profiles and a second distinct subtype associated with inactive enhancers and closed chromatin (figure 1). The active enhancer group with a stem-like phenotype was called the *NPM1*/*MLL* C2 subtype while the second, closed-chromatin subtype was termed the *RUNX1*/Splicesome C1 group. Such classification based on an integrated understanding of an active versus repressive epigenomic state has intriguing implications for potential therapies which could inhibit or promote global chromatin activity as a personalized approach to leukemia treatment. More challenging, however, will be integrating this type of global, chromatin-based AML classification into clinical practice as the proposed subtypes pair AML samples of favorable risk groups (mutated *NPM1* AML) with conflicting intermediate- or high-risk AML types (*MLL*- and splicesome-associated AML).

Currently, epigenetic therapies for leukemia have been limited to hypomethylating agents, 5-azacitidine and decitabine, which inhibit DNA-methyltransferase (DNMT) activity. Direct targeting of histone regulators has yet to show sufficient clinical efficacy to earn FDA approval for leukemia treatment, though, early-phase clinical trials are underway (Cai *et al.* 2015). Furthermore, current clinical management of leukemia does not incorporate direct analysis of histone modifications into diagnostic, prognostic, or therapeutic approaches. Molecular analysis, however, of somatic mutations in genes encoding histone regulators has begun to be utilized for assessing prognosis and potential response to treatments of acute myeloid leukemia (Cancer Genome Atlas Research, Ley *et al.* 2013; Papaemmanuil *et al.* 2016). In contrast to somatic DNA mutations, histone modifications are reversible and offer attractive therapeutic potential: aberrant chromatin states observed in leukemia are ultimately dynamic and can be altered through targeted, epigenetic therapies. Through further genome-wide analyses of histone signatures in the context of associated genetic mutations and DNA methylation status, biomedical researchers may begin to develop a better understanding of the dynamic chromatin states which are perturbed both globally and at specific loci in leukemia in order to better predict patient treatment outcomes in this new era of personalized medicine.

Acknowledgements

The authors are grateful to Edwin Smith for his critical review of this manuscript and to Nicole Ethen for her figure illustration. The Shilatifard laboratory is supported in part by an Outstanding Investigator Award through the NIH's NCI (R35CA197569).

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