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REVIEW ARTICLE

Disturbing the histone code in leukemia: translocations and mutations affecting histone methyl transferases

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Leukemia is characterized by increased numbers of blasts originating from transformed early hematopoietic stem and progenitor cells. Genetic alterations are widely recognized as the main drivers of oncogenic transformation. Of considerable interest are mutations affecting the writers of epigenetic marks. In this review, we focus on histone methyltransferases—enzymes that catalyze the methylation of lysine residues in core histones. Histone methylation is a tightly controlled mechanism that is responsible for both activating as well as repressing gene expression in a site-specific manner, depending on which lysine residue is methylated. Histone methyltransferases, including *MLL1*, *DOT1L*, *EZH2*, and *SETD2* are recurrently deregulated in human leukemia, either directly by gene mutations or balanced translocations, or indirectly as components of protein complexes that are disturbed in leukemia due to alterations of the other components in these complexes. Several small molecule inhibitors of histone methyltransferases are currently being clinically evaluated for their therapeutic potential in human leukemia. These drugs reverse some of the adverse effects of aberrant histone methylation, and can induce differentiation and cell death in leukemic blasts.

Keywords Cancer, epigenetics, histone methyltransferases, histone methylation, leukemia

Leukemia is a heterogeneous group of diseases originating from transformed hematopoietic cells and can be divided into four main types: acute lymphoblastic leukemia (ALL), chronic lymphoblastic leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML). Patients often present with symptoms related to bone marrow failure, such as recurrent infections, bruising, abnormal bleeding, or anemia (1). Despite a number of recent advances in targeted therapy (e.g., the introduction of tyrosine kinase inhibitors in CML (2)), most forms of leukemia are treated with high-dose chemotherapy and/or hematopoietic stem cell transplantation, and the prognosis is often rather poor (1).

Cellular transformation is frequently driven by mutations affecting genes that are key regulators of hematopoietic differentiation and proliferation. Mutations in genes involved in epigenetic pathways, including histone methyltransferases, contribute to the transformation of early hematopoietic stem and progenitor cells by changing the chromatin landscape that affects transcription factor binding and oncogene and tumor suppressor gene expression (3,4).

The histone code

In eukaryotes, DNA is arranged together with histone proteins into nucleosomes to form tightly packaged chromatin. A single nucleosome is composed of 146 DNA base pairs and an octameric histone complex that contains two histone H2A, H2B, H3, and H4 molecules each. This structure poses a major hindrance for gene transcription, and the packing and unpacking of chromatin is recognized as an important mechanism in the regulation of gene expression. Epigenetic modifications of both DNA bases and histone proteins are the basis of cell-type specific gene expression. DNA cytosine methylation is associated with transcriptional silencing, whereas post-translational modifications of histone proteins have different functions, depending on the modified histone protein, the affected amino acid, and the type of alteration

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itself (5,6). Acetylation and phosphorylation of histone proteins loosen the electrostatic interactions between basic histone proteins and the anionic charge of DNA, which directly contributes to the unpacking of heterochromatin to euchromatin, allowing for easier access of the transcription machinery to the DNA. A number of other covalent modifications do not alter the electric charge of histone proteins, but instead provide a readable mark for highly specialized protein complexes. One such modification is the methylation of lysine residues (7–9). The combination of different histone marks, also referred to as the histone code, shows a high correlation with the transcriptional state of a genomic region and most likely plays a fundamental role in determining transcriptional activity. Establishing, maintaining, and dynamically changing the histone code are tightly regulated processes involving numerous protein families, and the disruption of this code is implicated in various diseases, including cancer, neuropsychiatric disorders, and metabolic disorders (10,11).

Recent advances in sequencing and in techniques that help to decipher the histone code revealed that proteins involved in the writing, reading, and erasing of histone marks are common targets of genetic and genomic aberrations in a wide spectrum of cancers. Point mutations, deletions, amplifications, and genomic rearrangements affecting histone-modifying enzymes were recurrently identified in human malignancies. As a result, significant changes occur in global and/or local epigenetic marks and subsequent alterations in gene expression and cellular signaling, which ultimately alter differentiation and contribute to malignant transformation. As specialized enzymes that are often affected by gain-of-function mutations mediate these epigenetic perturbations, such proteins are promising targets for personalized cancer therapy.

In this review, we focus on the heterogeneous group of histone methyltransferases in human leukemia, how these enzymes contribute to leukemogenesis, and the recent advances that have been made to exploit these enzymes as therapeutic targets.

Histone methyltransferases

Protein methyltransferases catalyze methyl group transfer from the universal donor S-adenosyl-methionine (SAM) to N-terminal amino groups, carboxyl groups of C-terminal leucine or isoprenylated cysteine residues, or to the side-chain nitrogen atoms of lysine, arginine, and histidine residues (12). The occurrence of methylated lysine residues within histone proteins was recognized early (13), but we have only now begun to fully understand the consequences of these modifications.

A recent study identified 51 protein lysine methyltransferases in the human genome based on the conserved SET (suppressor of variegation, enhancer of zeste, trithorax) domain. The only known lysine methyltransferase that does not contain this domain is DOT1L (14). Histone lysine methyltransferases are highly selective enzymes that add one to three methyl residues to the ε-nitrogen atom of distinct lysine side chains within histone proteins. Most commonly, the tail regions of histones are modified in this way. Well-studied targets of lysine methylation are residues K4, K9, K27, and K36 (within the N-terminal tail of histone H3), K79 (within the globular domain of histone H3), and K20 (of histone H4) (8,15). These marks can be read by evolutionarily conserved recognition motifs (9) and target reader proteins and associated complexes to the modified chromatin regions.

Lysine methylation of histone proteins is an important mechanism for specifying whether a gene is transcriptionally active or silent (Table 1). Methylation at H3K9, H3K27, and H4K20 is generally associated with transcriptional repression, whereas methylation at H3K4, H3K36, and H3K79 correlates with active transcription. The eventual outcome of different methyl marks not only depends on the modified residue itself, but also on the context of other post-translational histone modifications on the same and neighboring nucleosomes (histone code) (15,16). To add a layer of complexity, recent research indicates that histone methylation may function in regulating gene expression by affecting nucleosome positioning (17,18). Furthermore, histone methylation is important for X inactivation, cell fate determination, and terminal differentiation (19,20).

Several histone methyltransferases are involved in human carcinogenesis and especially in leukemogenesis. These enzymes were found to be recurrently mutated or rearranged in leukemia and contribute to malignant transformation by both loss- and gain-of-function mechanisms.

Mixed lineage leukemia 1 (MLL1)

Structure and function of normal MLL1

The 3,969 amino acid large multidomain MLL1 protein encompasses the following: 3 DNA binding AT-hooks at the

Table 1 Characteristics of histone methylation marks

Histone	Lysine residue	Writer	Location	Effect
H3	K4	MLL1	Enhancers, promoters, transcriptional start sites	Transcriptional activation
	K9	Various	Constitutively repressed genes	Gene repression and silenced chromatin
	K27	EZH2	Facultatively repressed genes	Gene repression and silenced chromatin
	K36	SETD2, NSD	Body of actively transcribed genes	Transcriptional elongation
	K79	DOT1L	Body of actively transcribed genes	Transcriptional elongation
H4	K20	Various	Pericentric heterochromatin, telomeres, imprinted regions, and repetitive elements	Gene repression and silenced chromatin