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The role of DOT1L in the maintenance of leukemia gene expression

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Chromatin based (Epigenetic) mechanisms have been shown to play important roles in the regulation of gene expression during tumorigenesis and development. Mouse modeling suggests the methyltransferase DOT1L as a potential therapeutic target for MLL-rearranged leukemia. Epigenomic profiling indicates an abnormal H3K79me2 pattern on MLL-fusion targeted genes, but the molecular mechanism underlying this epigenetic dependency is not well understood. In this review, we will discuss recent advances in understanding the epigenetic mechanisms governed by DOT1L in the maintenance of gene expression. We will highlight the structural basis of chromatin targeting of DOT1L through its cofactors and the role of DOT1L in repelling transcription repressive complexes during leukemia development.

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Introduction

Posttranslational (PTM) modifications of histone proteins are common chromatin-based (epigenetic) mechanisms by which cells regulate gene expression. These modifications along with DNA methylation may influence how cells inherit lineage-specific transcription programs during development. Enzymes termed 'writers' catalyze covalent modifications of histones, and 'erasers' removes these modifications. Many histone writers and erasers have been shown to play important roles in developmental transitions and various diseases.

Disruptor of telomere silencing 1 (Dot1) is the only known methyltransferase that catalyzes mono-, di-methylation and tri-methylation of histone 3 lysine 79 (H3K79) [1,2,3]. No demethylase has been identified to remove H3K79 methylation, suggesting the regulation of Dot1 activity is likely to be the dominant determinant for H3K79 methylation. Dot1 was initially identified as a gene involved in the regulation of telomere silencing in *Saccharomyces cerevisiae* [4]. DOT1L, the mammalian homolog of Dot1, is required for the maintenance of *Mixed Lineage Leukemia* gene (*MLL*)-rearranged leukemias by facilitating the aberrant expression of *Homeobox (HOX)* genes and their cofactors [5,6]. DOT1L has also been shown to be important for somatic cell reprogramming and early embryonic development. Inhibition of DOT1L accelerates reprogramming of somatic cells to induced pluripotent stem cells [7]. Genetic deletion of Dot1l leads to cardiac defects and defects in erythropoiesis during early mouse development [8,9]. These data demonstrate an important role for DOT1L in the transition and maintenance of different developmental states. These roles are likely controlled, at least in part, by the modulation of developmental gene expression since epigenomic profiling of H3K79 methylation indicates that this modification is correlated with transcriptionally active gene bodies in different contexts [10–12]. However the exact role of DOT1L in the control of developmental gene expression remains incompletely characterized.

This review will focus on recent advances and important unresolved questions in understanding the role of DOT1L in the regulation of gene expression. First, we will discuss the role of DOT1L interacting partners, AF9 and AF10, which are proposed as readers of histone modifications that may recruit DOT1L complex to regions of open chromatin [13••,14•,15••]. We will then outline one of the functions of DOT1L in mammalian cells, which is to prevent SIRT1-mediated gene silencing in *MLL*-rearranged leukemia cells [16••]. Finally, we will discuss the role of H3K79 methylation as a mechanism to selectively modulate specific gene expression programs, and the potential of DOT1L as a therapeutic target.

DOT1L is targeted to specific chromatin regions through interaction with readers of histone modifications

DOT1L is found in multi-protein complexes with AF9, AF10, and ENL among other proteins, many of which are MLL-fusion partners [17–19]. In *MLL*-rearranged leukemia, these partners interact with DOT1L and thus recruit the DOT1L complex to MLL-fusion protein target genes. This recruitment explains the aberrantly high level of H3K79 methylation at MLL-fusion target loci

[5]. Loss of the DOT1L–AF9 interaction through point mutations abrogates the transformation ability of MLL-AF9 fusion [20]. However, in other contexts outside of *MLL*-rearranged leukemia, the recruitment of DOT1L complexes to chromatin remains not well defined.

Recent structural biology studies propose that AF9 and AF10 recognize histone peptides with specific modifications, and through interaction with these modifications enhance recruitment of DOT1L to loci with specific chromatin states [13••,15••]. These so-called readers of histone modifications are proteins that contain specific domains that recognize histone peptides with specific modifications. Through interaction with specific chromatin modifications, the reader proteins are believed to target histone modifying complexes like DOT1L, chromatin remodelers and/or transcription factors, and thereby help transmit histone modification information from chromatin to transcription (reviewed in [21]). For instance, another chromatin reading protein, Bromodomain-containing 4 (BRD4), recognizes histone acetylation and interacts with the general transcriptional Mediator complex [22]. Inhibition of BRD4 suppresses transcription of important oncogenes, such as *MYC*, in multiple cancers [22,23], indicating that readers of histone modifications play a critical role in the regulation of oncogene expression.

Multiple studies have demonstrated that the AF9 protein interacts with DOT1L in a stable complex through its C-terminus [19,24,25]. Structural and biochemical data show that AF9 interacts with acetylated histone 3 lysine 9 (H3K9ac) through its N-terminal YEATS domain [15••]. H3K9ac is enriched in transcriptionally active genes and more accessible chromatin regions. Chromatin immunoprecipitation assays followed by high-throughput sequencing (ChIP-seq) of AF9, H3K9ac, and H3K79me3 suggest the majority of the AF9 bound genes are enriched for H3K9ac and H3K79me3. Knockdown of AF9 leads to decreased H3K79 methylation on AF9 targeted genes[15••], which can be rescued by wild-type (WT) AF9, but not H3K9ac-binding deficient AF9 mutants. These studies suggest that the histone acetylation reader AF9 recruits the DOT1L complex to transcriptionally active genes decorated with H3K9ac, and thereby helps maintain the open chromatin state.

Another DOT1L binding partner AF10 has been shown to determine higher H3K79 methylation states (H3K79me2, H3K79me3) and maintain the expression level of DOT1L-dependent genes in multiple acute myeloid leukemia (AML) models [14•]. Structural studies of AF10 show that the PZP domain of AF10 recognizes unmodified H3K27, but not methylated H3K27 [13••], indicating a potential crosstalk may exist between H3K27 methylation and H3K79 methylation through AF10. H3K27me3 usually correlates with gene repression and does not often co-occur on the same genomic region with higher methylation states of H3K79. The transition from H3K79me2 and H3K79me3 to H3K27me3 happens on *HoxA* cluster during hematopoiesis as cells transition from hematopoietic stem cells (HSC) to more mature myeloid cell types [14•], and may act as an epigenetic mechanism for fine-tuning expression of important developmental genes during differentiation. The structural study of AF10 proposes an explanation for the relationship between H3K27 methylation and H3K79 methylation, indicating DOT1L might be repelled from chromatin covered with H3K27 methylation thus demonstrating why it is that H3K79 methylation and H3K27 methylation are generally mutually exclusive throughout the genome.

DOT1L antagonizes gene silencing mediated by the SIRT1 complex

One mechanism of gene silencing involves the establishment of an inaccessible chromatin state. Epigenetic modifications associated with gene silencing including H3K9 methylation and DNA methylation, which prevent accessibility of the transcriptional machinery to DNA. A well-studied example is the silencing of tumor suppressor genes in many cancer cells by the formation of heterochromatin and DNA methylation (reviewed in [26]). In *Saccharomyces cerevisiae*, Sir (Silent information regulator)-dependent gene silencing is important for the regulation of yeast homothallic mating (HM) type loci and telomeric loci. Sir2, an NAD (nicotinamide adenine dinucleotide) dependent enzyme that deacetylates histone lysines, forms the SIR complex with Sir3 and Sir4, and represses gene expression at HM loci and telomeric loci (reviewed in [27]). Dot1 is proposed to restrict genomic localization of Sir complex and prevent the Sir complex from spreading along the nucleosomes [1,2,28,29]. Loss of Dot1 accelerates the establishment of mating-type gene silencing [28,29], possibly because Dot1 has the ability to compete with Sir2 for binding sites on H4 [30].

In mammals, H3K79 methylation is associated with transcriptionally active gene regions, but the role of DOT1L in regulating gene expression has been less clear. In leukemia with rearrangements of the *MLL* gene, genetic deletion of Dot1l leads to differentiation, decreased cell growth and down-regulation of MLL-fusion target genes, including critical leukemogenesis regulators, such as the *HOXA* cluster genes and their cofactor *MEIS1* [5]. A recent study proposes that H3K79 methylation antagonizes SIRT1 and SUV39H1 binding to genomic regions that are targeted by MLL-AF9 fusion, and, therefore, facilitates aberrant transcription of *HOXA* cluster and other MLL-fusion target genes in *MLL*-rearranged leukemia [16••]. Inhibition of DOT1L leads to enhanced occupancy of SIRT1 and deacetylation of H3K9 on MLL-AF9 bound genes, resulting in accumulation of repressive histone modifications, such as H3K9 methylation, and down-regulation of *HOXA* cluster genes and *MEIS1* [16••]. Small molecule activators of SIRT1 synergize with DOT1L inhibitors and accelerate