**Hematological Oncology**
*Hematol Oncol* 2015; **33**: 42–45
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/hon.2215

**Supplement Article**

# VII. Are lymphomas driven by epigenetic lesions?

Isaac W. Boss and Ari M. Melnick*

*Sandra and Edward Meyer Cancer Center, Weill Cornell Medical College, New York, NY, USA*

**Correspondence to: Ari M. Melnick, Weill Cornell Medical College, 413 E. 69th Street, Suite 1430, New York, NY 10021, USA.*
*E-mail: amm2014@med.cornell.edu*

**Keywords: B cell; lymphoma; epigenetic regulation; epigenetic modifier mutations; KMT2D; CBP; P300; EZH2; clinical treatment strategies**

## Introduction

Epigenetic regulation of gene expression is a process driven by dynamic changes in chromatin architecture carried out by histone-modifying enzymes. In eukaryotic organisms, chromatin organization allows tight packaging of the genome while still allowing access to transcriptional machinery. The basic unit of chromatin is the nucleosome, which consists of DNA wrapped around an octamer of histone proteins. Histone tails are post-translationally modified by covalent modifications such as methylation or acetylation that occur at lysine and arginine residues. These histone modifications, or 'marks', result in changes to chromatin structure and serve as a histone code that directs the ordered recruitment of effector proteins that control gene transcription, replication and DNA repair [1]. A number of enzymes are responsible for establishing the histone code, by functioning as writers, readers and erasers of histone marks. These epigenetic modifiers are directed by transcription factors to histones located within DNA regulatory elements, including enhancers and promoters, allowing gene expression to be turned on or off. Histone marks associated with transcriptional activation include histone 3 lysine 4 methylation (H3K4me) and H3 lysine 27 acetylation (H3K27ac), whereas H3 lysine 27 methylation (H3K27me) is generally linked to repression. The functional state of enhancer regions that control cell context-specific gene expression can be defined from the chromatin standpoint as being in an active (H3K4me1/2 and H3K27ac), or poised (H3K4me1/2 and H3K27me3) configuration. Specific patterning of histone marks is context dependent and can change rapidly in response to external stimuli, providing a layer of epigenetic control to regulate cell fate and function decisions. Somatic mutation of histone-modifying enzymes is now recognized as a hallmark of certain forms of B-cell lymphomas. Here, we will focus on some of the more frequently affected histone-modifying enzymes in B-cell lymphomas and their potential mechanisms of action.

## Epigenetic mechanisms involved in B-cell lymphomagenesis

Next-generation sequencing of follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) genomes has uncovered frequent somatic mutations affecting histone-modifying proteins, pointing towards a significant contribution of altered epigenetic regulation in B-cell malignancy [2]. FL and DLBCL are germinal centre (GC)-derived B-cell lymphomas often featuring genetic lesions that induce constitutive expression of oncogenes (such as MYC, BCL6 and BCL2) or that inactivate canonical or novel tumour suppressors (p53, PRDM1 and A20). DLBCLs and FLs are among the tumour types with the highest burden of genetic lesions, which is likely the consequence of the genomic instability characteristic of B cells undergoing the GC reaction. During the T-cell-dependent immune response, resting naïve B cells undergo massive changes in transcriptional programming, resulting in their migration within lymphoid follicles and formation of GCs. Within the proliferative dark zone of these GCs, B cells undergo rapid replication and somatic hypermutation of their immunoglobulin loci to generate high affinity antibodies. This process results in DNA damage, which can lead to genetic mutations that induce transformation and outgrowth of malignant B cells. While extensive studies have characterized the role that genetic lesions play in promoting deregulated gene expression and transformation, only recently has the role of epigenetic mechanisms in lymphomagenesis been investigated.

It is notable that some of the most frequent somatic mutations occurring in lymphomas affect histone-modifying proteins *KMT2D* (also called MLL2 or MLL4), *EZH2*, *CREBBP* and *EP300* [2–4]. These mutations are mostly heterozygous. While mutations in the epigenetic transcriptional activators *KMT2D*, *CREBBP* and *EP300* are predicted to disable their catalytic activity; mutations in the epigenetic repressor *EZH2* were found to enhance its

activity, suggesting that transcriptional repression is the overall consequence of all of these mutations (Figure 1). Mutation of these four modifiers seems to be an early event during lymphomagenesis based on their allele variant frequency, indicating that they are potential driver mutations in these malignancies [5,6].

\EZH2 is a H3K27 methyltransferase, which silences gene expression through its secondary recruitment of Polycomb Repressive Complex 1 (PRC1). In embryonic stem cells, EZH2 plays an essential role in establishing cell lineage fate by keeping transcriptional regulator genes in a poised but inactive state of expression [7]. EZH2 is required for the development of GC B cells, through its direct repression of cell cycle checkpoint genes (e.g. *CDKN1A*) and genes that drive terminal differentiation of GC B cells (e.g. *IRF4* and *PRDM1*) [8]. Somatic mutations in *EZH2* occur in approximately 20–30% of FL and germinal center B cell (GCB)-DLBCL patients and mostly affect residue Y641 within its catalytic SET domain [2]. These mutations enhance the ability of EZH2 to mediate H3K27 trimethylation, resulting in a global increase of H3K27me3 and a hyper-repression of natural GC EZH2 target genes, such as *CDKN1A* and *IRF4* [8,9]. Conditional expression of mutant EZH2 in GC B cells leads to GC hyperplasia and cooperates with BCL2 to accelerate lymphomagenesis [8]. Recently developed specific EZH2 inhibitors have potent anti-lymphoma effects *in vitro*, in lymphoma xenograft studies, and in human patients with DLBCL [10,11].

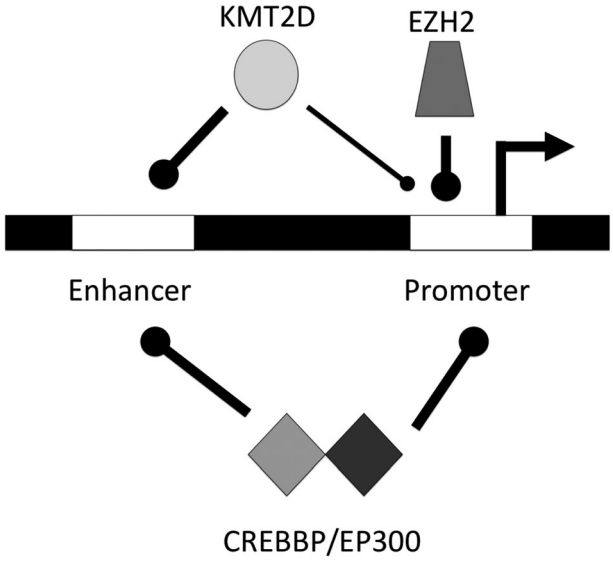


**Figure 1.** Epigenetic modifiers target regulatory regions of DNA to control gene expression. KMT2D promotes target gene expression by methylating histone tails found in enhancers and less frequently in promoters. CBP and P300 promote gene expression by acetylating histone tails found in enhancers and promoters. EZH2 represses gene expression by methylating histone tails mostly at promoters.

Inactivating mutations in the histone acetyltransferases, *CREBBP* and *EP300* were found to occur in approximately 30% of FL and DLBCL patients [2–4]. CREBBP and EP300 belong to the KAT3 family of proteins, which acetylate lysine residues in histones and many other proteins. Recent data suggest that the function of the BCL6 transcriptional repressor is to antagonize the activation of gene enhancers that are activated by *CREBBP/EP300*, in which case loss of function of these proteins might enable unopposed activity of BCL6 in transforming B cells [12]. In addition, *CREBBP/EP300* inactivating mutations could result in reduced acetylation of BCL6 and p53, which could facilitate the actions of the former and impair the functions of the latter [3]. Mutations in EP300 may also suppress expression of NFκB target genes, A20 and IκBa [13], and promote the oncogenic functions of Hsp90 in lymphoma cells [4]. These various scenarios are consistent with a tumour suppressor function for *CREBBP* and *EP300*.

KMT2D is a sub-unit of the COMPASS (Complex Of Proteins ASsociated with Set1) complex, which facilitates transcription through mono-methylation, di-methylation and tri-methylation of H3K4. While a B cell intrinsic function for KMT2D has yet to be defined, it is one of the most frequently mutated epigenetic modifiers in FL and DLBCL (~30–40%) [2,14]. The majority of *KMT2D* mutations are nonsense or frameshift and occur upstream of the catalytic C-terminal SET domain, which can lead to truncations that disable its methyltransferase activity. Unlike *EZH2* mutations, which only occur in GCB-DLBCLs, *KMT2D* (and *CREBBP/EP300)* mutations are also found in activated B cell (ABC)-DLBLCs [2]. Based on the frequent occurrence of inactivating mutations in cancer, KMT2D was proposed to act as a tumour suppressor. However, *KMT2D* shRNA-knockdown in a breast cancer cell line (MDA-MB-231) caused reduced proliferation and decreased expression of cell proliferation genes [15]. A second study found that *KMT2D* knockout, in murine hematopoietic progenitor cells, failed to form MLL-AF9 oncogene-driven leukaemia [16]. Both studies suggest that some types of cancers are dependent on normal KMT2D function during transformation and proliferation. Recently, KMT2D was found to be a major regulator of enhancers [17,18]. *KMT2D* deletion in human colon cancer cell line (HCT116) and mouse embryonic fibroblasts resulted in reduced H3K4me1 and H3K4me2, predominately at enhancers [17]. In a separate study, KMT2D was found to be recruited to enhancers by cell lineage transcription factors during adipogenesis and myogenesis, where it promotes activation of cell type specific genes involved in differentiation [18]. Therefore, it seems plausible that KMT2D may also regulate enhancer activation during B-cell differentiation, and loss of this function might facilitate lymphomagenesis.

A hallmark of FL and DLBCL is their inability to differentiate into memory or plasma cells, which increases their



DOI: 10.1002/hon

chance of further mutation and transformation because of persistence of the GC phenotype. This process requires the rapid switching of transcription programs in response to specific signalling pathways, such as CD40, B-cell receptor, and interleukins. Epigenetic mechanisms play a crucial role in this process by opening or closing chromatin to orchestrate gene expression in a stimulus-dependent manner [8,12]. While epigenetic regulatory mechanisms during B-cell activation and terminal differentiation have not been well characterized, evidence provided by studies in activated macrophages provides potential insights. For example, when macrophages are stimulated with the TLR agonist LPS, epigenetic remodelling rapidly occurs with lineage specific transcription factors PU.1 and NFκB-recruiting histone methyltransferases, including KMT2D, to approxiamtely 3000 de-novo enhancer sites where they deposit H3K4me1/2 marks[19]. In addition to KMT2D, p300 is also recruited to de novo enhancers upon LPS stimulation in macrophages[20]. In DLBCL cell lines, EZH2 mutations were found to promote repression of bivalent chromatin regions in a set of genes that activate the terminal differentiation programme [8]. Inactivating mutations in KMTD, which often co-occur with EZH2 mutations [2], could synergize to further deregulate enhancer poising and activation in GC B-cells. This could disable the ability of B cells to respond to signals that would normally induce terminal differentiation and cessation of the GC proliferation and survival programmes. Future studies are needed to determine how mutations in these histone-modifying proteins influence enhancer remodelling during B-cell stimulation and subsequent differentiation to promote malignancy.

## Conclusions

Genetic lesions that perturb histone-modifying enzymes are providing fundamental insights into mechanisms that determine the transcriptional programme and phenotype of lymphoma cells. The implications for lymphoma therapy are profound, because these aberrant epigenetic instructions are potentially reversible through the use of drugs that reverse the effects of these enzymes. While most DLBCL patients initially respond to the current standard R-CHOP regimen, approximately 30% are unresponsive to treatment and one-third undergo relapse. Treatment decisions in DLBCL are currently based on indirect biomarkers of clinical risk. Assigning patients to epigenetic therapies based on the nature of their somatic mutations could become a powerful new way to treat these patients. Because the effect of these somatic mutations is linked at least in part to aberrant transcriptional repression, it is intriguing to consider whether therapies that reverse repression could be useful. For example, it was demonstrated that histone deacetylase (HDAC) inhibitors can reverse the effect of KMT2D mutation on gene regulation and biological phenotype in a model of Kabuki syndrome [21]. Newly developed EZH2 inhibitors have activity in reversing aberrant repression mediated by this protein in patients with DLBCL in pre-clinical and clinical studies [8,10]. However, because epigenetic modifiers also play essential functions in normal cell biology, limiting their effects to malignant cells will be important. Because some cancer cells require normal KMT2D for proliferation, an intriguing idea is that small molecule inhibitors targeting KMT2D may preferentially kill lymphomas harbouring heterozygous *KMT2D* mutations. Conversely, inhibitors that block enzymes that oppose the action of KMT2D or CREBBP/EP300 (such as histone lysine demethylases or specific histone deacetylases) might be useful to re-establish the proper balance of epigenetic marks. Finally, given that epigenetic writers are downstream effectors of signalling pathways, their inactivation may also predict the efficacy of immunotherapies that require activation of specific downstream pathways in order to kill lymphoma cells. In order for these epigenetic-targeted strategies to work, a better understanding of the complex role that epigenetic modifiers play in regulating normal and malignant B cell signalling pathways will be essential.

### Conflict of interest

Dr. Melnick has been a consultant for Epizyme, Celgene, Roche, and Eli Lilly. All of these companies are engaged in development of epigenetic therapies. However, the current manuscript does not promote the use of any particular agent.

### Acknowledgement

I. W.B. is supported by the Sass Foundation. A. M. is supported by NCI R01 CA187109 and the LLS translational research programme.

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DOI: 10.1002/hon