IN THE SPOTLIGHT

Targeting HDAC3 in *CREBBP*-Mutant Lymphomas Counterstrikes Unopposed Enhancer Deacetylation of B-cell Signaling and Immune Response Genes

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Summary: The cellular phenotype of B-cell lymphomas arising from B cells undergoing germinal center reactions, such as follicular lymphoma and diffuse large B-cell lymphoma, is strongly shaped by mutations in chromatin-modifying genes. The work presented by Jiang and colleagues addresses how somatic mutations in CREBBP disable acetylation and cause unopposed deacetylation by BCL6/SMRT/HDAC3 complexes on enhancers of B-cell signaling and immune response genes. This opens a therapeutic avenue toward targeted inhibition of CREBBP-mutant lymphomas by HDAC inhibitors. *Cancer Discov; 7(1); 14–6. ©2017 AACR.*

See related article by Jiang et al., p. 38 (9).

The two most common non-Hodgkin lymphomas (NHL), follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL), are clinically characterized by the occurrence of multiple relapses and are still incurable in a substantial number of cases. Gene-expressing profiling of human B-cell lymphomas and normal B-cell subsets revealed a germinal center (GC) B-cell signature that was associated with FL, Burkitt lymphoma, and a subset of DLBCLs, suggesting the GC B-cell (GCB) origin of these lymphomas (1). FL and the GCB-like DLBCL subtype share their cellular origin but also similar pathogenetic mechanisms with regard to their chromosomal translocations. A cytogenetic hallmark in FL, but also in about 30% of *de novo* GCB-like DLBCL, is the chromosomal translocation (14;18)(q32;q21) of the *BCL2* oncogene next to the immunoglobulin heavy chain locus (1, 2).

However, these chromosomal translocations alone are not sufficient to drive lymphomagenesis, because they also occur in healthy individuals (2). Hence, in addition to cytogenetic alterations, factors such as antigen-mediated B-cell receptor (BCR) engagement as well as mutations of genes encoding proteins that modify chromatin have important roles in the pathogenesis of B-cell malignancies (3). Given the multifaceted roles of chromatin modifiers in cell fate decisions, including the development and activation of B cells, it is not surprising that mutations of chromatin modifiers have emerged as hallmarks of B-cell disorders.

Recent exome and transcriptome sequencing data revealed that genes with a role in histone modification were frequent targets of somatic mutations, including activating and inactivating mutations of genes that perform post-translational modification (PTM) of histones and organize the chromatin structure. PTMs of histones influence transcription initiation and elongation by directly affecting chromatin assembly or by serving as binding sites for effector proteins such as chromatin-modifying and chromatin-remodeling complexes. Histone PTMs are reversible, and enzymes mediating their addition or removal include histone acetyltransferases (HAT) and histone deacetylases (HDAC), lysine methyltransferases (KMT), and lysine demethylases, as well as ubiquitylation and deubiquitylation enzymes (3).

Two pioneering studies, by Pasqualucci and colleagues (4) and Morin and colleagues (5), identified frequent mutations of histone-modifying genes in DLBCL and FL. This included somatic mutations in genes such as *KMT2D/MLL2*, encoding for histone methyltransferases, or genomic deletions and somatic mutations that inactivate the HAT coding domains of *CREBBP* and *EP300*. Interestingly, structural alterations that inactivate CREBBP and EP300 are virtually absent in solid tumors but have been found in about 39% of DLBCL cases and in 41% of FL cases, identifying *CREBBP/EP300* mutations as a major pathogenetic mechanism in common B-NHLs. Moreover, these lesions are usually monoallelic, suggesting that a reduction in HAT dosage is crucial for malignant transformation (4). However, the exact mechanism for how these mutations drive malignant transformation has not yet been described. In this context, *CREBBP* mutations have been identified as early events within lymphoma cell progenitors, and it was suggested that these lesions might contribute to immune evasion via suppressed antigen presentation (6).

Similarly, the H3K4 methyltransferase *KMT2D* is frequently mutated in DLBCL and FL, and KMT2D loss has also been described as an early event preceding FL and DLBCL transformation (7). Zhang and colleagues first documented that FL- and DLBCL-associated *KMT2D* mutations disrupt KMT2D enzymatic activity, which leads to an impaired methylation of the enhancer H3K4 in GC B cells and DLBCL cells. Genetic ablation of *Kmt2d* in mice overexpressing BCL2 increased the development of GC-derived lymphomas. Thus, the role of KMT2D as a tumor suppressor gene was implied

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and a therapeutic approach that would combine inhibitors to BCL2 and histone methyltransferases or demethylases was suggested. A complementary study by Ortega-Molina and colleagues demonstrated that KMT2D controls the expression of multiple key regulators of CD40 and B-cell receptor activation and functions as a tumor suppressor *in vivo* (8).

In this issue of *Cancer Discovery*, Jiang and colleagues (9) suggested that although both CREBBP and KMT2D are enhancer regulators, distinct nonoverlapping functions partially exist. The authors addressed the downstream mechanisms of how loss of histone acetyl transferases could accelerate lymphomagenesis. First utilizing the *Vav-Bcl2* transgenic mouse model, they corroborated that the two interacting HAT genes, *Crebbp* and *Ep300*, also function as tumor suppressor genes and that *Crebbp* and *Ep300* deficiency promotes GC lymphomagenesis *in vivo*. The authors demonstrated alterations in transcriptional and epigenetic signatures after CREBBP knockdown in murine and human cells and concluded that these signatures are very similar to those found in human lymphomas with *CREBBP* mutations. Mechanistically, they revealed that CREBBP loss of function caused a preferential depletion of enhancer H3K27 acetylation. Importantly, they validated the murine model by demonstrating a significant enrichment of the murine CREBBP enhancer H3K27ac-loss gene signature in human lymphoma cell enhancer H3K27ac-loss genes with CREBBP knockdown.

To dive even deeper into the mechanisms how CREBBP loss of function affects enhancer function, Jiang and colleagues performed chromatin immunoprecipitation sequencing (ChIP-seq) for H3K4me2, H3K4me3, and H3K27ac to map the location of these enhancers in human GC B cells, the benign counterparts of DLBCL and FL. Interestingly, among the sites that lose H3K27 acetylation after CREBBP knockdown, substantial enrichment for genes that also lose enhancer activity in GC B cells was seen. Hence, lymphomagenesis somehow recapitulates aspects of the GC B-cell phenotype. Additionally, in human cells, an enrichment for antigen presentation and MHC class II genes could be detected. This is an important aspect because the ultimate phenotypic consequence of *CREBBP* mutations has not yet been well defined. In this line, a recent paper by Green and colleagues (6) reported that *CREBBP* mutations in early FL progenitors are associated with suppressed antigen presentation and by that contribute to immune evasion in FL. Together, both reports provide strong evidence that *CREBBP* mutations can contribute to immune evasion.

Furthermore, comparing CREBBP knockdown RNA sequencing and H3K27ac ChIP-seq profiles in mice and human cells to a database of B-cell ChIP-seq and transcriptional profiles, Jiang and colleagues found a substantial enrichment for genes whose enhancers are bound by the BCL6/SMRT transcriptional repressor complex. They proposed opposing effects of the CREBBP/EP300 and BCL6/SMRT complexes on enhancers that are silenced in GC B cells but activated upon GC exit and antigen stimulation, and implicated a novel function of BCL6 in downmodulating the MHC II locus during GC reactions. Next, the authors confirmed that human *CREBBP*-mutant lymphomas exhibited a GC/BCL6 target enhancer repression signature and thus proved that the experimental CREBBP signatures were also relevant in the human disease. One last important mechanistic point addressed here was whether *CREBBP*-mutant lymphoma cells are preferentially dependent on the histone deacetylase HDAC3. This hypothesis was raised because HDAC3 is known to be a component of the corepressor SMRT/NCOR complex in normal GC B cells and mediates H3K27 deacetylation of enhancers upon induction of BCL6. Utilizing transgenic mouse strains that express mutant forms of the corepressor complex or hold a deletion of *Hdac3* revealed that HDAC3 is indeed required by the BCL6/SMRT complex to establish GC responses. Because GC enhancer deacetylation is mediated by HDAC3, *CREBBP*-mutant lymphomas also become strongly dependent on HDAC3, as the authors proved in both the *CREBBP*-mutant DLBCL cells as well as in the CREBBP loss-of-function assays. Most intriguingly, Jiang and colleagues showed that CREBBP loss of function directly mediates loss of MHC class II expression and subsequently impairs the ability to activate T cells that the authors could effectively rescue by targeting HDAC3 with a selective inhibitor. The current study further supports recently published data by Green and colleagues (6), which provided ample evidence that *CREBBP* mutations were associated with a signature of decreased antigen presentation. Tumor B cells exhibited reduced MHC class II expression, and tumor-infiltrating T cells were diminished and less proliferative.

However, other factors most likely also contribute to lymphoma B-cell and tumor-infiltrating T-cell interactions, aside from *CREBBP* mutation–associated downregulation of MHC class II. For example, other target genes of BCL6 and CREBBP such as *CIITA* can also mediate MHC class II expression and can by themselves be mutated in B-cell malignancies. On the other hand, Pasqualiucci and colleagues described that *CREBBP* mutations additionally introduce other specific defects in acetylation-mediated inactivation of the BCL6 oncoprotein and in activation of the p53 tumor suppressor (4). Hence, further studies are warranted to unravel alternative effects of *CREBBP* mutations in lymphomagenesis.

Altogether Jiang and colleagues provide an intriguing mechanistic model of how *CREBBP* mutations disable acetylation and cause unopposed deacetylation by BCL6/SMRT/HDAC3 complexes at enhancers of B-cell signaling and immune response genes (Fig. 1), and by that promote lymphomagenesis. This and other related studies have direct implications for the use of drugs targeting acetylation/deactylation mechanisms in B-NHL. The data presented here provide a rational basis for the use of HDAC inhibitors in B-NHL, because they may contribute to reestablishing physiologic acetylation levels and subsequently lead to the restoration of tumor immune surveillance. Based on the growing number of specific epigenetic targets, the efficacy of applied HDAC inhibitors should be reevaluated based on the presence of HAT defects critical for disease pathogenesis. Curated databases from cancerous and normal genomes, such as the database of epigenetic modifiers (dbEM), maintain extensive detailed information on each epigenetic modifier and may help to identify altered epigenetic proteins that have a role in oncogenesis and could be explored as therapeutic targets (10).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

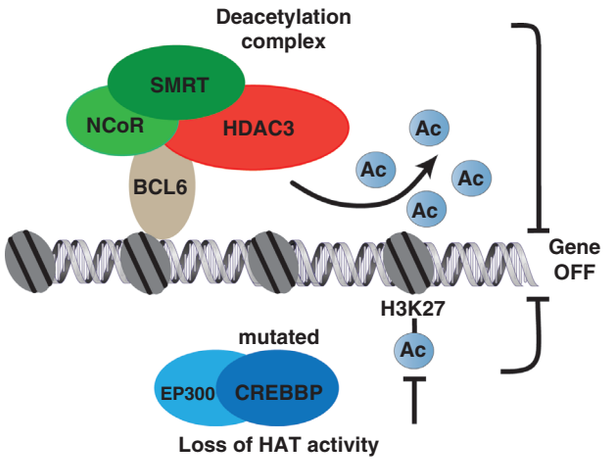

Figure 1. CREBBP inactivation disables acetylation and results in unopposed deacetylation by the BCL6/SMRT/HDAC3 complex, which disrupts enhancer activity in B cells. Ac, acetyl groups; HDAC3, histone deacetylase 3; NCoR, nuclear receptor corepressor; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor.

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