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Chromatin modifying gene mutations in follicular lymphoma

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Follicular lymphoma (FL) is an indolent malignancy of germinal center B cells. Although the overall survival of FL patients has recently improved with the introduction of novel therapies, there is significant heterogeneity in patient outcome and a need for rationally designed therapeutic strategies that target disease biology. Next-generation sequencing studies have identified chromatin modifying gene (CMG) mutations as a hallmark of FL, highlighting epigenetic modifiers as an attractive therapeutic target in this disease. Understanding the complex roles of these mutations will be central to identifying and adaptively targeting associated vulnerabilities. Recent studies have provided insight into the functional consequences of the most frequently mutated CMGs (*KMT2D*, *CREBBP*, and *EZH2*) and point to a role for these events in modifying normal B-cell differentiation programs and impeding germinal center exit. However, the majority of FL tumors serially acquire multiple CMG mutations, suggesting that there is a level of cross talk or cooperation between these events that has not yet been defined. Here, I review the current state of knowledge on CMG mutations in FL, discuss their potential as therapeutic targets, and offer my perspective on unexplored areas that should be considered in the future. (*Blood*. 2018;131(6):595-604)

Introduction

Follicular lymphoma (FL) is an indolent malignancy of germinal center B (GCB) cells, and the second most common form of non-Hodgkin lymphoma (NHL). It is characterized by a unique histology, in which tumor B cells form follicle-like structures with large numbers of nonmalignant immune cells infiltrating within the follicular and interfollicular regions.[1] As such, the tumor microenvironment of FL is central to disease etiology and affects response to therapy and patient outcome.[2-5] There is considerable heterogeneity in patient outcome; ~20% of patients have rapid disease progression following treatment and a poor prognosis.[6] Advancing our understanding of FL disease biology and harnessing this information to develop rationally targeted therapies is therefore a priority.

The most frequent genetic event in FL is the t(14;18)(q32;q21) translocation that places *BCL2* under control of the immunoglobulin heavy-chain enhancer. This occurs in ~90% of FL patients[7] but is also observed within a subset of cells in healthy individuals. The presence of *BCL2* translocation is associated with a significantly increased risk, particularly in those individuals with a high frequency of translocation-positive cells in the blood, but the majority of these individuals never develop FL.[8,9] Furthermore, *BCL2* translocations are likely acquired in pro- or pre-B cells,[10] and in healthy individuals these cells can terminally differentiate via the GC to become memory B cells.[8] Together, this suggests that secondary alterations are required for FL disease genesis, and these may play a role in stalling B-cell development at the GCB stage. In 2011, Morin et al described the frequent mutation of *KMT2D* (aka *MLL2*) and other chromatin modifying genes (CMGs) in FL.[11] Since then, CMG mutations have emerged as a hallmark of FL and have been the subject of recent studies that have provided insight into their function. Here I will discuss the role of CMG mutations in FL, with a focus on *EZH2*, *KMT2D*, and *CREBBP*.

The unique genetic landscape of FL

Epigenetic deregulation is a salient feature of multiple hematologic malignancies, but those derived from GCB cells are unique. Myeloid malignancies are in part characterized by somatic mutations that alter DNA methylation status.[12] Among others, these include *IDH1/2*, *TET2*, and *DNMT3A* mutation.[13-15] Interestingly, mutations in these genes have also been observed in some T-cell malignancies,[16,17] despite these cells aligning to a different hematopoietic lineage. The homeostatic control of DNA methylation is therefore important in both myeloid and T-lymphoid malignancies, but mutations affecting these processes are largely absent from GCB-derived malignancies such as FL. In contrast, FL preferentially acquires mutations in genes with a role in catalyzing the posttranslational modification of histones, such as the histone H3 lysine 4 (H3K4) methyltransferases *KMT2D* and *KTM2C*, the histone acetyltransferases *CREBBP* and *EP300*, and the histone H3 lysine 27 (H3K27) methyltransferase *EZH2*. Other CMGs that are mutated at a lower frequency in FL include those that control higher-order chromatin structure, such as the Switch/sucrose nonfermentable (SWI/SNF) complex components and multiple genes in the *HIST1H1/2* linker histone family.[18-20] Taken together, the somatic genome of FL shows a highly significant enrichment for mutations in CMGs.[19] This makes

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understanding the functional role of these genes and mutations critical to the understanding of FL etiology and highlights this axis as a potentially attractive therapeutic target in this disease.

FL is not the only NHL subtype to align with a GCB differentiation state, nor is it the only subtype to acquire CMG mutations. Burkitt lymphoma (BL) and the GCB-like subtype of diffuse large B-cell lymphoma (DLBCL) also align with the GCB cell differentiation state and acquire mutations in CMGs.[21,22] Table 1 shows the frequencies of CMG mutations from whole genome sequencing (WGS) and whole-exome sequencing (WES) studies of 71 FL,[19,23-26] 82 BL,[27-29] and 1046 DLBCL,[30-32] including a subset of 331 GCB-like DLBCL.[32] Notably, there are key contrasts between FL and other GCB-derived malignancies. For example, BL more commonly acquires mutations in components of the SWI/SNF chromatin remodeling complex (SMARCA4 [~21%] and ARID1A [~7%]) than it does in genes that post-translationally modify histones[22,27,29] (Table 1). A recent targeted sequencing study suggests that SWI/SNF complex components may be recurrently mutated in FL,[20] including frequent mutation of *BCL7A*. However, many of the other genes encoding the complex were mutated only within a single tumor and analogous approaches have not been applied to BL and DLBCL that would allow direct comparison of mutation frequencies across diseases. Furthermore, WES/WGS studies have only identified a low frequency of mutations in genes such as *SMARCA4* (~1% of tumors) in FL. The CMGs that are recurrently mutated in DLBCL show a similar rank order to those mutated in FL.[11,33,34] However, even when restricting the analysis to the GCB-like subtype of DLBCL, the frequency of CMG mutations is markedly lower in this disease. The final characteristic that distinguishes FL from other GCB-derived malignancies is the high rate at which FLs acquire multiple CMG mutations in the same tumor, at variant allele frequencies which indicate that they co-occur within the same cell.[19] This could be ascribed to a greater degree of genetic homogeneity in FL compared with other GCB-derived malignancies, but my alternative hypotheses are that (1) CMG mutations have a unique role in the context of FL compared with other GCB-derived malignancies, and/or (2) the microenvironment that is so distinctive of FL may provide selective pressure for the acquisition of a unique spectrum of mutations. Each of these scenarios may be influenced by the interplay of multiple CMG mutations within the same tumor cell, which is yet to be investigated in murine models or other experimental systems. Recent studies have, however, cast light onto the role of individual alterations of *EZH2*, *KMT2D*, and *CREBBP* in B-cell lymphomagenesis and are briefly discussed subsequently.

What we know (and do not know) about the function of CMG mutations in lymphomagenesis

EZH2

The *EZH2* gene encodes a lysine methyltransferase enzyme that catalyzes trimethylation of H3K27 (H3K27me3) as part of the polycomb repressor 2 (PRC2) complex. The H3K27me3 mark is transcriptionally repressive and has an important role in the control of developmentally regulated genes through the formation of bivalent promoters. These promoters possess both the activating H3K4 trimethylation (H3K4me3) mark and the repressive H3K27me3 mark and can be rapidly activated through loss of H3K27me3 or stably repressed through loss of H3K4me3. *EZH2* and other components of the PRC2 complex are highly expressed in GCB cells,[35-37] and conditional deletion of *Ezh2* within this compartment significantly attenuates GC development.[38] The normal function of EZH2 in GCB cells is to repress the expression of a set of genes that are highly transcribed in naïve B cells, either through addition of H3K27me3 to H3K4me3-marked promoters to create bivalency or through addition of H3K27me3 to promoters that are not marked with H3K4me3. Genes with bivalent promoters in GCB cells include those involved in terminal differentiation such as *PRDM1*, *IRF4*, and *XBP1*,[38,39] and negative regulators of the cell cycle such as *CDKN1A* and *CDKN1B*.[38] Silencing of these genes by EZH2 occurs in cooperation with BCL6 and BCOR[40] and temporarily suspends the B-cell differentiation program during the GC reaction. Notably, silencing of the BCL6 target gene, *CDKN1A*, by EZH2 is particularly important for promoting B-cell proliferation and GC formation, and deletion of *Cdkn1a* is sufficient to rescue GC formation in *Ezh2* conditional knockout mice.[41]

Mutations of *EZH2* were the first recurrent CMG mutation to be reported in FL[42] and were the first to be investigated using murine models.[38-40] These are most often missense mutations of tyrosine 641 (Y641) within the Su(var)3-9, Enhancer-of-zeste and Trithorax (SET) domain of *EZH2*, resulting in a neomorphic change with increased activity for catalyzing the addition of the third methyl group to H3K27me2.[43,44] However, the mutant protein has reduced activity for addition of the first and second methyl groups, potentially explaining why it is always found as a heterozygous mutation in patient tumors. This is believed to be because wild-type and mutant *EZH2* act cooperatively to promote H3K27me3, but there is contrasting evidence about whether maintenance of the wild-type *EZH2* allele is necessary for increased H3K27me3 and tumor development.[39,40,45] However, it is clear that B-cell–specific expression of *Ezh2* Y641 point mutants can cooperate with *Bcl2* overexpression to promote B-cell lymphoma.[38,39] B-cell–specific expression of mutant *Ezh2* resulted in increased H3K27me3 at promoter and promoter-proximal regions of genes (Figure 1) that are normally repressed in GCB cells, such as *PRDM1* and *IRF4*. As many of these genes are involved in GC exit and terminal differentiation, their stable repression likely contributes to lymphomagenesis at least in part by stalling B-cell differentiation at the GCB stage.[38,39]

Murine models of *Ezh2* Y641 mutations have characterized a relatively strong role for these events in promoting B-cell lymphoma, especially in comparison with models of *Kmt2d* or *Crebbp* loss (discussed subsequently). However, the majority of *EZH2* mutations are subclonal events in FL at diagnosis[19,23,24,26] (Table 1) and remain subclonal at relapse.[19] In contrast, other subclonal drivers such as *TP53* mutations in chronic lymphocytic leukemia are subject to strong selection and expand to become clonal at disease progression.[46] This difference may be related to a stronger role for *TP53* mutations in promoting therapeutic resistance. However, an alternative hypothesis is that there is a yet unexplored contextual advantage of *EZH2* mutations within the background of *KMT2D* and/or *CREBBP* mutations. This may result in a preferential acquisition of *EZH2* mutations at a later stage of genomic evolution after the early driver mutations have been acquired. Alternatively, there may be an advantage to *EZH2* mutation heteroclonality. In this scenario, only a minor number of *EZH2* mutant cells would be required to benefit the

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Table 1. Characteristics of select CMG mutations

CMG	Function	Mutation frequency			Percent missense: disruptive*	Percent occurring early in evolution† (no. early: no. late)	Murine models
		FL, %	DLBCL, % (GCB-like, %)	BL, %			
KMT2D	H3K4 methyltransferase	72	24 (28)	2	17:83	62 (43:26)	Knockout,[49] knockdown[50]
CREBBP	Lysine acetyltransferase	65	11 (16)	6	81:19	89 (42:5)	Knockout,[56-58,60] knockdown[61]
EZH2	H3K27 methyltransferase	25	6 (12)	2	100:0	41 (9:13)	Point-mutation knock-in[38,42]
EP300	Lysine acetyltransferase	15	6 (6)	0	100:0	57 (8:6)	—
KMT2C	H3K4 methyltransferase	13	5 (5)	1	75:25	60 (3:2)	—
HIST1H1E	Linker histone	14	17 (17)	0	100:0	21 (3:11)	—
ARID1A	SWI/SNF component	11	9 (10)	7	0:100	31 (4:9)	—
SMARCA4	SWI/SNF component	1	8 (10)	21	‡	‡	—

*Missense mutations include single amino acid substitutions and coding indels. Disruptive mutations include premature stop codon and frameshift mutations. Data are from FL WES/WGS studies with available information[19,24,25] and are represented as relative percentage.

†The percent of mutations occurring early in evolution is calculated from studies of clonal ancestry across 65 patients with paired diagnosis and relapse biopsies.[19,24-26] Early events are defined as those shared between all biopsies from the same patient. Late events are defined as those not present in all paired biopsies from the same patient.

‡Only 1 evaluable mutation (disruptive and clonal).

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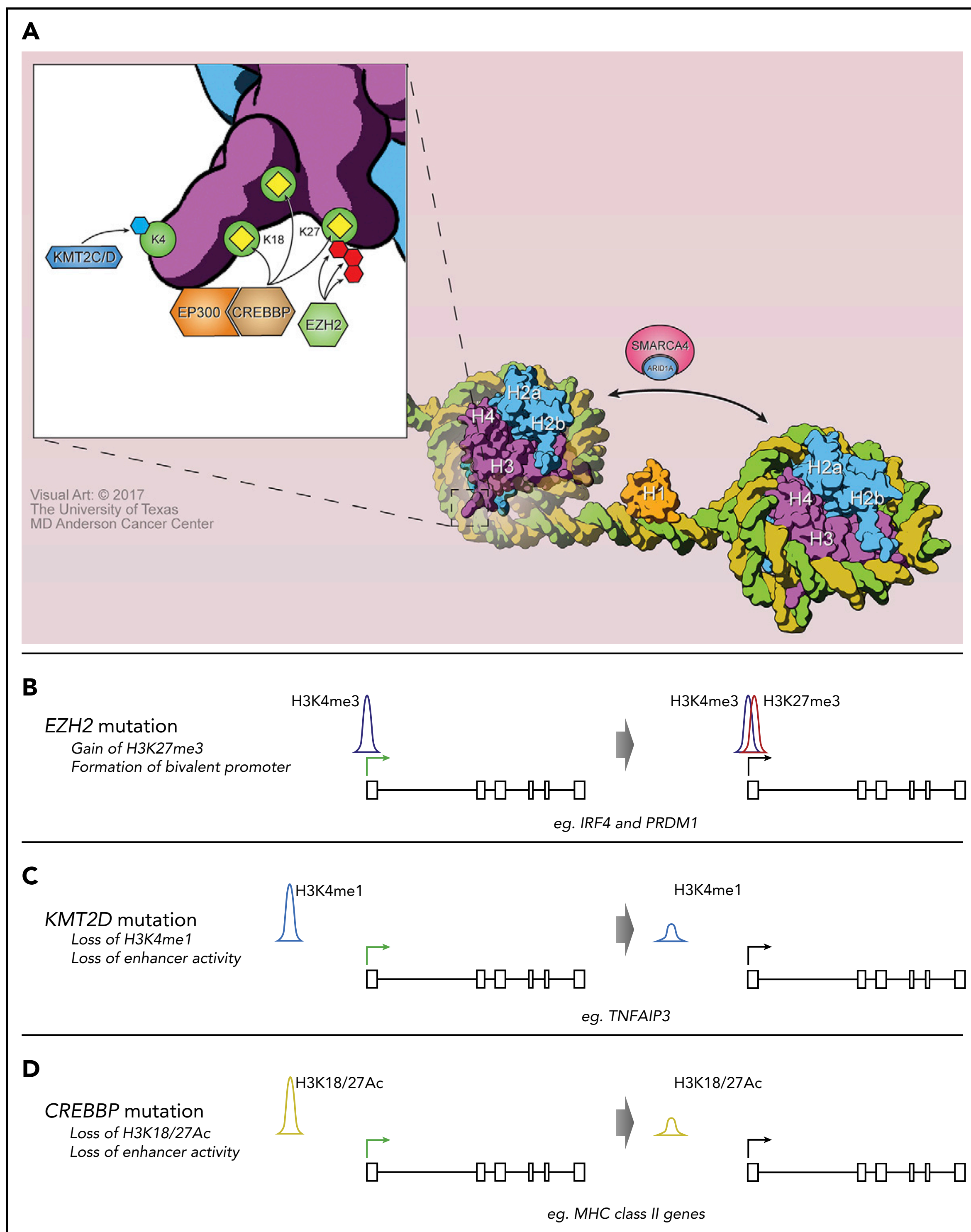

Figure 1. The consequences of CMG mutations on histone posttranslational modifications. (A) A depiction of the normal role of CMGs that are recurrently mutated in FL. KMT2C/D methylates H3K4, CREBBP and EP300 acetylate multiple residues including H3K18 and H3K27, and EZH2 catalyzes trimethylation of H3K27. (B) EZH2 Y641 mutation was predominantly associated with the gain of H3K27me3 at promoters already marked with H3K4me3, to form bivalent promoters. (C) *KMT2D* loss was associated with reduced H3K4 methylation at enhancer elements, including that for the *TNFAIP3* gene. (D) *CREBBP* loss was associated with reduced H3K18Ac/H3K27Ac at enhancer elements, including those of major histocompatibility complex (MHC) class II genes.

broader tumor population, and overpopulation by *EZH2* mutant cells may be deleterious. Understanding the context-specific roles of *EZH2* mutation and heteroclonality will be particularly important because of the emerging potential for EZH2 inhibitors as a novel therapy for FL.

KMT2D

The *KMT2D* gene (aka *MLL2*) encodes an SET domain–containing lysine methyltransferase that is part of the KMT2 family. *KMT2D* and *KMT2C* are conserved members of this family that are recruited to genomic loci by nuclear coreceptors

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or transcription factors and are thought to be responsible for the majority of H3K4 monomethylation at enhancer elements.[47] In addition to their ability to catalyze H3K4 methylation independently, KMT2D and KMT2C can also perform this function as part of multiprotein complex of proteins associated with Set1 (COMPASS)–like complexes that are predominantly associated with developmentally regulated genes.[48] The monomethylation of enhancer elements leads to the recruitment of other coactivators that culminate in enhancer-promoter looping, activation of RNA pol II, and gene transcription. Knockout studies have pointed to a partial genetic redundancy between *Kmt2d* and *Kmt2c*, although many of their functions are nonredundant.[49]

The *KMT2D* gene is the most recurrently mutated CMG in FL (~72% of cases) and is also recurrently mutated in a lower frequency of DLBCL (~24% of cases). The majority of these mutations are nonsense or frameshift events that result in a loss of KMT2D protein[50] (Table 1). The remaining coding mutations predominantly affect the C-terminal portion of the gene containing the SET domain and reduce methyltransferase activity.[50] Two recent studies have interrogated the functional consequence of *Kmt2d* loss in murine models.[50,51] Knockout or knockdown of *Kmt2d* in murine B cells resulted in no obvious changes in normal B-cell development but led to a marked increase in the frequency of GCB cells and reduced numbers of class-switched B cells following immunization. This is indicative of a defect in B-cell maturation and/or class-switch recombination in the absence of *Kmt2d*. These studies also showed a loss of H3K4 methylation at GCB-specific enhancers that were bound by Kmt2d (Figure 1) and confirmed a role for *Kmt2d* loss in promoting lymphomagenesis in the context of *Bcl2* overexpression.

Notably, *KMT2C* is also mutated in ~13% of FL. These mutations do not mutually exclude *KMT2D* mutations, suggesting that these genes may function nonredundantly in FL. However, the question of redundancy between *KMT2D* and *KMT2C* activity remains controversial because of contrasting results with respect to the effect of *Kmt2d* loss on the global abundance of H3K4 methylation.[50,51] In addition, it is not clear whether C-terminal missense mutations that reduce KMT2D methyltransferase activity, but maintain the protein expression, would have the same functional consequence as nonsense or frameshift mutations that result in a loss of protein. This is because KMT2D methyltransferase activity has been found to be dispensable for the transcription of enhancer RNAs, which indicate enhancer activity.[52] The scaffolding function of KMT2D, in addition to its methyltransferase activity, is therefore very important for enhancer activation. This may explain why FLs select for a higher rate of nonsense/frameshift mutations in *KMT2D* than in other CMGs and also points to a possible functional discrepancy between loss-of-protein and missense mutations. The role of *KMT2C* mutation, and the function of *KMT2D* SET domain missense mutations in FL, will be important areas to address in future studies.

CREBBP

The *CREBBP* gene (aka CBP) encodes a lysine acetyltransferase (KAT) that acetylates histone 3 at lysines 18 (H3K18Ac) and 27 (H3K27Ac), as well as acetylates nonhistone proteins. It functions as a transcriptional coactivator by acetylating histones at regulatory elements following its recruitment by DNA-binding transcription factors and other coactivator complexes.[53] Histone acetylation alters their charge and loosens their association with DNA to make it more accessible to transcription factors. In addition, the acetyl mark is also recognized by bromodomain-containing proteins such as adenosine triphosphate–dependent chromatin remodelers and factors that promote transcriptional elongation.[53,54]

Mutations of *CREBBP* occur in ~65% of FLs and ~11% of DLBCLs.[22] Approximately 80% of the mutations observed in FL create missense changes in the KAT domain, with 26% of all mutations altering a single KAT domain amino acid (R1446).[19,55-57] These lead to reduced acetyltransferase activity.[55,56] Multiple studies including our own have shown that *CREBBP* mutations arise as early events during FL disease genesis and likely reside within tumor cell progenitors that propagate disease relapse.[19,23-26] A recent study also showed in a single patient that a *CREBBP* mutation could be detected within the hematopoietic stem and progenitor cell pool,[58] although this requires further validation. In primary human FL tumors, these mutations are associated with a marked reduction in MHC class II expression and reduced tumor-infiltrating T-cell number and function.[19] Importantly, reducing tumor cell MHC class II expression was found to enhance lymphomagenesis,[59] suggesting *CREBBP* mutation–associated reductions in antigen presentation may be important for disease pathogenesis.

Recent murine studies found that loss of *Crebbp* promotes B-cell lymphoma,[58,60] particularly in cooperation with *Bcl2* overexpression,[57,61,62] and that regions of reduced histone acetylation associated with *Crebbp* loss were primarily located at distal enhancer elements[57,58,61,62] (Figure 1). This included regulatory elements of MHC class II genes and was also associated with reduced MHC class II expression.[59,61] However, the magnitude of this change was less than that observed in primary human FLs.[19] In addition, regions of reduced H3K27Ac associated with *Crebbp* loss were found to be enriched for loci that are also bound by BCL6[61,62] and repressed by the recruitment of HDAC3.[61] In this context, loss of *CREBBP* may promote B-cell lymphoma by interfering with the derepression of BCL6 target genes and thereby preventing GC exit and terminal differentiation.

Reduced acetylation of nonhistone proteins such as p53 and BCL6 were also previously described as important features associated with *CREBBP* mutation.[56] A recent study expanded on this observation by showing that Crebbp knockout led to a significant attenuation of p53 acetylation in response to DNA damage, and reduced DNA damage response.[58] Although it is likely that the reduced acetylation of nonhistone proteins plays a contributing role in disease pathogenesis, the relative importance of these changes and their possible interplay with patterns of reduced histone acetylation remains to be explored. In addition, our recent study suggests that *CREBBP* mutations show a different spectrum in FL compared with DLBCL.[57] Loss of *CREBBP* was associated with induction of *MYC* expression,[57,58] which is more characteristic of DLBCL than FL.[57] It is likely that the expression of a catalytically inactive CREBBP protein resulting from KAT domain missense mutations has different functional consequences than mutations resulting in the loss of CREBBP protein expression. It will therefore be important to characterize the functional consequence of *CREBBP* KAT domain mutations that are more prevalent in FL and compare this with loss-of-protein events that were modeled in recent studies[57,61,62] and are more frequent in DLBCL.[57]

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Coassociation of CMG mutations

Our recent study using deep-sequencing of purified tumor cells from FL revealed that ~70% of tumors possess ≥2 different CMG mutations.[19] This same pattern has been noted in other studies utilizing whole tumor specimens, although at a lower frequency.[24-26] We and others have expanded on this observation by analyzing paired biopsies from diagnosis and relapse and showing that CMG mutations are serially acquired during the genomic evolution of FL.[19,23-26] It is therefore clear that the majority of FLs undergo genomic evolution that culminates in multiple CMG mutations. Some might suggest that coassociation of CMG mutations within individual FL tumors is a random consequence of their high individual gene mutation rates. In contrast, my perspective is that the individual gene mutation rates in this disease are high because it is of evolutionary benefit for FLs to acquire multiple CMG mutations.

There is considerable cross talk between epigenetic marks and complex interactions among different CMGs. Although the purpose of this article is not to review these interactions, some are particularly pertinent to our consideration of the possible function of cosegregating CMG mutations. For example, there is a well-defined antagonistic relationship between H3K27Ac and H3K27me3.[63,64] This suggests that *CREBBP* mutations that result in a loss of H3K27Ac may also alter the landscape of H3K27me3, because of normal PRC1/2 activity with wild-type *EZH2* and/or enhanced PRC2 activity resulting from *EZH2* mutation. KMT2D and KMT2C can also indirectly influence both H3K27Ac and H3K27me3. The COMPASS-like complexes formed by KMT2D and KMT2C recruit the UTX histone demethylating enzyme to enhancers, thereby facilitating the removal of H3K27me3.[48] This indicates that loss of KMT2C/D may lead to the accumulation of H3K27me3 at enhancers that are normally bound by these proteins. Although many of the changes in H3K27me3 catalyzed by mutant *EZH2* were observed at promoter-proximal elements, a loss of *KMT2D* and COMPASS-like complex occupancy may allow PRC1/2 to additionally silence enhancer elements and may open a new dimension of epigenetic changes catalyzed by mutant *EZH2* in the presence of *KMT2C/D* loss-of-protein mutations.[38,39] Enhancer occupancy by KMT2C/D, but not their methyltransferase activity, is also required for the recruitment of cofactors such as CREBBP and EP300 that catalyze H3K27Ac and promote enhancer activation.[65] This suggests that the distribution of CREBBP and EP300 at enhancers, and therefore the functional consequence of their mutation or loss, may be different in the context of *KMT2C/D* nonsense/frameshift mutations than it would be in the presence of *KMT2C/D* protein expression. Importantly, ~50% of FLs possess mutations in both *KMT2D* and *CREBBP*.[19] These observations again raise the questions of whether KMT2C and KMT2D can act redundantly to recruit UTX or CREBBP/EP300, and whether inactivating missense mutations of *KMT2D* would be functionally equivalent to nonsense/frameshift mutations.

Mutations in HIST1H1/2 linker histone family genes are also likely to have significant cross talk with mutations in genes that control histone posttranslational modification. Linker histones bind to DNA at the entry and exit sites of the nucleosome and are required for the stability of higher-order chromatin structure. Recent data suggest that the histone tails of oligonucleosomes that are assembled with linker histones are poorer substrates for a range of posttranslational modifiers including CREBBP and EZH2,[66] although alternative findings suggest that they are better substrates for EZH2.[67] In addition, the most frequently mutated linker histone, *HIST1H1E*, can be methylated at lysine 26 by EZH2 to create a docking site for HP1 and facilitate heterochromatin formation. Linker histones can also recruit DNA methyltransferases, and recruitment DNMT3B is impaired by somatic mutation.[18] In many cases, linker histones therefore facilitate heterochromatin formation, possibly in tandem with EZH2, and their inactivation by somatic mutation is inconsistent with the promotion of heterochromatin formation by activating *EZH2* mutations and loss-of-function *KMT2D* and *CREBBP* mutations. However, acetylation of HIST1H1E at lysine 34 by GCN5 can also lead to recruitment of TAF1 and promote transcriptional activation, meaning that loss-of-function mutations may have a transcriptionally repressive effect. As the biology of linker histones is not as well defined as core histones, and each family member plays a unique role in gene-specific activation and inactivation,[68] detailed functional studies will be required to understand the precise role of these mutations in FL and their cross talk with other CMG mutations.

Despite these interactions only representing the "tip of the iceberg" with respect to epigenetic cross talk, they clearly indicate a potential for CMG mutations in the same tumor cell to interact and promote unique epigenetic phenotypes. This would suggest a level of codependency between CMG mutations wherein the product of the mutations is greater than (or different from) the sum of the parts, and their serial acquisition progressively "locks in a phenotype." That is, the critical genes that are altered by each CMG mutation may be largely overlapping, and serial acquisition of multiple CMG mutations may therefore lead to progressively deeper repression of these common genes that is more difficult to reverse. An alternative viewpoint may be that, despite epigenetic cross talk, each CMG mutation has a unique and mutually exclusive functional consequence and their serial acquisition acts to "build a phenotype." That is, the critical genes that are altered by each CMG mutation may be nonoverlapping, and serial acquisition of multiple CMG mutations may therefore lead to the repression of progressively larger sets of genes. I would suggest that the truth is probably somewhere between these 2 scenarios.

Targeting FL with epigenetic modifying agents

Therapeutic targeting of epigenetic deregulation in FL is a theoretically attractive concept. However, the most common CMG mutations (ie, those in *KMT2D* and *CREBBP*) are loss-of-function/loss-of-protein events, which are difficult to drug. This has made targeting activating *EZH2* mutations the lowest hanging fruit. Multiple companies have developed inhibitors for EZH2,[69-71] and these have entered early phase clinical trials in NHL and other settings (www.clinicaltrials.gov; #NCT02082977, #NCT02395601, #NCT01897571). Cell lines bearing *EZH2* mutations are more sensitive to EZH2 inhibitors than wild-type cell lines,[69,70] providing some credence to precision targeting of *EZH2* mutations. However, genetic studies of FL have shown that *EZH2* mutations are late events in disease evolution and are present only in a subclonal population.[19,23,24,26] In this respect, *EZH2* mutations may not be a good therapeutic target, because they only reside in a subset of tumor cells. Despite this, a recent update of a phase 2 study of an EZH2 inhibitor, tazemetostat, reported a 92% objective response rate in FL patients bearing *EZH2* mutations,[72] which was higher

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BOX 1

Potential future avenues of exploration:

1. Investigate the role of combinations of CMG mutations using patient samples, cell lines, and animal models.
2. Related to the first avenue, perform single-cell analysis of genotype/phenotype relationships for combinations of CMG mutations and single low variant allele frequency mutations.
3. Discriminate between the functional consequences of missense and disruptive mutations in frequently mutated CMGs.
4. Interrogate the relationship between the tumor microenvironment and somatic mutations using comparisons of both inter- and intrapatient heterogeneity.
5. Identify mechanisms of sensitivity and resistance to epigenetic modifying agents and rational therapeutic combination strategies.

than *EZH2* wild-type patients (26%). These trends are based on early observations in a small cohort of patients and require further follow-up and validation, but we should also be careful to discriminate between targeting EZH2 vs targeting *EZH2* mutations in FL. Although there may be flaws in the rationale for targeting *EZH2* mutations, multiple studies have demonstrated that wild-type EZH2 is a critical regulator of GCB development[35,38,40,73] and therefore also represents an attractive therapeutic target. The acquisition of an *EZH2* mutation may be restricted to cases in which the EZH2-driven epigenetic program has a strong oncogenic role. This would mean that even tumor cells with wild-type EZH2 may be sensitive to its inhibition, and responses will be observed regardless of the clonal representation of the *EZH2* mutation. In other words, *EZH2* mutational status may be a biomarker for the likelihood and/or depth of response to EZH2 inhibition, rather than a strict criterion for whether EZH2 inhibition should be used. Alternatively, the association between *EZH2* mutation and better clinical outcome[74,75] may mean that EZH2 mutation is associated with superior responses to a range of therapies, and the use of *EZH2* mutation as a predictive biomarker for response may not be restricted to EZH2 inhibitors.

As previously discussed, there may also be a role for *KMT2D* and/or *CREBBP* mutations in altering the landscape of EZH2-catalyzed H3K27me3 in FL tumors through epigenetic cross talk. It is currently unclear how this may modify sensitivity to EZH2 inhibition. In addition, EZH2 plays an important role in the development of other immune cells, including T cells[76-79] and natural killer cells.[80] Inhibiting EZH2 may therefore have additional tumor cell–independent therapeutic activity through the alteration of T-cell polarization or natural killer cell development/activation, similar to that described for ibrutinib.[81,82] The notion that EZH2 may be a good therapeutic target in FL, independent of its mutational status, is supported by some of the most pronounced responses to tazemetostat being observed in EZH2 wild-type patients.[72] The complexity of epigenetic cross talk and the potential role of nonmalignant immune cells may make it difficult to fully predict the efficacy of EZH2 inhibition in FL using genotypic information alone.

Histone deacetylase (HDAC) inhibitors are also a logical avenue for continued exploration in FL, because of their potential to counteract the loss of histone acetylation that results from *CREBBP* or *EP300* mutations.[55,56,62] Pan-HDAC inhibitors have shown some promise in FL, with objective responses rates of 47% to 49% and 64% for vorinostat and abexinostat, respectively.[60,83,84] These response rates are similar to the reported frequency of *CREBBP* mutations,[19,24,56] but this may be purely coincidental as the mutational status of *CREBBP* was not reported in these studies. It will be interesting to evaluate whether histone acetyltransferase gene mutations are predictive of response to pan-HDAC inhibitors. Isoform-specific inhibitors, such as those that are HDAC3-specific,[61] may also provide a similar therapeutic benefit while limiting the toxicity associated with pan-HDAC inhibition.[85] HDAC3 participates in nuclear receptor corepressor 1 (NCOR) and 2 (SMRT) complexes that are recruited by BCL6 to repress target genes in GCB cells via deacetylation of H3K27,[86] and it was hypothesized that mutation of *CREBBP/EP300* impedes the reversal of this HDAC3-mediated target-gene repression in GCB cells. As a proof of concept, knockdown or chemical inhibition of HDAC3 was capable of inducing the expression of genes that were silenced by *Crebbp* loss, including MHC class II genes.[61] Specific inhibition of HDAC3 may therefore be efficient in targeting *CREBBP* or *EP300* mutations and may have efficacy in FL.

Although epigenetic modifying agents represent a logical and exciting avenue for treatment of FL, the complete response rates for these therapeutics as single agents have been low.[60,72,83,84] This may be a product of the relapsed/refractory population used in the trials but might also indicate that their most effective use may be as part of a combination regimen. An area of importance is understanding the functional implications of these therapeutics, including both sensitizing pathways and mechanisms of resistance, so as to identify rational combination strategies.

Conclusion

There is strong evidence that *KMT2D*, *CREBBP*, and *EZH2* mutations share a common function in controlling GCB-cell differentiation programs. This likely contributes to lymphomagenesis by stalling FL tumor cells within the GC, where they can persist as a result of *BCL2* overexpression and are subject to continuous activation-induced cytidine deaminase activity that promotes further genomic evolution. However, I find it unlikely that CMG mutations act redundantly in FL, because of the large degree of cosegregation of these mutations within the same tumor and the huge potential for epigenetic cross talk. Serial mutation of unique CMGs likely "builds" a complex epigenetic program by altering discrete sets of genes that would normally be deleterious to the prolonged residence of a B cell within the GC, with a subset of their effects complementarily "locking in" 1 or more critical epigenetic programs. This includes programs that (1) drive germinal center exit, (2) are required for terminal differentiation, or (3) control interactions with cells in the microenvironment. The latter may modulate B-cell deletion from the GC resulting from neglect by T follicular helper (T_{FH}) cells[87] or promote antigen-driven antitumor immunity.[88] Box 1

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highlights some suggested avenues of exploration to provide greater understanding of the role of CMG mutations in FL and how we may target them therapeutically. Although it is difficult to model, it will be imperative to somehow account for the complex role of the tumor microenvironment in FL etiology and its interplay with the tumor cell–intrinsic phenotypes that are governed by CMG mutations. This is an incredibly challenging task ahead of us, but one that may unravel a targetable biology of FL and improve the lives of countless patients.

Acknowledgments

The figure was created with assistance from The University of Texas MD Anderson Cancer Center Medical Graphics Department.

M.R.G. receives funding from the National Cancer Institute, National Institutes of Health (grant R01CA201380).

Authorship

Contribution: M.R.G. wrote the paper.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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Footnote

Submitted 31 July 2017; accepted 15 November 2017. Prepublished online as *Blood* First Edition paper, 20 November 2017; DOI 10.1182/blood-2017-08-737361.

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