



Epigenetic Mechanisms in Leukemias and Lymphomas

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Although we are just beginning to understand the mechanisms that regulate the epigenome, aberrant epigenetic programming has already emerged as a hallmark of hematologic malignancies including acute myeloid leukemia (AML) and B-cell lymphomas. Although these diseases arise from the hematopoietic system, the epigenetic mechanisms that drive these malignancies are quite different. Yet, in all of these tumors, somatic mutations in transcription factors and epigenetic modifiers are the most commonly mutated set of genes and result in multilayered disruption of the epigenome. Myeloid and lymphoid neoplasms generally manifest epigenetic allele diversity, which contributes to tumor cell population fitness regardless of the underlying genetics. Epigenetic therapies are emerging as one of the most promising new approaches for these patients. However, effective targeting of the epigenome must consider the need to restore the various layers of epigenetic marks, appropriate biological end points, and specificity of therapeutic agents to truly realize the potential of this modality.

Epigenetic instructions are the equivalent of software programs that instruct cellular phenotypes, including those of tumor cells. Along these lines, it has been shown that aberrant epigenetic programming occurs universally in hematologic malignancies, and somatic mutations in genes encoding epigenetic modifiers and transcription factors are the most abundant class of genetic lesions in these tumors. Therefore, hematological malignancies provide an excellent viewpoint for exploring how the many layers of epigenetic mechanisms interact to mediate transformed phenotypes. Herein, we focus on, and provide the basis to compare and contrast, epigenetic mechanisms underlying patho-

genesis of acute myeloid leukemia (AML) and the common lymphomas derived from germinal center (GC) B cells.

EPIGENETIC MECHANISMS IN AML

Aberrant Cytosine Methylation Profiles Are a Hallmark of AML

A significant subset of recurrent mutations in AML affect epigenetic modifications of DNA and/or histones (Fig. 1) (Abbas et al. 2010; Marcucci et al. 2010, 2012; Paschka et al. 2010; Holink et al. 2011; Shen et al. 2011; Gaidzik et al. 2012; Patel et al. 2012; Weissmann et al. 2012;

Editors: Michael G. Kharas, Ross L. Levine and Ari M. Melnick
Additional Perspectives on Leukemia and Lymphoma: Molecular and Therapeutic Insights available at
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Cite this article as *Cold Spring Harb Perspect Med* 2020;10:a034959

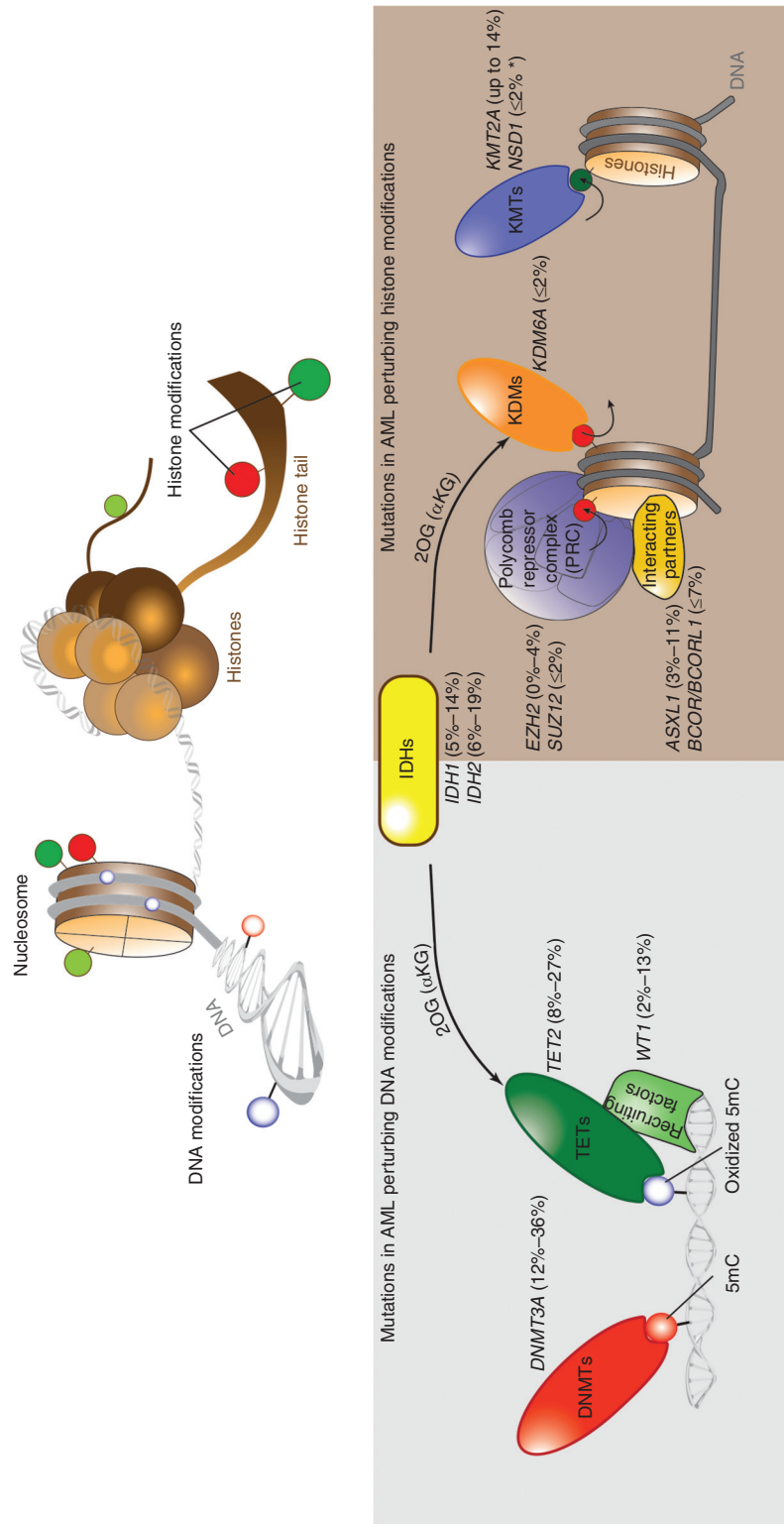


Figure 1. Frequent mutations of epigenetic regulators in acute myeloid leukemia (AML). *Upper panel* illustrates epigenetic modifications on the DNA and histone layer of the epigenome. *Lower panel* shows gene mutations that impact DNA and/or histone modifications in at least 1% of AML cases. Ranges of reported mutation frequency are indicated in parentheses (Abbas et al. 2010; Marcucci et al. 2010, 2012; Paschka et al. 2010; Hollink et al. 2011; Shen et al. 2011; Gaidzik et al. 2012; Patel et al. 2012; Weissmann et al. 2012; Cancer Genome Atlas Research 2013; Gao et al. 2013; Metzeler et al. 2016; Papaemmanuil et al. 2016; Terada et al. 2018). The asterisk indicates frequency in adult AML. KMT, lysine methyltransferase; KDM, lysine demethylase; 2OG, 2-oxoglutarate, also called α -ketoglutarate (α KG).

Cancer Genome Atlas Research 2013; Gao et al. 2013; Metzeler et al. 2016; Papaemmanuil et al. 2016; Terada et al. 2018). Cytosine methylation (5mC) is critical for gene silencing, imprinting, X-chromosome inactivation, genome stability, and cell fate determination (Bird 2002). Tissue-specific 5mC patterns are established primarily by de novo DNA methyltransferases DNMT3A and DNMT3B (Okano et al. 1999) and are subsequently maintained by DNMT1 in a replication-dependent manner (Fig. 2A) (Robert et al. 2003). CpG islands (CGIs), comprising <10% of all CpGs, are found in ~70% of promoters and are predominantly unmethylated, whereas a majority of remaining CpGs (60%–80%) are methylated in human cells (Saxonov et al. 2006; Smith and Meissner 2013). Disruption of cytosine methylation patterning at gene promoters is a hallmark of AML and is clearly linked to aberrant gene silencing (Figuroa et al. 2010b; Cancer Genome Atlas Research 2013). However, more recent studies indicate that critical 5mC changes in AML are also present at gene enhancers, in which their effects on gene expression are more nuanced (Glass et al. 2017). Importantly, 5mC profiles allow AMLs to be classified into biologically defined subtypes with distinct clinical outcomes (Figuroa et al. 2010a,b). Some of these profiles are linked to specific somatic mutations, whereas others are independent of mutations and are dependent on underlying disease-driving mechanisms such as overexpression of EVI1 (Lugthart et al. 2011) or silencing of myeloid lineage transcription factors such as CEBP α (Figuroa et al. 2009). Importantly, a subset of genes appears to be almost universally aberrantly methylated and silenced in AML patients regardless of somatic mutations (Figuroa et al. 2010b). It is possible that these epigenetic alleles are “epi-drivers” that are required for normal hematopoietic cells to manifest a leukemic phenotype in cooperation with somatic mutations and may explain why AMLs manifest relatively few genetic lesions. 5mC redistribution plays a critical role in myeloid differentiation (Bröske et al. 2009; Ji et al. 2010; Bock et al. 2012) and might be prone to disruption by such epi-drivers.

AMLs Feature Highly Recurrent Mutations of Proteins That Modify Cytosine Residues

DNMT3A Mutations

Most mutant *DNMT3A* AML show either a heterozygous missense (R882H/C) mutation that affects the catalytic domain or truncating mutations (Cancer Genome Atlas Research 2013; Yang et al. 2015). R882 hotspot mutations impair *DNMT3A* methyltransferase activity and are linked to hypomethylation at specific CpGs (Russler-Germain et al. 2014). *Dnmt3a* knockout mice manifest severe differentiation block and enhanced self-renewal potential of hematopoietic stem cells (HSCs) (Challen et al. 2011). This is associated with DNA hypomethylation at borders of so-called “canyons” at regulatory regions of self-renewal genes (Jeong et al. 2014) consisting of large hypomethylated regions that contain histone activating marks (H3K4me3), repressive marks (H3K27me3), or both (Xie et al. 2013; Jeong et al. 2014). Non-competitive transplantation experiments reveal that loss of *DNMT3A* predisposes murine HSCs to malignant transformation (Mayle et al. 2015), consistent with findings in humans, in which *DNMT3A* mutations establish a reservoir of preleukemic stem cells that can evolve to AML (Shlush et al. 2014). In addition, mutant *DNMT3A*^{R882} impairs chromatin remodeling and nucleosome eviction during chemotherapy, conferring resistance against anthracyclines that could explain the poor prognosis of *DNMT3A*^{mut} AML (Ley et al. 2010; Shen et al. 2011; Guryanova et al. 2016).

Somatic Mutations of TET2 in AML

Removal of 5mC is initiated by members of the ten-eleven translocation (TET) family of dioxygenases through successive oxidization of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (Fig. 2A) (Tahiliani et al. 2009; He et al. 2011; Ito et al. 2011). Apart from DNA demethylation by passive dilution following cell replication, 5fC and 5caC can be actively excised by thymine-DNA glycosylase (TDG) and repaired by the base excision repair (BER) pathway to regen-

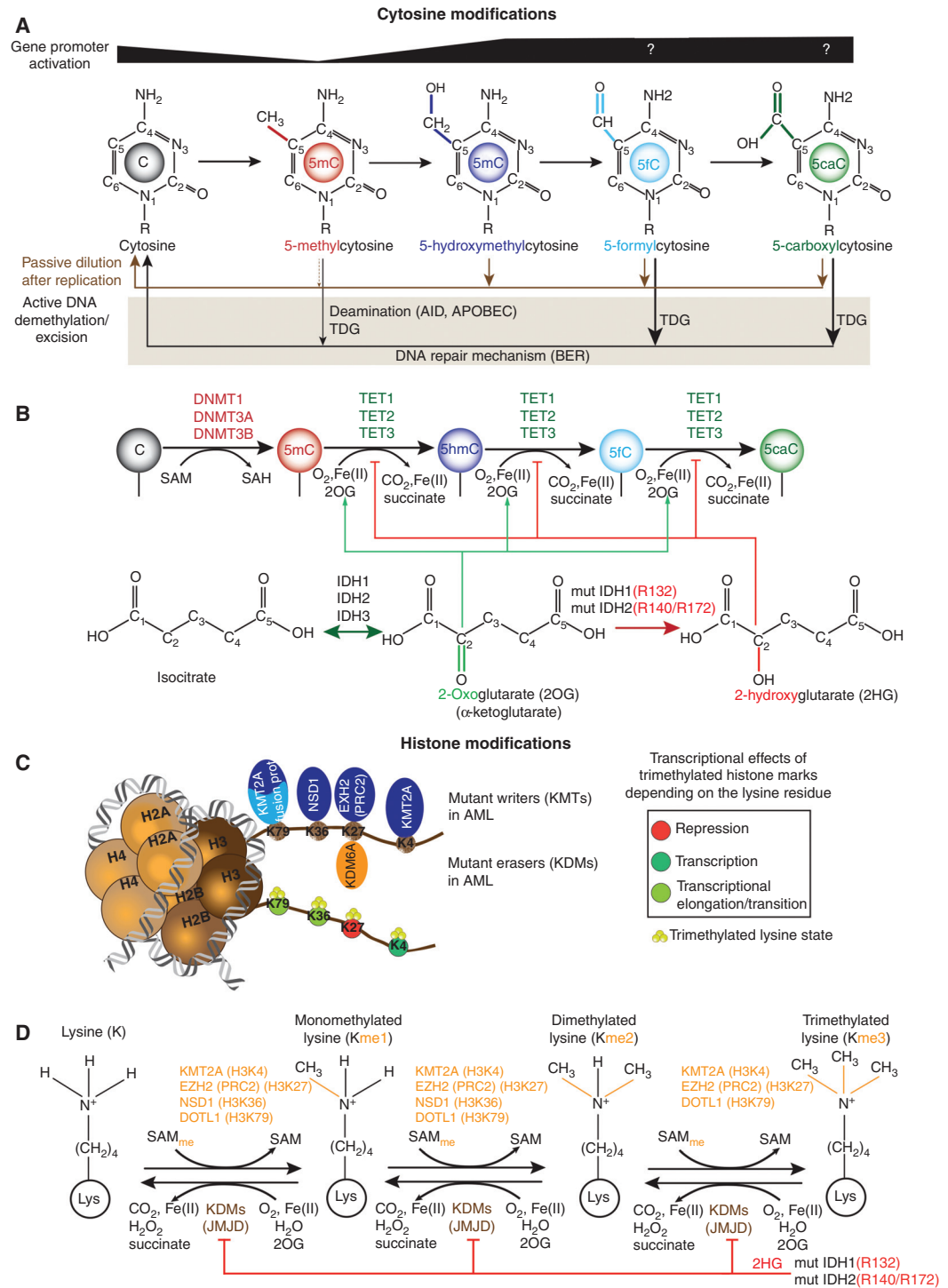


Figure 2. (See legend on following page.)



erate unmodified cytosines (Maiti and Drohat 2011; Rasmussen and Helin 2016). In addition, activation-induced cytidine deaminase (AID) and APOBEC can deaminate 5mC but not 5hmC, contributing to 5mC erasure (Fig. 2A) (Nabel et al. 2012). Recent findings suggest that genomic 5hmC might also play an active role as an epigenetic mark that regulates transcription through modulation of chromatin accessibility and recruitment/hindrance of specific factors (e.g., MBD proteins binds to 5hmC DNA with weaker affinity than to 5mC DNA) (Hashimoto et al. 2012; Shen et al. 2013; Bachman et al. 2014; Ngo et al. 2016). In murine embryonic stem cells (ESCs), 5hmC function is involved in maintaining the active state of gene enhancers (Hon et al. 2014). Importantly, TET2 is primarily localized at CpG-sparse distal regulatory elements, suggesting a main function in enhancer regulation (Rasmussen et al. 2019). In agreement with this, deletion of TET2 in hematopoietic cells reduces 5hmC primarily at enhancers leading to down-regulation of tumor-suppressor genes (Rasmussen et al. 2015; Duy et al. 2019). *TET2* is frequently mutated in AML, largely by frameshift or nonsense mutations (Chou et al. 2011; Weissmann et al. 2012). Loss of TET2 impairs differentiation and increases self-renewal potential of HSCs but requires cooperating mutations such as FLT3-

ITD for full leukemic transformation (Delhommeau et al. 2009; Moran-Crusio et al. 2011; Quivoron et al. 2011; Shih et al. 2015). Notably, loss of TET2 alone has little effect on 5mC, but TET2 can synergistically induce DNA hypermethylation in the presence of a cooperating oncogene such as FLT3-ITD (Shih et al. 2015). Transformed cells derived from *Tet2*-deleted *Flt3*-ITD mice or human AML cells show DNA hypermethylation and suppression of GATA2, a hematopoietic master regulator, whose reconstitution impairs leukemia growth (Shih et al. 2015; Duy et al. 2019). Recruitment of TET2 to chromatin depends on transcription factors such as WT1 (Rampal et al. 2014; Wang et al. 2015). TET2 functions as a tumor suppressor in AML and requires the interaction with WT1 to suppress leukemia growth (Rampal et al. 2014; Wang et al. 2015). Overexpression of WT1 increased global levels of 5hmC, whereas reduced 5hmC levels were observed when WT1 was silenced. Recurrent missense mutations of TET2 can compromise its binding to WT1 and thereby fail to inhibit leukemia proliferation (Wang et al. 2015). Finally, loss of TET2 inactivates enhancers not only by reducing 5hmC but also through reduced enhancer histone marks such as H3K4me1 mediated through LSD1 (KDM1A) (Duy et al. 2019). Hence, loss of TET2 impairs multiple layers of the epigenome.

Figure 2. Epigenetic features and perturbations in acute myeloid leukemia (AML). (A) Diagram showing the structure of methylated cytosine (5mC) and oxidized mCs (5hmC, 5fC, 5caC) as well as their transcriptional association at promoters. Active DNA demethylation is indicated by black arrows below modified cytosines. 5fC and 5caC can be excised directly by the thymine DNA glycosylase (TDG), resulting in an abasic site that is eventually replaced with unmethylated cytosine by the base excision repair (BER) machinery. Activation-induced cytidine deaminase (AID) or APOBEC can deaminate 5mC to thymine that is subsequently removed by TDG and repaired by BER. On the other hand, passive demethylation (brown arrows) is an alternative process that can occur through dilution following cell replication. DNMT1 can directly replicate the 5mC pattern onto the newly synthesized daughter strand unlike the pattern of oxidized mCs. (For review, see Rasmussen and Helin 2016.) (B) Enzymatic processes involved in cytosine methylation and oxidation of 5mC. DNMTs catalyze the addition of a methyl group to cytosine using S-adenosyl methionine (SAM) as methyl donor. Ten-eleven translocation (TET) proteins oxidize methylated cytosines to 5hmC, 5fC, and 5caC in an iterative manner using 2OG (α KG) as co-substrate. Mutant IDH1/2 generates 2HG that inhibits competitively the enzymatic activity of TET proteins. (C) Nucleosome model shows lysine substrate residues on histone H3 for writers (KMTs) and erasers (KDMs) that are found mutated in AML. Chromosomal translocations involving KMT2A (KMT2A-re) often result in fusion proteins that associate with the H3K79 histone methyltransferase DOT1L. (D) Enzymatic processes of lysine (de)methylation for selected KMTs. Besides TET proteins, 2HG can also impair the enzymatic activity of Jumonji domain (JMJD)-containing KDMs.

Somatic Mutations of IDH1 and IDH2

AML somatic mutations in the *IDH1* and *IDH2* genes are virtually always heterozygous and affect *IDH1* on R132 or *IDH2* on the R140, R172 residues (Mardis et al. 2009; Abbas et al. 2010; Paschka et al. 2010). *IDH1* (cytosolic protein) and *IDH2* (mitochondrial protein) are metabolic enzymes that catalyze the interconversion of isocitrate to α -ketoglutarate (α KG) (Fig. 2B). α KG is necessary for oxidation of 5mC by TET enzymes (Fig. 2B). *IDH1/2* hotspot mutations yield an enzymatic gain of function that instead favors the synthesis and accumulation of (R)-2-hydroxyglutarate (2HG) (Dang et al. 2009; Gross et al. 2010; Ward et al. 2011). 2HG competitively inhibits α KG-dependent dioxygenases such as TET enzymes and the Jumonji domain histone lysine demethylases, resulting in loss of 5hmC, gain of 5mC, and gain of histone methylation (Fig. 2B,C) (Figueroa et al. 2010a; Chowdhury et al. 2011; Xu et al. 2011; Lu et al. 2012; Rampal et al. 2014). Mutant *IDH1/2* impairs HSC differentiation and it is notable that *IDH1/2* mutations are almost entirely mutually exclusive with TET2 mutations in AML, suggesting that their effects on 5hmC and/or 5mC are dominant transforming effects in myeloid cells (Figueroa et al. 2010a). However, unlike *Tet2* knockout mice, mutant *IDH1*^{R132} knock-in mice show reduced long-term (LT) HSCs and an altered DNA damage response caused by down-regulation of the ATM kinase independent of TET2 (Inoue et al. 2016). It was proposed that ATM down-regulation was mediated by 2HG inhibition of KDM4, resulting in a repressive chromatin environment suppressing ATM (Inoue et al. 2016). Moreover, 2HG can impair all TET enzymes, thereby causing a more pleiotropic effect than just mutant TET2 alone. Indeed, *IDH*^{mut} AML shows a greater DNA hypermethylation phenotype than *TET2*^{mut} AML (Rampal et al. 2014). Altogether, this suggests that the leukemogenic mechanisms are not equivalent and may contribute to different therapeutic responses of *IDH*^{mut} AML compared to *TET2*^{mut} (Patel et al. 2012; Duy et al. 2019). Moreover, *IDH*^{mut} and *DNMT3A*^{mut} AMLs feature opposing cytosine methylation profiles, and

double-mutant *IDH1/DNMT3A* AML show almost complete loss of these profiles, thus questioning the relevance of DNA methylation (Glass et al. 2017). How these mutations cooperate is still unknown, but double-mutant *IDH1/DNMT3A* AML features up-regulation of RAS signatures and unique sensitivity to MEK inhibition *ex vivo* relative to AMLs with either single mutation (Glass et al. 2017).

Somatic Mutation of Chromatin Modifiers in AML

Loss-of-function and missense mutations have been reported in core components of the Polycomb repressive complex 2 (PRC2) *SUZ12* and *EZH2*, which trimethylates histone H3 lysine 27 (H3K27me3) to induce transcriptional repression (Figs. 1 and 2C,D) (Ernst et al. 2010, 2012; Cancer Genome Atlas Research 2013). PRC2 induces stable silencing of early development genes, as well as transiently repressed bivalent chromatin promoters (H3K4me3, H3K27me3) (Margueron and Reinberg 2011). This complexity is exemplified by the finding that *Ezh2* deletion preceding transduction of oncogenes like MLL-AF9 or AML1-ETO9a accelerates leukemia progression, whereas deletion of *Ezh2* in already established AMLs with these oncogenes had the opposite effect and, in this case, attenuated disease progression (Basheer et al. 2019). Thus, EZH2 has a tumor-suppressive function at initiation of AML but a tumor-supportive one in maintenance of AML. Epigenetic changes induced by impaired function of PRC2 allows AML cells to tolerate increased stress including chemotherapy treatment (Göllner et al. 2017; Duy and Melnick 2018; Maganti et al. 2018). PRC2 functions can also be disrupted in the presence of inactivating somatic mutations in *ASXL1*, which directs PRC2 to target loci (Fig. 1) (Abdel-Wahab et al. 2012). Disrupting mutations in *BCOR* and *BCORL1* have been reported and likely impact Polycomb-like complexes (Grossmann et al. 2011; Metzeler et al. 2016; Chittock et al. 2017). Mutations in KDM6A (UTX), which demethylates H3K27me3, have been reported in a small cohort of AML (Figs. 1 and 2D) (Cancer Genome Atlas Research

2013; Metzeler et al. 2016). Knockout of UTX induces spontaneous AML and results primarily in bidirectional changes of H3K27Ac as well as chromatin remodeling that is associated with inactivation of tumor-suppressive GATA programs and activation of oncogenic ETS programs (Gozdecka et al. 2018).

The histone methyltransferase KMT2A (MLL) is frequently mutated by partial tandem duplications (PTDs) or chromosomal rearrangements, the latter resulting in loss of the catalytic SET domain (Caligiuri et al. 1998; Grimwade et al. 2001; Krivtsov and Armstrong 2007). KMT2A methylates H3K4, which plays a role in the formation and activity of enhancers and promoters (Fig. 2C,D) (Ernst et al. 2011). The duplicated region in MLL-PTD includes the CXXC domain, which preferentially binds to nonmethylated CpGs (Schichman et al. 1994; Birke et al. 2002) that can potentially perturb coordination with DNA methylation. Mice with *MLL-PTD* show dysregulation of homeobox (*Hox*) gene expression but require additional mutations such as the *Fli3-ITD* for full leukemogenesis (Dorrance et al. 2008; Zorko et al. 2012). MLL rearrangements (KMT2A-re) result frequently in fusion with genes encoding super elongation complex proteins like AF9, AF10, and ENL (Krivtsov and Armstrong 2007). This complex associates with the histone methyltransferase DOT1L that targets H3K79 (Fig. 2C,D). MLL-transformed AML samples show aberrant H3K79 methylation, leading to ongoing expression of *Hox* genes. The NSD1 H3K36 histone methyltransferase is sometimes fused with NUP98, which could affect several gene regulatory functions (Fig. 2C,D) (Wagner and Carpenter 2012).

An additional layer of epigenetic regulation in AML is conferred by the 3D conformation of chromatin that brings distant genes into spatial proximity with each other or that forms loops between enhancers and promoters (Kagey et al. 2010). Many of these interactions are mediated by the cohesin complex, a ring-like structure composed of *SMC1A*, *SMC3*, *RAD21*, and *STAG1/STAG2*. The cohesin complex is responsible for connecting sister chromatids, regulation of transcription, and DNA repair (Haarhuis et al. 2014; Kim et al. 2016). Mutations in the

cohesin complex are found between 6% and 18% in AML (Ding et al. 2012; Cancer Genome Atlas Research 2013; Kon et al. 2013; Thol et al. 2014; Thota et al. 2014; Tsai et al. 2017) and induce expansion of hematopoietic stem/progenitor cells and impair myeloid differentiation (Mullenders et al. 2015; Viny et al. 2015; Tothova et al. 2017). ASXL1 also interacts with the cohesin complex for proper gene regulation and might indicate a potential role in chromatin conformation (Li et al. 2017).

Epigenetic Therapy of AML

Targeting Specific Epigenetic Mechanisms

Aberrant DNA hypermethylation can be removed by DNMT inhibitors (DNMTi) (Fig. 3A) (Jones and Baylin 2007), although these drugs have pleiotropic effects and impact methylation on a genome-wide level. Specific inhibitors were recently developed against mutant IDH1/2 (AG120/AG221) and show a promising overall response rate of ~40% in patients with relapsed/refractory IDH-mutant AML (Stein et al. 2017; DiNardo et al. 2018); however, their impact on epigenetic reprogramming and remodeling in patient samples remains to be delineated. Despite the initial response to IDH inhibitors, multiple drug resistance pathways have been reported including isoform switching between mutant IDH1 and mutant IDH2, second-site target mutations, co-occurring NRAS mutations, and selection of ancestral or terminal clones (Amatangelo et al. 2017; Harding et al. 2018; Intlekofer et al. 2018; Quek et al. 2018). Although protein loss (e.g., truncating *TET2* mutations) cannot be targeted directly, approaches to compensate its function present an option. This has been shown with vitamin C, which enhances the catalytic activity of the residual wild-type TET proteins and suppresses leukemia growth (Fig. 3A) (Cimmino et al. 2017). A related approach was proposed for mutant DNMT3A by increasing intracellular levels of SAM (Fig. 3A) (Adema et al. 2017).

Targeting Epigenetic Mechanisms on Histones

LSD1 has emerged as a promising therapeutic target in AML (Fig. 3B) (Harris et al. 2012;

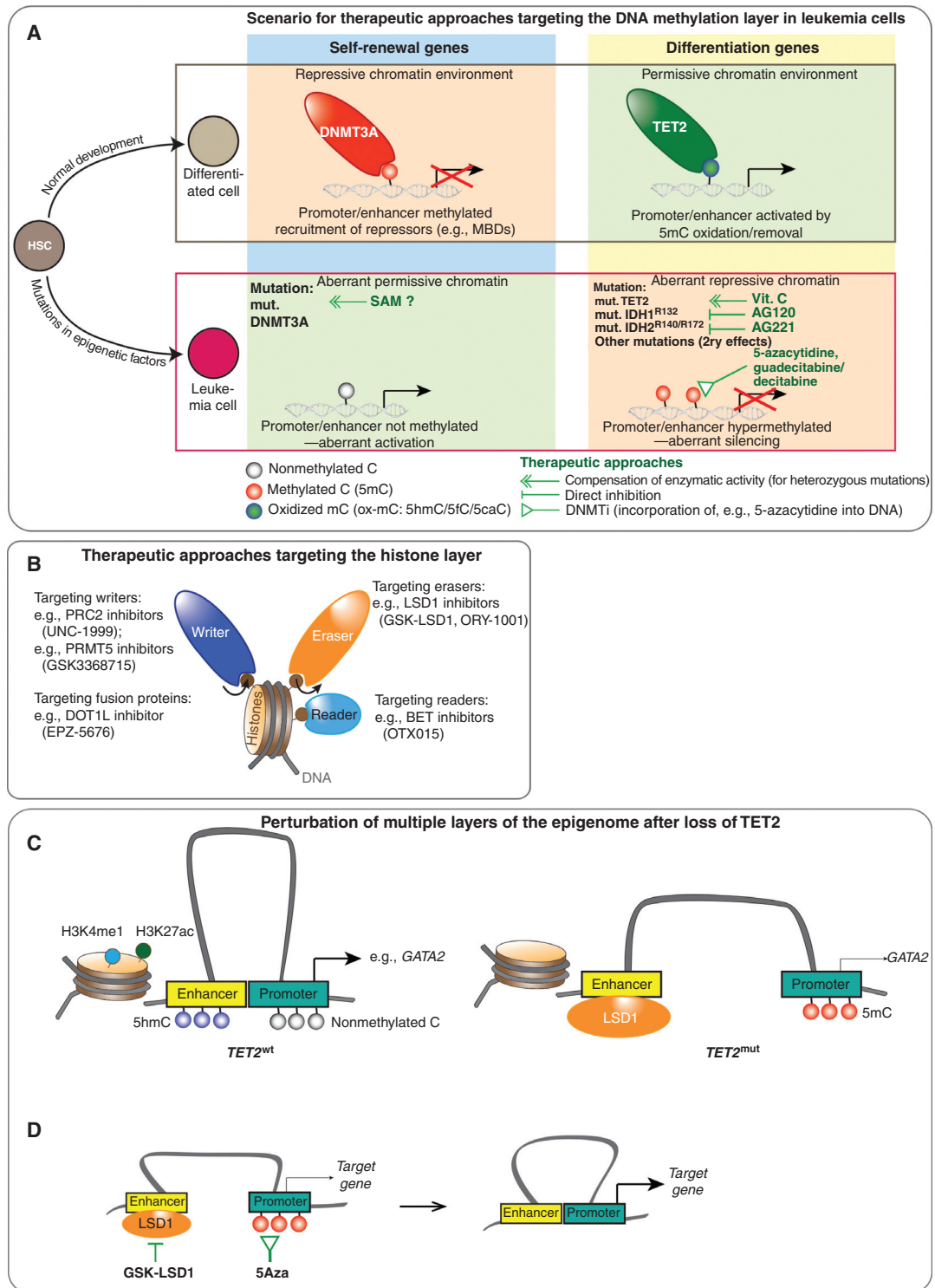


Figure 3. (See legend on following page.)



Schenk et al. 2012; McGrath et al. 2016; Maes et al. 2018). LSD1 inhibitors (LSD1i) present also another option in *TET2^{mut}* AML, in which it reverses aberrant repression of LSD1-inactivating enhancers (Duy et al. 2019). Moreover, inhibition of LSD1 can overcome nongenetic acquired drug resistance against BET inhibitors by modulating enhancer dependencies of key survival genes, showing further the critical role of enhancers in drug tolerance in AML (Bell et al. 2019). BET proteins can bind to acetylated lysines on histones (Fig. 3B) and facilitate transcriptional activation and promoter–enhancer interactions (Florence and Faller 2001; Wu and Chiang 2007). BRD4, a BET family member, has been implicated in AML, perhaps linked to maintaining MYC expression (Zuber et al. 2011). The role of PRC2 (EZH2) in AML is complex and not clear (Duy and Melnick 2018; Basheer et al. 2019). The use of UNC1999, a dual inhibitor of EZH1/2, impaired the growth of *MLL(KMT2A)*-rearranged leukemia cell lines suggesting that inhibition of PRC2 is detrimental in these leukemias (Fig. 3B) (Xu et al. 2015). Inhibition of DOT1L (EPZ-5676)

also targets *MLL*-rearranged AML (Fig. 3B) (Chen et al. 2016). In addition, inhibition of protein arginine methyltransferase 1 (PRMT1) and 5 (PRMT5) displays an antileukemic effect in certain AMLs (Fig. 3B) (Shia et al. 2012; Tarighat et al. 2016; Fedoriw et al. 2019). Overall, it is important to note that all these histone-regulating proteins are not frequently mutated or overexpressed in AML and also play distinct roles in normal cells. Thus, targeting these factors requires a careful approach in a therapeutic window that will preferentially interfere with key nononcogene dependencies underlying the malignant program of AML.

Epigenetic Combination Therapy

Because the epigenome consists of many functionally interdependent mechanisms, it is inherently challenging to fully correct aberrant epigenetic programming by hitting only a single epigenetic target. This consideration may explain in part the relatively modest activity of epigenetic therapies to date (Fennell et al. 2019). Combining epigenetic therapies is chal-

Figure 3. Epigenetic therapy and combination therapy targeting cooperating layers of the epigenome in acute myeloid leukemia (AML). (A) Scenario illustrating the effect of distinct epigenetic mutations (indicated in bold letters) in leukemia cells versus normal differentiated cells derived from hematopoietic stem cells (HSCs). Epigenetic therapy and strategies targeting aberrant DNA methylation are shown by green lines. Loss of DNA methylation at self-renewal genes in *DNMT3A^{mut}* AML may be potentially reversible by increasing intracellular levels of SAM (methyl donor). Loss of TET2 function can be compensated with vitamin C treatment. Specific inhibitors (AG120, AG221) against mutant IDH1/2 can block production of the TET inhibitor 2HG. Direct DNA hypermethylation (mutant TET2 or IDH1/2) or indirect hypermethylation due to secondary effects from other disease-driving mutations can be treated with DNMT inhibitors like 5-azacytidine or guadecitabine/decitabine (Issa et al. 2015; Gardin and Dombret 2017). (B) Drugs to target histone-modifying enzymes (writers+erasers) as well as readers (e.g., BET inhibitors) that are critical for AML maintenance and present vulnerabilities of the disease. Specific LSD1 inhibitors include GSK-LSD1 and ORY-1001 (Mohammad et al. 2015; Maes et al. 2018). Although PRC2 is deleted in a subset of AML, other AML subtypes such as *KMT2A-re* (*MLL*-rearranged) leukemias depend on functional PRC2. UNC1999, a dual inhibitor of EZH1/2, impaired the growth of *KMT2A-re* in preclinical studies (Xu et al. 2015). DOT1L inhibitors (e.g., EPZ-5676) were also developed to target *KMT2A(MLL)*-re AML (Chen et al. 2016). The BRD 2/3/4 inhibitor OTX015 induces apoptosis in a variety of AMLs (Coudé et al. 2015). Inhibition of PRMT1 and PRMT5 show an antileukemia effect in distinct AMLs (Shia et al. 2012; Tarighat et al. 2016; Fedoriw et al. 2019). (C) Scheme illustrating the disruption of multiple layers of the epigenome in *TET2^{mut}* cells. Loss of *TET2* facilitates recruitment of the H3K4me1/2 histone demethylase LSD1 that inactivates enhancers at target genes. In addition, loss of *TET2* results in promoter methylation at target genes such as *GATA2*. (D) Concept of targeting cooperating layers of the epigenome at enhancers and promoters in *TET2^{mut}* AML to reconstitute enhancer–promoter interactions. Removal of 5mC promoter methylation by 5Aza treatment combined with LSD1 inhibition (GSK-LSD1) facilitates interactions of the LSD1-occupied enhancer and its target promoter, resulting in up-regulation of target genes like *GATA2*.

lenging, however, because they may antagonize in vivo, such as occurred with the combination of DNMT inhibitors (DNMTi) + histone deacetylase inhibitors (HDACi) in AML patients (Prebet et al. 2014), and certain compounds such as HDACi have profoundly pleiotropic effects and hence significant toxicity in humans. Moreover, because little is known yet about how epigenetic mechanisms play into AML, it is difficult to predict which patients might respond to a given therapy, with rare exceptions such as HDACi. On the other hand, it is difficult to assess the efficacy of epigenetic therapies in the laboratory because AML cell lines do not reflect the genetic and epigenetic spectrum of AML cases. However, recent studies deploying organoid type cultures of primary AML cells are better suited to test epigenetic agents, many of which mediate their effects through slow and gradual effects. The unexpected enhanced activity of LSD1i+DNMTi against TET2 leukemia was discovered through such an approach, in which a large cohort of genetically characterized primary AMLs were screened using an organoid system (Duy et al. 2019). In *TET2^{mut}* AML, full activation of aberrantly silenced tumor suppressors such as GATA2 could only be restored by simultaneously reversing gene promoter hypermethylation using DNMTi and restoring active enhancer marks for the same genes using LSD1i (because loss of TET2/5hmC results in LSD1-mediated enhancer repression; Fig. 3C,D).

Combination of Epigenetic with Nonepigenetic Drugs

AML mutations in nonepigenetic factors (e.g., FLT3) provide a rationale to combine epigenetic with specific nonepigenetic drugs. Supporting this, combination of DNMTi and FLT3 inhibitors improved therapeutic response in *FLT3-ITD*-mutant AML (Ravandi et al. 2013; Muppidi et al. 2015; Strati et al. 2015; Chang et al. 2016; Shih et al. 2017). Also, combination of DNMTi with drugs targeting nonmutant proteins like BCL2 (venetoclax) improved therapeutic efficacy particularly in elderly patients with AML (Tsao et al. 2012; Bogenberger et al. 2015; Shih et al. 2017; Aldoss et al. 2018; Di-

Nardo et al. 2019). However, it is unclear whether this combination therapy is driven by epigenetic effects or rather by partial DNA damage caused by DNMTi treatment that results in apoptosis in leukemia cells after inhibition of BCL2. Because DNMTi also show immunomodulatory effects like induction of PD-1 and IFN- γ signaling, clinical trials exploring the combination of DNMTi with immune checkpoint inhibitor (ICI) drugs (e.g., nivolumab) are underway (Daver et al. 2019). Although CTLA-4 and PD-1 immune checkpoint drugs have revolutionized the management of certain cancer types like melanoma (Postow et al. 2015; Robert et al. 2015), other cancer types (e.g., pancreatic cancer) remain unaffected by ICI drugs (Brahmer et al. 2012). Given that the mutational burden of AML is one of the lowest compared with other cancers (Lawrence et al. 2013), the recognition of AML cells by the immune system is presumably less efficient compared with cancers with high mutational burden such as melanoma.

EPIGENETIC MECHANISMS IN B-CELL MALIGNANCIES

Most Lymphomas Arise from GC B Cells, Which Are Epigenetically Programmed to Resemble Tumor Cells

B-cell lymphomas arise from a wide variety of functionally distinct B-cell subsets to yield a bewildering number of lymphoma subtypes. Some of the more common B-cell neoplasms, their mutations, and their cell of origin are depicted in Figure 4. However, a majority of B-cell lymphomas (diffuse large B-cell lymphomas [DLBCLs] and follicular lymphomas [FLs]) arise from B cells transiting the GC reaction, which will be the focus of this section. GCs are transient structures that form in response to T-cell-dependent antigen (Hatzi and Melnick 2014). Naive B cells are epigenetically “primed”—that is, feature active chromatin marks—to induce expression of genes required for plasma cell differentiation, on receiving activation signals. However, after T-cell-directed activation, a subset of naive B cells is able to transiently silence these terminal differentiation genes and instead migrate within

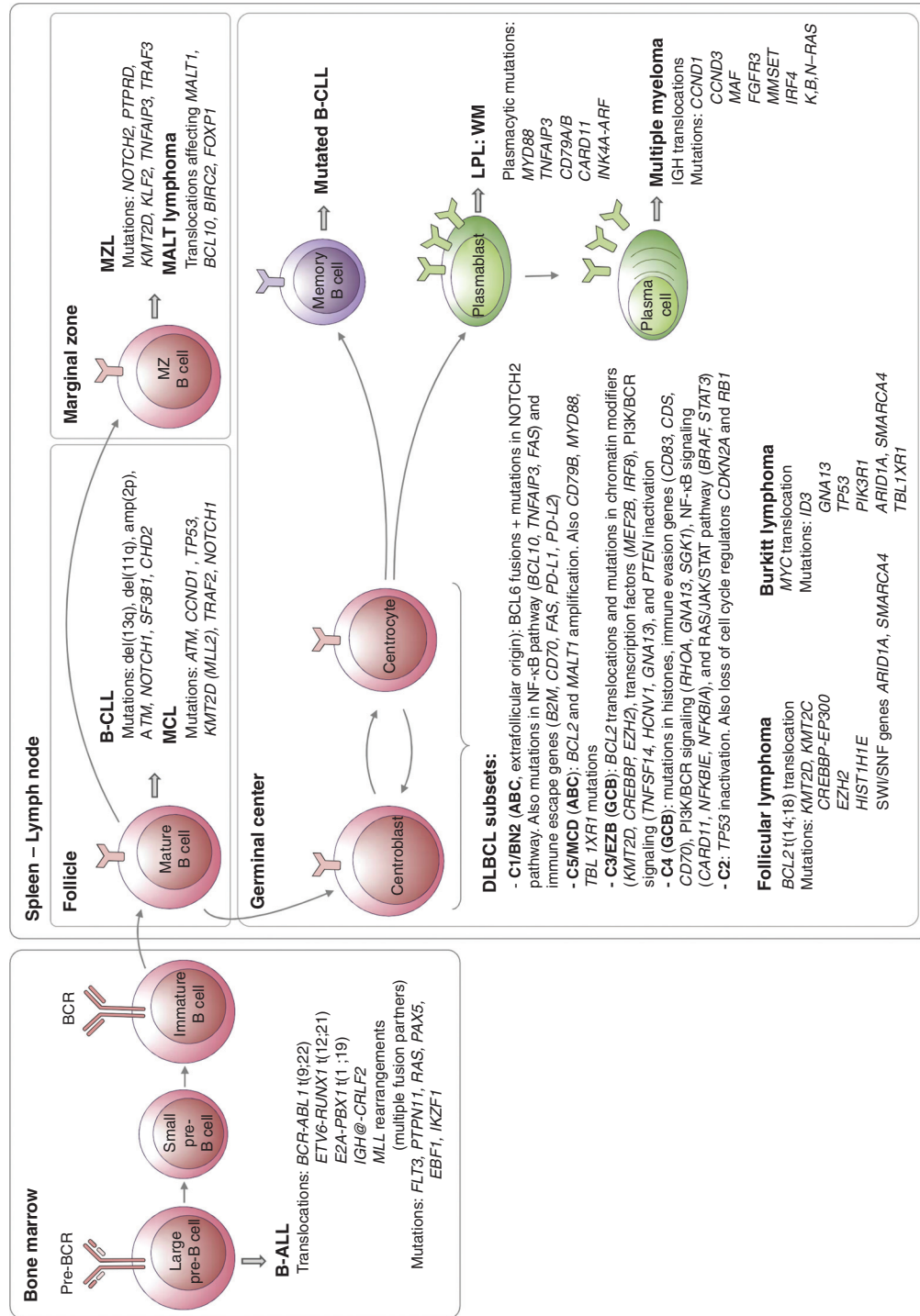


Figure 4. (See legend on following page.)

lymphoid follicles to form nests of highly proliferative GC B cells called “centroblasts,” which undergo somatic hypermutation of their immunoglobulin genes because of the actions of AICDA. After several rounds of division these GC B cells stop proliferating and compete to interact with a limiting number of T follicular helper (TFH) cells to survive. These nonproliferating GC B cells are called “centrocytes,” and interact with TFH cells through a number of receptor–ligand processes collectively called the “immune synapse” (Papa and Vinuesa 2018). Only the few

GC B cells that generate high-affinity B-cell receptors interact strongly with TFH cells and can then restore their original epigenetic programming to differentiate into plasma or memory B cells or undergo further somatic hypermutation. However, the vast majority of GC B cells undergo apoptosis (Fig. 5A) (Mesin et al. 2016).

Importantly, the phenotype of GC B cells features many hallmarks that are considered pathognomonic for cancer (Hanahan and Weinberg 2011). These include (1) sustained proliferation and self-renewal (through repression of

Figure 4. Frequent mutations during B-cell development. In the bone marrow, rearrangements of the immunoglobulin genes of B-cell precursors to form a B-cell receptor (BCR) generate DNA breaks that are occasionally resolved aberrantly, leading to chromosomal translocations (Fugmann et al. 2000). These are the most common genetic alterations in B-precursor acute lymphoblastic leukemia (B-ALL) (Mullighan 2012). The most frequent mature B-cell neoplasms that have their origin outside the germinal center (GC) are B-cell chronic lymphocytic leukemia (B-CLL), mantle cell lymphoma (MCL), marginal zone lymphoma (MZL), and mucosa-associated lymphoid tissue (MALT) lymphoma. B-CLL and MCL differ in their molecular pathways, genomic alterations, and clinical behavior, being more aggressive in naive-like- than memory-like-derived tumors. The pathogenesis of the two malignancies involves the BCR signaling, tumor cell microenvironment interactions, genomic alterations, and epigenome modifications (Zhang et al. 2014; Landau et al. 2015). MALT lymphoma is the commonest MZL type and presents recurrent chromosomal translocations, which usually lead to activation of the NF- κ B pathway. Nodal and splenic MZLs share recurrent mutations affecting the Notch pathway and the transcription factor *KLF2*, but differ for the inactivation of two tumor-suppressor genes, detected exclusively (*PTPRD*) or much more commonly (*KMT2D/MLL2*) in the nodal type (Rossi et al. 2012; Spina et al. 2016). The presence of immunoglobulin mutations is evidence that the cell of origin of the tumor passed through the GC microenvironment. Follicular lymphomas, Burkitt lymphomas, and DLBCLs express GC B cell signature genes. In the GC, two molecular processes remodel DNA: immunoglobulin class switch recombination (CSR) and somatic hypermutation (SHM), mechanisms that predispose to chromosomal translocations and mutations (Muramatsu et al. 2000). DLBCL is a clinically and genetically heterogeneous disease and accounts for 35% of non-Hodgkin lymphomas. Based on transcriptional profiles, DLBCL is further classified into activated B-cell (ABC) and germinal center B-cell (GCB) subtypes (Alizadeh et al. 2000; Rosenwald et al. 2002). ABC-DLBCLs derive from B cells that are committed to plasmablastic differentiation (Victora et al. 2012). These tumors have increased NF- κ B activity, genetic alterations in NF- κ B modifiers and components of the BCR pathway, and perturbed terminal B-cell differentiation (Lenz et al. 2008; Ngo et al. 2011). GCB-DLBCLs originate from light-zone GC B cells (Alizadeh et al. 2000; Victora et al. 2012). These tumors have frequent alterations in chromatin-modifying enzymes, PI3 K signaling, and genetic alterations of *BCL2* (Pfeifer et al. 2013; Basso and Dalla-Favera 2015). Modifications in these pathways could favor epigenetic reprogramming and escape from cellular immunity. Recent genomic profiles have identified sub-ABC and GCB-DLBCL clusters: C1-C5 in one study (of which two are GCB-, two are ABC-subtypes, and the fifth is mostly characterized by genomic instability and *TP53* mutations) (Chapuy et al. 2018), BN2, MCD, N1 (mostly ABC), and EZB (mostly GCB) in a different study (Schmitz et al. 2018). Follicular lymphoma (FL) 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 inter-follicular regions (Kridel et al. 2012). The most frequent genetic event is the t(14;18) translocation that places *BCL2* under control of the immunoglobulin heavy-chain enhancer, which occurs in 90% of FL patients. Mutations in epigenetic modifiers (*KMT2D*, *CREBBP*, and *EZH2*) are also a hallmark of FL (Green 2018). These mutations result in altering normal B-cell differentiation programs and impeding GC exit (Green et al. 2015). Burkitt lymphoma is characterized by deregulation of the *MYC* gene through its translocation to one of the immunoglobulin loci (Love et al. 2012). LPL, lymphoplasmacytic lymphoma; WM, Waldenstrom macroglobulinemia.

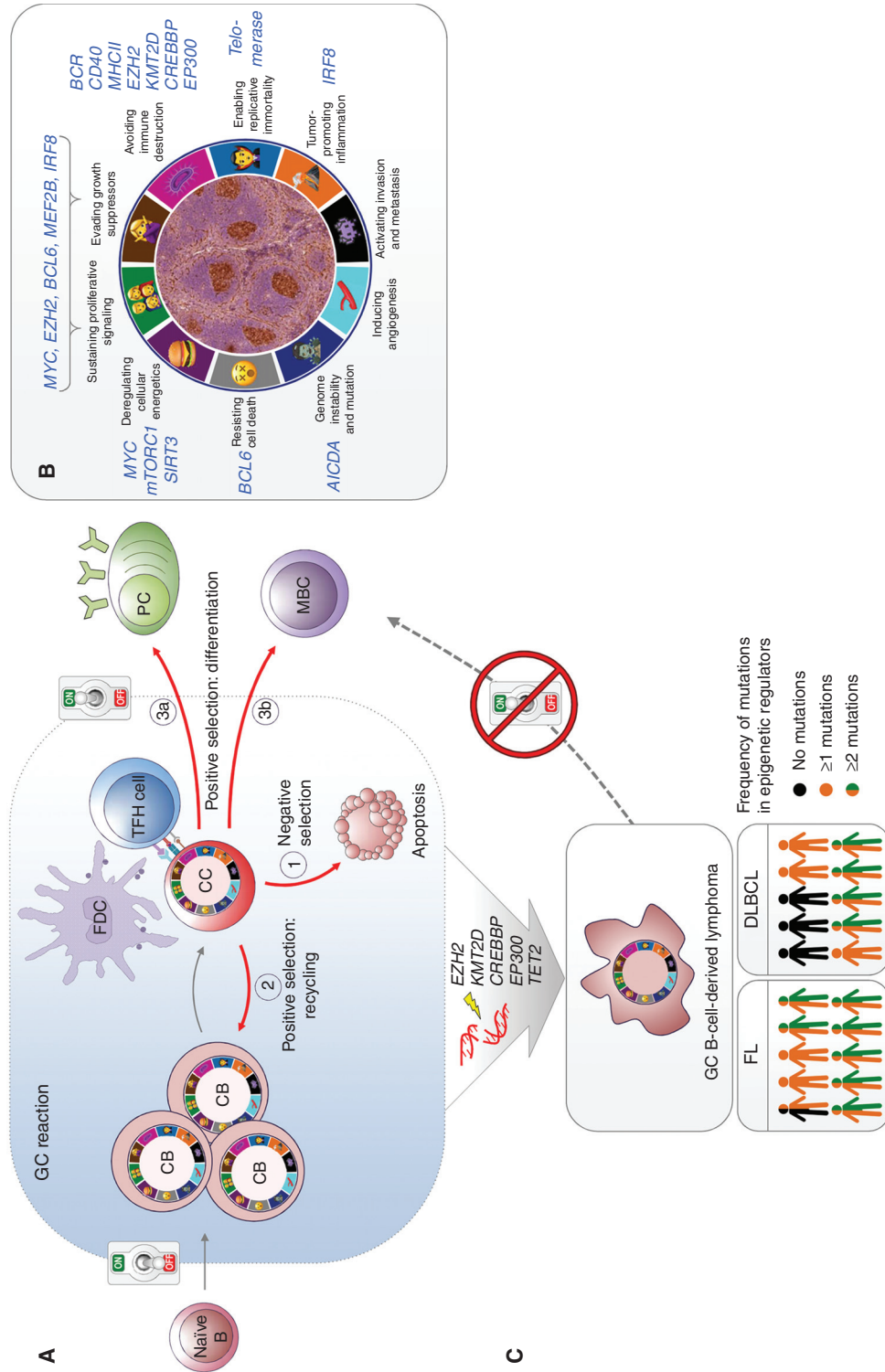


Figure 5. (See legend on following page.)



cell cycle checkpoint genes) (Phan and Dalla-Favera 2004; Phan et al. 2005; Cato et al. 2011; Béguelin et al. 2013, 2017); (2) potential immortalization (induction of telomerase) (Hu et al. 1997; Herrera et al. 2000; Norrback et al. 2001); (3) silencing of DNA damage checkpoint genes (repression of *ATR*, *CHEK1*, and *TP53*) (Phan and Dalla-Favera 2004; Ranuncolo et al. 2007, 2008); (4) genome instability and mutagenesis (caused by AICDA) (Muramatsu et al. 2000; McHeyzer-Williams et al. 2015; Teater et al. 2018); (5) resistance to cell death (induction of stress response pathways) (Cerchiatti et al. 2009; Fernando et al. 2019); (6) deregulated energetics (efficient anaplerosis to support massive growth needs) (Doughty et al. 2006; Jellusova et al. 2017; Waters et al. 2018); (7) blockade of terminal differentiation (Béguelin et al. 2013, 2016); and (8) evasion from immune surveillance (down-regulation of antigen presentation and immune synapse genes) (Fig. 5B) (Basso et al. 2004; Good-Jacobson et al. 2010).

However, unlike cancer cells in which the transformed phenotype is primarily irreversible and caused by somatic mutations, in GC B cells this phenotype is solely induced through epigenetic reprogramming, which is reversible once B cells exit from the GC reaction (Bunting et al. 2016; Rivas and Melnick 2019). One of the more prominent epigenetic features of the GC reaction is the transient silencing of promoters and enhancers for genes involved in proliferation checkpoints, BCR signaling, CD40 signaling, in-

terferon response, antigen presentation, and plasma cell differentiation (Béguelin et al. 2013, 2016; Hatzi et al. 2013; Ortega-Molina et al. 2015; Jiang et al. 2017). Most of this effect is coordinated by the transcriptional repressor BCL6, which is induced in the GC reaction and silenced on GC exit. Many of the mutations occurring in DLBCL and FL result in strengthening this effect (Fig. 5C).

Somatic Mutations in Epigenetic Modifiers Are a Hallmark of GC-Derived B-Cell Lymphomas

More than 70% of the genes mutated in DLBCL and FL are epigenetic modifiers and components of transcription factor complexes (Green et al. 2015; Ortega-Molina et al. 2015; Reddy et al. 2017; Chapuy et al. 2018; Schmitz et al. 2018). These include the H3K4 methyltransferase *KMT2D*, the H3 acetyltransferases *CREBBP* and *EP300*, the H3K27 methyltransferase *EZH2*, and *TET2*. Approximately 95% of FL patients manifest at least one of these mutations (Green et al. 2015), as do a majority of patients with GCB-DLBCL (Reddy et al. 2017; Chapuy et al. 2018; Schmitz et al. 2018). Each one of these genes is required to either establish the GC phenotype or to facilitate exit from the GC reaction. All are acquired early during pathogenesis and are considered founder mutations (Green et al. 2015; Pasqualucci and Dalla-Favera 2015). Mutations in *TET2* are the earliest and only DLBCL

Figure 5. Vulnerable points of germinal center (GC) B cells that give advantage to lymphomagenesis. (A) Chromatin-based epigenetic switches transiently poise the active plasma/memory B-cell program to enable the GC phenotype to emerge in a reversible manner. Cell fate decisions during a normal GC reaction: (1) negatively selected centrocytes (CC) undergo apoptosis; positively selected centrocytes may (2) recycle to centroblast (CB) and reenter the dark zone, or (3) differentiate to (3a) plasma cells (PC) or (3b) memory B cells (MBC). (B) GC B cells feature the typical hallmarks of transformed cells through epigenetic mechanisms, without requiring somatic mutations. This “pseudo-malignant” state can be reversed to normal state also through epigenetic switching mechanisms. (Sketch adapted from Hanahan and Weinberg 2011.) An immunohistochemistry (IHC) picture of GCs identified with peanut agglutinin (PNA) stain in a murine splenic section is shown in the middle. (C) Mutations in epigenetic modifiers maintain B cells in the GC phenotype, allowing the development of GC-derived B-cell lymphomas. Although mutations of one or more chromatin modifier genes occur within 96% of follicular lymphoma (FL) and ~70% diffuse B-cell lymphoma (DLBCL) patients, 76% FL, and ~40% DLBCL cases feature at least two mutations in epigenetic regulators (Green et al. 2015; Ortega-Molina et al. 2015; Reddy et al. 2017; Chapuy et al. 2018; Schmitz et al. 2018).

mutations that originate in HSCs (Quivoron et al. 2011; Dominguez et al. 2018).

Wild-Type and Mutant EZH2 in the GC Reaction

EZH2 is induced and required for the formation of GC B cells (Velichutina et al. 2010; Béguelin et al. 2013; Caganova et al. 2013), where it deposits the H3K27me₃ mark at active promoters (marked with H3K4me₃) to form bivalent chromatin (Béguelin et al. 2013). The H3K27me₃ mark then attracts the repressive Polycomb-like BCOR complex (Béguelin et al. 2016). However, a second tether is needed for BCOR recruitment and is provided by BCL6 (Huynh et al. 2000; Ghetu et al. 2008; Béguelin et al. 2016). This EZH2-BCOR-BCL6 combinatorial tethering process explains how EZH2 represses GC context-specific target genes, thanks to DNA sequence-specific binding of BCL6. Heterozygous somatic mutation of *EZH2* occurs in up to 30% of FL and GCB-DLBCL patients and primarily affects the EZH2 SET (histone methyltransferase) domain at residue Y641 (Morin et al. 2010; Bödör et al. 2013; Okosun et al. 2014; Reddy et al. 2017). Unlike AML, in which *EZH2* mutations result in loss of function, these mutations confer a gain of function that makes EZH2 more efficient at H3K27 trimethylation, but less efficient at H3K27 monomethylation (Sneeringer et al. 2010; Yap et al. 2011; McCabe et al. 2012). Mice engineered to express heterozygous mutant *Ezh2* in GC B cells develop hyperplasia and B-cell lymphoma (Béguelin et al. 2013, 2016). This may be a consequence of more profound and less reversible silencing of *EZH2* target genes involved in cell cycle checkpoints and GC exit. In contrast homozygous expression of mutant *Ezh2* phenocopies the *EZH2* knockout phenotype, showing that the WT *EZH2* allele is required to cooperate with the mutant allele (Béguelin et al. 2016).

Somatic Mutations of CREBBP and EP300

These often manifest as missense mutations that inactivate their histone acetyltransferase do-

main, or truncations leading to loss of the allele (Cerchietti et al. 2010; Morin et al. 2011; Pasqualucci et al. 2011; Green et al. 2015; Jiang et al. 2017). *CREBBP* and *EP300* maintain H3K27 acetylation mark at enhancers for genes involved in immune synapse and antigen presentation functions in B cells (Jiang et al. 2017). However, during the GC reaction BCL6 toggles these enhancers to a poised configuration by recruiting the SMRT/HDAC3 complex (Hatzi et al. 2013) (different than the function of BCL6 at promoters with BCOR and EZH2). *CREBBP* and p300 reactivate these enhancers when B cells are induced to exit the GC reaction. However, their loss-of-function mutation in lymphoma impairs this function and results in unopposed repression of these genes by HDAC3 and biological dependence on this protein (Jiang et al. 2017; Mondello et al. 2019). Loss of *CREBBP* or *EP300* in GC B cells accelerates lymphomagenesis in mice (Garcia-Ramírez et al. 2017; Hashwah et al. 2017; Jiang et al. 2017; Zhang et al. 2017). The MHC class II genes are critical *CREBBP* targets that are aberrantly and persistently silenced by HDAC3 in *CREBBP*-mutant lymphoma cells (Hashwah et al. 2017; Jiang et al. 2017; Zhang et al. 2017).

Somatic Mutations of KMT2D

KMT2D has functions analogous to *CREBBP* in maintaining H3K4 monomethylation of gene enhancers in B cells. During the GC reaction, BCL6 represses KMT2D regulated enhancers in part through direct recruitment of LSD1, which in turn recruits the CoREST complex (Hatzi et al. 2019). In B cells, exiting the GC reaction KMT2D is required to enable the functionality of enhancers that normally respond to CD40 and BCR signaling. Lack of KMT2D leads to aberrant repression of these genes, failure of B cells to differentiate and exit the GC reaction, and eventually lymphomagenesis (Ortega-Molina et al. 2015; Zhang et al. 2015).

TET2 Mutations in the GC Context

TET2 mutations in DLBCL are similar in nature to those occurring in myeloid neoplasms (Quiv-

C. Duy et al.

oron et al. 2011). Loss of *TET2* in GC B cells results in loss of enhancer 5hmC including at the *PRDM1* locus, which is a master regulator of plasma cell differentiation. In addition, there is repression of many of the same genes that are normally induced by CREBBP on GC exit. Indeed, *TET2* and *CREBBP* mutations are generally mutually exclusive, suggesting they represent a similar pathogenic hit. Along these lines, *TET2* loss of function results in failure of CREBBP to mediate H3K27 acetylation at gene enhancers and makes lymphoma cells biologically dependent on HDAC3 (Dominguez et al. 2018).

The Immune Synapse as a Focal Point for Epigenetic Deregulation in Lymphoma

Reversion of the GC B-cell phenotype is essential to immune homeostasis. This effect is largely dependent on the immune synapse and interaction with TFH and follicular dendritic cells, involving CD40, BCR, MHC class II, IL21, and other signaling pathways (Fig. 6A) (Papa and Vinuesa 2018). Through unknown signals, the immune synapse reverses the poised state of gene promoters and enhancers driven by BCL6 with BCOR, EZH2, HDAC3, and LSD1 during the GC reaction. This is impaired by somatic mutations of *EZH2*, *CREBBP*, *EP300*, *KMT2D*, and *TET2*. Mutant *EZH2* mediates aberrant silencing of cell cycle checkpoint inhibitors, MHC I and MHC II, and other GC exit genes (Béguelin et al. 2013; Ennishi et al. 2019). *CREBBP*, *KMT2D*, *EP300*, and *TET2* loss of function disables enhancers that respond to CD40, BCR, and MHC class II genes (Ortega-Molina et al. 2015; Zhang et al. 2015, 2017; Hashwah et al. 2017; Jiang et al. 2017; Dominguez et al. 2018). Thus, the primary effect of epigenetic founder mutations in DLBCL and FL is mostly about “maintaining” GC B cells in an inherently oncogenic state by suppressing their ability to interface with the immune microenvironment. This also likely explains how mutant GC B cells evade immune surveillance to give rise to DLBCLs and FLs (Nicholas et al. 2016). Moreover, it is possible that aberrant immune synapse interactions with mutant

GC B cells could epigenetically reprogram components of the immune microenvironment to form a lymphoma-permissive cell niche (Fig. 6B).

Cytosine Methylation Patterning in Lymphomagenesis

Unlike the case of AML, 5mC patterning is not established as a driver of disease pathogenesis in GC-derived lymphomas. To date, DNA methylation profiling studies in DLBCL and FL have failed to identify robust and specific patterns of cytosine methylation indicative of underlying pathogenesis or with links to particular somatic mutations, with the possible exception of *TET2*. As compared with naive B cells, human and murine GC B cells manifest a characteristic hypomethylation signature (Dominguez et al. 2015, 2018; Teater et al. 2018). This appears to most likely represent a genomic “scar” effect of AICDA-mediated cytosine deamination of methylated residues that are replaced with unmethylated cytosines during replication, because *AICDA* knockout GC B cells fail to manifest this phenotype (Dominguez et al. 2015). Maintenance of DNA methylation is clearly important because, as expected, reduction of DNMT1 levels in mice severely impair GC formation (Shaknovich et al. 2011). DNA methylation profiling studies in cohorts of DLBCL patients have shown that progressive shifting of 5mC distribution from the normal GC pattern is linked to inferior clinical outcomes (Chambwe et al. 2014). Moreover, aberrant methylation and silencing of genes such as *SMADI* have been causally linked to chemotherapy resistance in DLBCL and can be overcome by administering DNA methyltransferase inhibitors (Clozel et al. 2013).

The Role of Epigenetic Therapy in GC-Derived Lymphomas

Nonspecific Epigenetic Therapies

The most experience to date with “epigenetic” therapy in lymphoma involves first-generation, pan-HDAC inhibitors. By and large these stud-

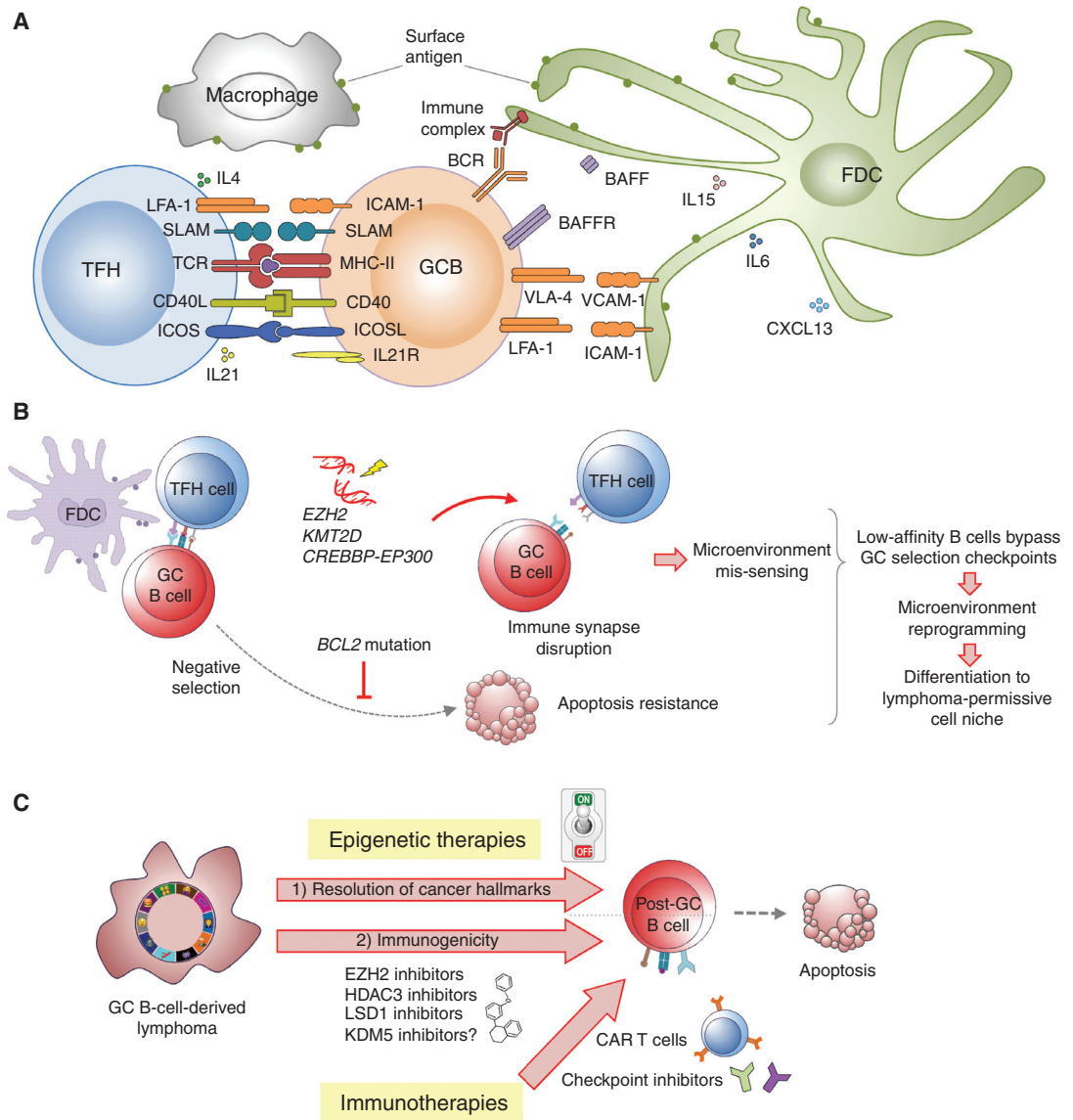


Figure 6. The immune synapse and epigenetic therapy. Like germinal center (GC) B cells, the microenvironment in B-cell malignancies is crucial for the provision of survival and proliferation signals. (A) In both cases, normal GC B cells and malignant B cells interact with T cells, dendritic cells, macrophages, and lymphoid stromal cells (follicular dendritic cells) (Papa and Vinuesa 2018). (B) Mutations in epigenetic regulators and oncogenes such as *BCL2* allow low-affinity B cells to survive, leading to the initiation of a pro-survival, immunosuppressive microenvironment in the lymphoid tissue. (C) Although epigenetic therapy can overcome the effect of founder mutations, the microenvironment makes critical contributions to both disease progression and drug resistance/disease relapse. The combination of epigenetic therapy with checkpoint inhibitor therapy can lead to potent antilymphoma effects.

ies have been disappointing (Sermer et al. 2019). This may not be entirely surprising because these drugs are pleiotropic in their actions, have toxic side effects and there are no definitive, causal data indicating that they affect lymphoma cells through a particular epigenetic effect. The one bright spot has been in the setting of T-cell lymphomas, albeit for unknown biological reasons. BET inhibitors, which block binding of BRD4 to acetylated lysines, have shown toxicity against lymphoma cells in laboratory models but modest effects in patients (Chapuy et al. 2013; Amorim et al. 2016). On the other hand, DNMTis are well-tolerated in lymphoma patients in combination with chemo-immunotherapy and yielded favorable outcomes in phase I/II studies (Clozel et al. 2013). DNMTis were shown to reverse chemotherapy resistance in patients through the ex vivo study of sequential lymphoma biopsies obtained on patients enrolled in DNMTi trials (Clozel et al. 2013), warranting the expansion of these studies into the phase III setting.

Precision Epigenetic Therapy

Given the specific role of aberrant epigenetic modifiers in DLBCL and FL, there is great interest to deploy therapeutic agents that could reverse these effects. The only such approach to reach the clinic are EZH2 inhibitors, based on the strong rationale that EZH2 is essential to GC B cells and its mutant forms further enhance dependency on EZH2. Early clinical trials (albeit in patients with aggressive disease) show that EZH2i are well-tolerated and confer a significant clinical benefit (Gulati et al. 2018). Although there are a number of PRC2 inhibitors moving into clinical trials, most data available to date come from studies of the selective EZH2 inhibitor tazemetostat. A phase I, dose-escalation study in DLBCL patients previously treated with R-CHOP (NCT02889523) reported favorable safety results. However, in April 2018, the Food and Drug Administration (FDA) placed a temporary hold on the enrollment of new U.S. patients into this clinical trial because of a pediatric patient who developed a T-cell lymphoma while taking the drug. Another phase II clinical

trial enrolls subjects with DLBCL and FL for the determination of efficacy and safety of tazemetostat monotherapy in combination with prednisolone (NCT01897571). For FL, it was reported that tazemetostat achieved objective response rates of 71% in patients with EZH2 mutations and 33% in patients with wild-type EZH2. These data are encouraging because the patients enrolled in this study had already undergone at least two previous therapies.

Despite achieving this milestone, there are currently no biomarkers that can predict whether a given patient will be responsive nor is the optimal timing and duration of dosing established. The fact that DLBCLs with *EZH2* mutations manifest silencing of MHC I, MHC II, and reduced infiltrating CD4 and CD8 cells has pointed toward a potential immunomodulatory effect of EZH2i (Ennishi et al. 2019). However, it will be necessary to assess whether EZH2i might suppress antitumor immunity, given that PRC2 also plays critical roles in T-cell activation.

Because *CREBBP*- and *TET2*-mutant DLBCLs are addicted to HDAC3, there is a rationale for deploying selective HDAC3i to the clinic. HDAC3 has been biochemically purified as a component of the SMRT complex, >90% of which is bound with BCL6 in lymphoma B cells, pointing to the likely highly selective impact of HDAC3 inhibitors (Hatzi et al. 2013). Indeed, treatment of DLBCL cell lines or primary patient specimens with HDAC3i resulted in strong induction of MHC class II and triggered T-cell-mediated killing of lymphoma cells (Mondello et al. 2019). In contrast to pan-HDACis, HDAC3is lack toxicity against hematopoietic and T cells (Mondello et al. 2019) and hence represent a promising approach to restore T-cell-mediated immune surveillance against *CREBBP*-mutant DLBCL or FL cells. In the case of *KMT2D*-mutant lymphomas, there may be a rationale for targeting LSD1, although it would be required to use compounds that degrade LSD1 because purely enzymatic inhibition of this histone demethylase is insufficient to counteract its full affect in suppressing *KMT2D* target genes (Hatzi et al. 2019). It is also proposed that compounds that target the KDM5

family of H3K4 demethylases might also offer selective activity in the setting of *KMT2D*-mutant patients (Mondello et al. 2018).

Although these more precise epigenetic therapies represent a logical and promising opportunity, their most effective use will be in combination regimens, especially with immunotherapies. The success of immunotherapies such as checkpoint inhibitors and even CAR T cells is generally mixed in the setting of FL and DLBCL, perhaps linked to their epigenetic down-regulation of genes needed for B cells to effectively interface with T cells. Epigenetic therapies that reverse these effects might potentially synergize with these immunotherapy modalities (Fig. 6C).

EPIGENETIC ALLELES, CLONAL EVOLUTION, AND THERAPY RESISTANCE IN HEME MALIGNANCIES

AMLs and lymphoma are composed of genetically distinct clones, and clonal complexity is linked to inferior clinical outcome, presumably because this endows tumors with greater population fitness. Because 5mC patterning is maintained with 1000-fold less fidelity than genome sequencing during replication, there is far more opportunity to develop clonal variance in epigenetic marks than in genetic composition. Indeed, foci of CpGs with variable methylation with populations of cells were identified by two initial studies (Hansen et al. 2011; Shaknovich et al. 2011), one in solid tumor cells and one in normal GC B cells. Epigenetic heterogeneity in GC B cells was considered as a possible precursor to malignant transformation: Perhaps sampling of epigenetic states during the GC reaction could yield selection advantages and the formation of premalignant clonal precursor cells (Shaknovich et al. 2011). Indeed, the first study of epigenetic heterogeneity was performed in GC-derived lymphomas (FL and DLBCL) and showed more severe 5mC heterogeneity in these tumors as compared with GC B cells (De et al. 2013). The extent of 5mC heterogeneity was associated with shorter survival, suggesting that it contributes to lymphoma population fitness (De et al. 2013).

Linking together the concepts of epigenetic heterogeneity and tumor clonality, several groups developed the concept of “epigenetic alleles,” defined as the DNA methylation status of groups of four consecutive CpGs present on the same DNA strand (Li et al. 2016). Analysis of DLBCL patients indicated that epigenetic allele diversity is linked to risk for relapse (Pan et al. 2015). Relapsed DLBCLs showed selection epigenetic allele states, which suggests that particular 5mC patterns were best suited to tolerate exposure to chemotherapy (Pan et al. 2015). Epigenetic allele studies in CLL also showed a link between 5mC diversity and outcome (Landau et al. 2014). Loci that manifested epigenetic allele diversity in CLL patients were also associated with deregulated transcription, providing further evidence that epigenetic heterogeneity has functional implications (Landau et al. 2014). Notably, 5mC heterogeneity in the context of normal GC B cells and GC-derived lymphomas was shown to be caused at least in part by AICDA (Dominguez et al. 2015; Teater et al. 2018). This is likely due to stochastic deamination of 5mCs in B cells, which eventually leads to AICDA dose-dependent 5mC heterogeneity (Dominguez et al. 2015; Teater et al. 2018).

A large-scale integrative analysis of exomes, whole-genome sequencing, methylomes, and transcriptomes in matched paired AML specimens showed that epigenetic allele diversity was an independent risk factor for time to relapse and, most remarkably, was completely independent of genetic clonality (Li et al. 2016). Genes linked to promoters with epigenetic allele diversity manifested diversity in their transcriptional output, and after relapse there was also evidence of selection for distinct epigenetic clonal states (Li et al. 2016). Taken together, epigenetic allele diversity or 5mC heterogeneity have emerged as critically important biological features of lymphoid and myeloid neoplasms that cannot be predicted based on tumor genetics. It is intriguing to speculate that DNMT inhibitors might actually suppress epigenetic clonal diversity, perhaps explaining in part how these drugs enhance the effect of chemotherapy drugs in AMLs and DLBCLs.



CONCLUDING REMARKS

It is evident that epigenetic mechanisms play a fundamental role in the pathogenesis of hematologic malignancies. However, as of yet, we only have a very superficial view of the depth and breadth of epigenetic mechanisms and very few therapeutic approaches that are amenable for clinical translation. We need to dig deeper into how epigenetic marks connect with each other and the role of novel layers of the epigenome. It is also necessary to consider the potential for epigenetic heterogeneity among these layers and within given patients. There is a need to understand bidirectional epigenetic programming between tumor and microenvironment and the host immune system, and how epigenetic drugs could have beneficial or deleterious effects on these various cellular compartments. This is an important and richly rewarding avenue of research that will require concerted effort to truly harness from the therapeutic standpoint.

ACKNOWLEDGMENTS

A.M. is supported by National Cancer Institute (NCI) R35 CA220499, NCI 1UG CA233332, NCI R01 CA198089, the Leukemia & Lymphoma Society (LLS) TRP 6572-19, LLS SCOR 7013-17, the Follicular Lymphoma Consortium, and the Chemotherapy Foundation. W.B. is supported through LLS SCOR 7012-16, LLS TRP 6572-19, and NCI R35 CA220499. C.D. is a recipient of the LLS Fellow Award LLS 5486 and is supported by NCI R01 CA198089.

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