An epigenetic view of B-cell disorders

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REVIEW

An epigenetic view of B-cell disorders

Federica Alberghini[1], Valentina Petrocelli[1], Mahshid Rahmat[1] and Stefano Casola

B-cell development is a multistep process sustained by a highly coordinated transcriptional network under the control of a limited set of transcription factors. Epigenetic mechanisms, including DNA methylation, histone posttranslational modifications and microRNAs act in concert with transcription factors to promote lineage commitment, define and sustain cell identity and establish heritable cell-type- and stage-specific gene expression profiles. Epigenetic modifiers have recently emerged as key regulators of B-cell development and activation. Central to B-cell-mediated immunity are germinal centers, transient structures formed in secondary lymphoid organs where antigen-specific B cells undergo intense proliferation, immunoglobulin somatic hypermutation and isotype switching, to generate ultimately long-lived memory B cells and terminally differentiated plasma cells expressing high-affinity antibodies. Deregulation of one or more epigenetic axes represents a common feature of several B-cell disorders arising from germinal center B cells, including autoimmunity and lymphoma. Moreover, the hijacking of epigenetic determinants is central to the ability of the B-lymphotropic Epstein–Barr virus (EBV) to establish, via the germinal center reaction, life-long latency and occasionally contribute to malignant B-cell transformation. In the light of recent findings, this review will discuss the relevance of epigenetic deregulation in the pathogenesis of B-cell diseases. Understanding how specific epigenetic alterations contribute to the development of lymphomas, autoimmunity and EBV-associated disorders is instrumental to develop novel therapeutic interventions for the cure of these often fatal pathologies.
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INTRODUCTION

In multicellular organisms, homeostasis relies on the coordinated function of different cell types whose identity is defined by gene expression patterns imposed by cell-type-specific transcription factors (TFs). Accessibility of TFs to *cis*-regulatory regions of target genes, which is necessary to promote (or repress) their transcription, is regulated by the local state of the chromatin. Chromatin is the complex formed by DNA and histones. Core histones assemble to form the nucleosome unit, which comprises two copies of each histone subtype, namely H2A, H2B, H3 and H4, around which 146 base pairs (bp) of DNA are bound. The N terminus of histone tails can be subjected to different kinds of posttranslational modifications, including acetylation, methylation and ubiquitylation, which affect their affinity for DNA and therefore the degree of chromatin accessibility to the transcriptional machinery. Histone modifications, together with DNA methylation and miRNAs, belong to an essential regulatory axis, also known as epigenetics, which contributes to ensure stable gene expression patterns through cell division.

DNA methylation is an active process that promotes the addition of a methyl group to cytosine, converting it into 5-methylcytosine. This modification occurs mainly at CpG-rich genomic regions, with the exception of CpG islands that reside in close proximity to promoters of actively transcribed genes. DNA methylation is catalyzed by DNA methyltransferases (DNMTs). DNMT3A and DNMT3B mediate *de novo* methylation, whereas DNMT1 acts on hemimethylated DNA to ensure inheritance of DNA methylation patterns through cell division.[1]

Regulatory modifications of histone tails are exerted via acetylation, methylation, SUMOylation and ubiquitylation of specific amino-acid residues of core histones subunits. Acetylation of lysine residues correlates with active transcription and is controlled by the opposing activities of histone acetyltransferases (HATs) and deacetylases (HDACs).[2] Histone methylation has different effects depending on the specific residue that is modified. Trimethylation of lysine-4 on histone H3 adjacent to the transcriptional start site is associated with transcription of the target gene. It is carried out by the Thritorax (Trx) complex, as a result of the activity of members of the mixed lineage leukemia (MLL) family of methyltransferases. Dimethylation of lysine-36 on histone H3 is also associated with active transcription and its deposition within gene bodies relies on the activity of *N*-methyltransferase family members NSD1, NSD2 and NSD3. On the other hand, methylation of lysine-9 of histone H3 and lysine-20 of histone H4 is associated with gene silencing.[3] Addition of these covalent modifications is catalyzed by the methyltransferases SUV39H1/2 and SUV420H1/2, respectively. Trimethylation of lysine-27 of histone H3 (H3K27me3) also represses transcription. It is catalyzed by the polycomb group protein enhancer of zeste homolog 2 (EZH2) as part of the polycomb repressive complex (PRC) 2.[4] Chromobox-containing proteins allow recognition of H3K27me3 by PRC1, a multiprotein complex that catalyzes ubiquitylation of

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lysine-119 on histone H2A through the Ring domain-containing proteins RING1A and RING1B.[4] Through deposition of H3K27me3 and ubiquitylation of lysine-119 on histone H2A, PRC2 and PRC1 cooperate to promote gene silencing. This model of sequential and concerted activity of the two complexes has been challenged by recent lines of evidence suggesting the existence of diverse PRC1 complexes with different subunit composition, some of which display H3K27me3-independent chromatin binding.[4–6] Trimethylation of H3K27 is counteracted by the Jumonji C domain-containing proteins, ubiquitously transcribed tetratricopeptide repeat gene, X chromosome (UTX/KDM6A) and the Jumonji domain-containing protein 3 (JMJD3/KDM6B).

Both UTX and JMJD3 are recruited into chromatin remodeling complexes to facilitate transcription of target genes. This can be achieved through both H3K27me3 demethylase-dependent and -independent mechanisms.[7–10] Despite sharing similar catalytic activity, UTX and JMJD3 may exert opposing functions in both physiological[11–14] and pathological settings.[15,16] Methylation can also occur on arginine (R) residues within histone tails and is carried out by different members of the protein arginine methyltransferases family. Importantly, depending on the methylation symmetry, the mark can either activate (for example, symmetric H3R2me2) or repress (for example, asymmetric H3R2me2) gene transcription.[17]

MicroRNAs (miRNAs) are 21- to 25-nucleotide-long RNAs that are generated through subsequent cleavage steps of a precursor form called pri-miRNA. Processing of the pri-miRNA starts in the nucleus by a complex containing the RNAse III Drosha and Pasha/DGCR8. Approximately 70-nucleotide-long pre-miRNAs are then translocated to the cytoplasm where they get further cleaved by the double-strand RNAse III Dicer to generate 19- to 22-bp-long double-stranded pre-miRNAs. Finally, slicing of pre-miRNAs that occurs within the RNA-induced silencing complex gives rise to the mature form of the miRNA. Interaction between the miRNA and its mRNA targets occurs within RNA-induced silencing complex through incomplete base pairing. This leads to translational repression, deadenylation and decay of the target mRNA, which depend on the action of Argonaute proteins.[18,19]

Epigenetic mechanisms have important roles in controlling gene expression during embryogenesis and postnatal development. Alterations in patterns of epigenetic modifications are associated with several pathological conditions, including cancer, autoimmune diseases (ADs) and viral infections.

B-cell lymphopoiesis is a tightly coordinated process where identity specification and cellular maturation are under the control of genetic programs centered on lineage-specific TFs that act in a hierarchical manner.[20] In mammals, postnatal B-cell development starts in the bone marrow (BM). Following hematopoietic stem cell commitment, common lymphoid progenitors give rise to progenitor B cells that activate the expression of recombination activation gene (*RAG*)*-1* and *-2*, to generate immunoglobulin (Ig) heavy- (H) and light- (L) chain variable (V) region genes through a process called VDJ recombination. Expression of functional IgH and IgL chains and their pairing to form a functional Ig receptor (also called B-cell receptor, or BCR) is a prerequisite for the transition from the progenitor to the immature B-cell stage.[21] Newly formed immature B cells undergo a selection process that prevents further maturation of cells whose BCR recognizes self-antigens with high affinity. Expression of a functional, non-autoreactive BCR allows B cells to leave the BM and reach secondary lymphoid organs, where they complete their differentiation to become B-2/follicular (Fo), marginal zone or B-1 B cells. Marginal zone and B-1 B cells mostly contribute to innate immune responses by differentiating into short-lived, low-affinity, antibody-secreting plasma cells (PCs) upon encounter with foreign antigens often derived from blood-borne pathogens. Fo B cells represent the majority of mature B cells present in secondary lymphoid organs. Fo B cells are predominantly recruited into T-cell-dependent (TD) immune responses upon antigen recognition through the BCR.[22] During a TD immune response, antigen-specific Fo B cells, supported by T-cell help, are recruited to form germinal centers (GCs). During the GC reaction, intense B-cell proliferation is accompanied by somatic mutation of Ig variable region genes and Ig isotype switching, both dependent on enzymatic activity of activation-induced cytidine deaminase (AID).[23] B cells expressing mutated Ig receptors undergo stringent antigen-dependent selection, allowing only few cells, namely those expressing high-affinity BCRs, to exit the GC after differentiation into long-lived high-affinity memory B cells or antibody-secreting PCs.[21] The high rate of somatic mutations coupled to the frequent occurrence of DNA double-strand breaks as a consequence of Ig isotype switching render GC B cells highly susceptible to malignant transformation. Indeed, most B-cell malignancies, including Hodgkin disease (HD), the most aggressive subtypes of non-Hodgkin B-cell lymphoma (NHL) and multiple myeloma (MM), originate from B cells that have transited through the GC.[23] The GC reaction represents also an elective site for the generation and expansion of autoreactive B cells that drive severe forms of ADs similiar to systemic lupus erythematosus through the production of autoantibodies.[24] Finally, through the GC reaction, B-cell lymphotropic Epstein–Barr virus (EBV) acquires life-long persistence in the host organism by accessing the pool of long-lived memory B cells.[25]

Recent studies have shed light on the importance of different epigenetic determinants, including DNA methylation, specific histone modifications and miRNAs in the regulation of B-cell lymphopoiesis and immunity (for further reading refer to Barneda-Zahonero *et al.*[26]). This review will summarize evidence of the contribution of aberrant epigenetic regulation of B-cell development and function to the pathogenesis of B-cell disorders ranging from autoimmunity to lymphomas.

EPIGENETICS OF B-CELL AUTOIMMUNITY

ADs are pathogenic conditions characterized by aberrant immune responses triggered by the recognition of self-antigens that lead to tissue destruction and organ failure. B cells contribute to autoimmunity through the production of autoantibodies, by presenting autoantigens to T cells and by secreting proinflammatory cytokines.[27] Autoreactive B cells arise as a result of failures of checkpoint mechanisms ensuring self-tolerance mainly at the immature and GC B-cell stages.[24,27] In the BM, immature B cells expressing newly formed BCRs that recognize self-antigens are either eliminated or, more frequently, lose the ability to respond to BCR-mediated antigenic recognition (this state is also called anergy).[28,29] Interference with the establishment of self-tolerance or subversion of anergy is among the driving forces of autoimmunity. Although several genetic mechanisms predisposing to ADs have been identified,[24,27] the contribution of epigenetics to the pathogenesis of these diseases has only started to be appreciated. Exposure of BM B cells to hydralazine, which inhibits DNA methylation, followed by adoptive cell transfer into syngeneic mice, leads to a disorder resembling human systemic lupus erythematosus.[30] This condition resulted from the failure of hydralazine-treated B cells to induce *RAG-2* expression, which is critical for editing of autoreactive BCRs.[30,31] DNA methylation also has a role in preserving anergy. Hypomethylation of the *CD5* gene, which encodes for a negative regulator of BCR signaling, induces the

expression of a non-functional isoform generated through alternative splicing. The latter condition predisposes to increased signaling from autoreactive BCRs.[32]

Histone modifications also contribute to B-cell tolerance. The finding that mice carrying a B-cell-specific mutation of the HAT *Ep300* develop a systemic lupus erythematosus-like disease suggests that checkpoints preventing the selection of autoreactive B cells are strictly dependent on the correct acetylation of histone and/or non-histone substrates.[33] However, the molecular mechanisms through which defective p300 promotes B-cell autoimmunity remain poorly understood.

MiRNA-dependent regulation of B-cell selection is crucial for the prevention of B-cell autoimmune disorders. Conditional gene targeting studies have assigned to the miRNA-processing enzyme Dicer a critical function in the establishment of B-cell tolerance.[34] Specifically, the absence of processed miRNAs in *Dicer* mutant BM B cells resulted in the selection of an aberrant primary antibody repertoire enriched with self-reactive specificities. This contributed to the development of antibody-based autoimmunity in aged animals.[34] B-cell autoimmunity may also occur as a result of aberrant selection of B cells recruited into the GC reaction during a TD immune response.[24] The miR-17-92 cluster has emerged as an important determinant of GC B-cell selection. Xiao *et al.*[35] reported that overexpression of miR-17-92 restricted to B and T lymphocytes resulted in enlarged GCs. Increased miR-17-92 expression caused resistance of B cells to Fas-mediated apoptosis. This was associated with impaired T-cell tolerance as a result of downregulation of miR-17-92 targets PTEN and proapoptotic factor Bim. Such alterations promoted chronic GC reactions accompanied by the selection of autoreactive IgG class-switched PCs secreting pathogenic antibodies.[35] In accordance with its positive role in sustaining the GC B-cell response,[36,37] inactivation of *miR-155* in a mouse model of systemic lupus erythematosus alleviated clinical symptoms of the disease and reduced production of GC B-cell-derived pathogenic IgG autoantibodies.[38] MiR-155 inhibition also decreased autoantibody production in a mouse model of myasthenia gravis, a disease characterized by muscular weakness due to the selection of pathogenic B cells expressing autoantibodies against the acetylcholine receptor at neuromuscular junctions.[39] Similar to miR-155, silencing of miR-146a also decreased disease burden in mouse models of myasthenia gravis. This effect correlated with fewer B-1, PC and memory B cells, limited B-cell activation and impaired Ig isotype switching.[40] Deregulated expression of miR-146a and miR-155 has been in the mean time reported in several additional forms of AD.[41]

EPIGENETICS OF EBV INFECTION AND ASSOCIATED DISORDERS

EBV is a γ-herpesvirus that infects the human population worldwide. Human B lymphocytes represent the main target of EBV infection, which typically proceeds in an asymptomatic manner. EBV can occasionally promote serious diseases, including infectious mononucleosis and hemophagocytic lymphohistiocytosis. As its original identification in endemic Burkitt lymphoma (BL) cells, EBV has been implicated in the pathogenesis of several B-cell malignancies, including HD and aggressive forms of B-cell lymphomas arising in immunocompromised patients (that include posttransplant lymphoproliferative disorders).[42] EBV infection of B cells triggers an active and protective T-cell- and NK cell-mediated immune response, which ultimately results in the clearance of most infected B lymphocytes. The lytic form of EBV infection requires full activation of the viral transcriptional program, which is necessary for the production of new viral particles. In sharp contrast, life-long persistence of EBV in B cells depends on general silencing of virus-encoded genes with the exception of EBV nuclear antigen-1 (EBNA-1), which is required for replication and partitioning of the viral episome in dividing cells.[43] External stimuli and specific activation states of host B cells influence the establishment of three alternative latency programs that sustain life-long persistence of the virus in infected individuals and occasionally trigger lytic cycle activation.[43] The study of EBV-associated malignancies has been instrumental to dissect the molecular mechanisms through which different sets of EBV proteins, active during specific latency programs, affect the transcriptional status of the host cell. In particular, genome-wide analyses have shown that, following EBV infection, B cells undergo substantial epigenetic reprogramming. Specifically, histone marks associated with both constitutive (H4K20me3) and facultative (H3K9me3 and H3K27me3) heterochromatin were considerably reduced following EBV infection.[44] This epigenetic profile was associated with increased genome-wide accessibility to endonucleases, suggesting increased transcriptional competence of large genomic regions following EBV infection. This hypothesis is confirmed by the marked changes in transcriptome profiles of naive B cells following EBV infection, predominantly consisting of host genes that were upregulated. Importantly, such epigenetic changes did not correlate with the ability of the virus to induce B-cell proliferation.[44] The contribution of specific EBV proteins to the epigenetic resetting of EBV-infected B cells has only recently started to be revealed.

Latent membrane protein 1 (LMP1) is an EBV oncoprotein that, alone, is sufficient to transform B cells.[45] LMP1, together with latent membrane protein 2 (LMP2) and EBNA-1, represents the only EBV protein expressed in malignant Hodgkin Reed–Sternberg cells of HD. LMP1 is also expressed in a different latency program (latency III) that is active in malignant B cells of patients affected by posttransplant lymphoproliferative disorders.[42,46] LMP1 mimics a constitutively active CD40 receptor, promoting both cell proliferation and survival through concomitant modulation of the NF-κB, MAPK and JNK signaling pathways.[43] LMP1 is expressed during acute infection of naive B cells and, transiently, in GC B cells to favor the recruitment of EBV-infected cells into the pool of long-lived memory B cells.[25] LMP1 exerts a significant influence on epigenetic regulation of gene expression in normal and malignant B cells. In particular, it induces the expression of the demethylase JMJD3 to facilitate the removal of H3K27me3 mainly from genes specifying the GC B-cell program, which are usually repressed in naive B lymphocytes.[47] At the same time, LMP1 can recruit EZH2 to the promoter of tumor suppressor genes, including *DOK1*, mediating epigenetic silencing via H3K27me3 deposition.[48] LMP1 can also influence gene expression through the regulation of DNA methylation via DNMT1.[49] The contribution of EBV proteins to the resetting of the host DNA methylome needs careful evaluation, as large genomic areas undergoing DNA demethylation soon after infection are coupled to specific regions that acquire *de novo* methylation.[49,50] The latter process likely depends on DNMT3A, whose expression is upregulated soon after infection of the target cell.[49] Beside LMP1, other EBV proteins hijack the epigenetic machinery and influence the transcriptome profile of host cells to allow viral persistence and propagation. EBNA-3A, -3B and -3C are DNA-binding proteins that have an essential function during the early stages of EBV infection and are required for *in vitro* B-cell immortalization.[51] Expression of EBNA-3 factors is also consistently found in malignant posttransplant lymphoproliferative disorder B cells. Induction of EBNA-3 proteins correlates with substantial silencing of the B-cell transcriptome, suggesting a role as transcriptional repressors.[52] This is, at least partially, achieved through

PRC2-dependent recruitment and deposition of H3K27me3 (and hence silencing) onto EBNA-3 target genes including tumor suppressors *BIM* and *CDKN2A*.[53]

EBV infection and its persistence within normal and malignant B cells is accompanied by marked changes in host miRNA expression. Moreover, the EBV genome itself encodes for several miRNAs, which are expressed in specific latency stages to fine tune expression of both host and viral mRNA targets.[54] EBV-dependent rewiring of the proteome of infected B cells through the action of miRNAs is relevant to sustain host cell proliferation, survival and for immune evasion. MiR-155 is one of the host miRNAs that is most strongly upregulated in response to EBV infection.[55,56] Induction of miR-155 by LMP1 may facilitate the entry of the virus into the pool of long-lived memory B cells by sustaining the transit of infected B cells through the GC reaction. Through miR-155 induction, EBV (and in particular LMP1) may support both B-cell survival and proliferation.[57] MiR-155 may also help infected B cells to evade the immune system via down-modulation of B-cell-autonomous anti-viral responses.[58] The functional relevance of EBV modulation of cellular miRNA expression may in some instances appear difficult to interpret, as the same miRNA is subjected to both positive and negative regulation by different viral proteins. This is the case for miR-146a, which is strongly induced by LMP1 and instead repressed by EBV nuclear protein EBNA-2A.[59,60] Modulation of cellular miRNA expression by EBV can contribute to malignant B-cell transformation. Interestingly, clusters of different miRNAs show aberrant expression in different types of EBV-associated lymphomas.[61] Whether such differences depend on the particular B-cell type that is targeted by malignant transformation and/or the specific EBV latency program active in the precursor tumor B cell remains yet to be understood.

EPIGENETIC DEREGULATION DRIVES B-CELL TRANSFORMATION

Alterations in the mechanisms driving B-cell development and that specify lymphocyte identity and/or function can lead to tumor development. B-cell malignancies are grouped into two major types, HD and NHL. MM, originating from terminally differentiated PCs, represents the third major group of B-cell malignancies. Common NHL subtypes (including diffuse large B-cell lymphoma, DLBCL, follicular lymphoma, FL and BL), HD and MM originate from B cells that have transited through the GC reaction. Within GCs, intense proliferation coupled to AID-induced single- and double-strand DNA breaks render B cells susceptible to the acquisition of chromosomal translocations and somatic mutations of non-Ig genes that may eventually drive lymphomagenesis.[23] Whole genome and/or exome sequencing efforts have contributed in recent years to improve our understanding of the genetic bases of NHL and MM. Such analyses have highlighted that epigenetic dysregulation represents a recurrent alteration associated with malignant B-cell transformation.[62,63]

Loss-of-function mutations affecting HAT genes *CREBBP* and *EP300* have been reported in 35% of GC-derived DLBCL and in 40% of FL cases, respectively. Mutations in these genes targeted the catalytic domain, disrupting in most cases one of the two alleles.[64] Functional studies have started to unravel the consequences of such mutations. DLBCL carrying mutations in *CREBBP* and *EP300* featured reduced acetylation of the tumor suppressor p53 and that of the proto-oncogene and master regulator of the GC reaction BCL6. Importantly, whereas reduced acetylation of p53 impaired its transcriptional activity, HAT haploinsufficiency exerted a stabilizing effect on BCL6 protein levels, thereby possibly sustaining its oncogenic function.[65] Whether *CREBBP*/*EP300* mutations also affect other molecular functions (including the accessibility of pioneering TFs to enhancers via modulation of H3K27 acetylation) to sustain lymphoma growth remains to be established. The high frequency of heterozygous mutations in *CREBBP* and *EP300* identified in GC-derived DLBCL and FL may reflect an exquisite sensitivity of (GC) B cells to changes in acetyltransferase activity provided by the two HATs. In line with this hypothesis, simultaneous inactivation of CREBBP and EP300 in the mouse model interfered with B-cell development.[66] Importantly, *Ep300*;*Crebbp* double-deficient animals failed to spontaneously develop B-cell lymphomas, indicating that loss of function of the HATs requires additional genetic hits to promote malignant B-cell transformation.[66] The high frequency of DLBCL and FL cases featuring *CREBBP*/*EP300* haploinsufficiency has provided the rationale to start clinical trials based on HDAC inhibitors for the treatment of these malignancies.[67]

Another major epigenetic axis that is commonly deregulated in NHL is centered on the H3K4 methyltransferase MLL2/KMT2D. Monoallelic mutations disrupting MLL2 catalytic activity have been identified in 30% of DLBCL and in 89% of FL cases. *MLL2* paralogue, *MLL3*, is also mutated in 15% of DLBCL, supporting a scenario whereby the MLL2/MLL3 axis has a central role in the pathogenesis of DLBCL and FL.[64] MLL2/MLL3 may participate in B-cell malignant transformation by acting at multiple levels. As components of the Trx complex, MLL proteins catalyze H3K4 trimethylation at the promoter of target genes, thereby facilitating their expression. This activity is exerted by antagonizing the function of PcG proteins at shared targets.[68] In activated B cells, AID recruitment to *Ig* loci is modulated by local deposition of trimethylation of lysine-4 on histone H3 by members of the MLL family.[69,70] MLL proteins also influence cell-type- and stage-specific transcriptional programs by regulating chromatin accessibility at enhancer regulatory sequences.[71] MLL2/MLL3 haploinsufficiency may contribute to lymphomagenesis by altering the fine balance between Trx and PcG complexes, thereby facilitating the persistence of B cells within the GC and sustaining AID mutagenesis.[72,73] MLL function, within the Trx complex, is supported by the action of H3K27me3 demethylase UTX.[7,74] Biallelic inactivating mutations of *UTX* are commonly observed in MM,[75] and have recently been also reported in DLBCL.[76] *UTX* mutations in MM predominantly target the Jmj-C containing demethylase domain, possibly leading to impaired enzymatic activity. Importantly, *UTX* and *MLL2/MLL3* mutations are mutually exclusive in MM, suggesting that altered Trx function, through either MLL haploinsufficiency or UTX inactivation,[62,75] represents a critical step in malignant PC transformation. Haploinsufficiency of *MLL2/MLL3* was shown to be also mutually exclusive with inactivating mutations of the other H3K27me3 demethylase *JMJD3*, in DLBCL cases.[15]

Increased and/or deregulated expression of the H3K27 methyltransferase EZH2 is commonly observed in HD and NHL, in particular DLBCL, FL and BL.[77,78] Importantly, *EZH2* gain-of-function mutations have been described as recurrent genetic events in GC-derived DLBCL and FL, pointing to a critical contribution to malignant GC B-cell transformation.[79] In support of this, studies in a mouse model of NHL and whole genome sequencing analyses of DLBCL and FL cases have identified *EZH2* gain-of-function mutations as possible driver mutations.[15,72,80] Evidence highlighting the role of EZH2 in GC B-cell development helped to understand the molecular mechanisms underpinning its possible transforming activity in these cells. *Ezh2* conditional mutant mice showed impaired GC B-cell proliferation, survival and increased tendency to undergo terminal differentiation.[72,73] Importantly, *Ezh2* mutant GC B cells suffered from heightened apoptosis due to AID mutagenesis, indicating a critical role

exerted by the polycomb protein in the protection against genotoxic stress.[73] Elucidation of genome-wide H3K27me3 distribution in GC B cells revealed the existence of a selected set of TFs, including PC determinant and tumor suppressor *BLIMP1*, whose repression by Ezh2 is crucial to retain B cells in the GC reaction.[73] Importantly, enforced H3K27me3-dependent silencing of *BLIMP1* by an EZH2 gain-of-function mutant was necessary to support DLBCL growth *in vitro*.[72,73] It is important to mention that contribution of polycomb deregulation to hematological malignancies appears to be cell-type-specific. Indeed, in tumors of the myeloid lineage and in T-cell precursor acute lymphoblastic leukemia, PRC2 function is frequently inactivated via mutations of genes coding for its essential subunits EZH2, SUZ12 and EED.[81–84] A similar tumor suppressor role for Ezh2 has been proposed in progenitor B-cell lymphomas driven by deregulated c-MYC expression.[85] Opposing functions in tumorigenesis have also been observed for the H3K27 demethylase JMJD3. Indeed, whereas inactivating mutations are identified in DLBCL and FL, a tumor-promoting function for JMJD3 has been proposed in T-cell precursor acute lymphoblastic leukemia.[15,16]

The histone code of malignant B cells is often altered by concomitant deregulation of two or more histone writers exerting opposing functions. H3K36 methyltransferase *MMSET/NSD2* is constitutively overexpressed in over 20% of MM cases as a result of the t(4;14),chromosomal translocation. Activating mutations in *NSD3* have also been observed in MM.[62] Increased H3K36 di- and trimethylation in MM cells bearing the t(4;14) translocation are commonly accompanied by a concomitant reduction in genome-wide H3K27me3 levels, in accordance with a model whereby H3K27me3 and dimethylation of lysine-36 on histone H3/3-specific histone methyltransferase complexes compete at promoter sites to regulate target gene expression.[86] Interestingly, a selected list of genes retained H3K27me3 at the transcriptional start site in NSD2-overexpressing MM cells. The repression of the latter genes, including miR-126* (which targets the c-MYC proto-oncogene), appears critical to sustain the transformed phenotype, as pharmacological inhibition of EZH2 caused a substantial growth retardation of MM cell lines bearing the t(4;14) translocation.[86] The dependence of MM cases carrying the t(4;14) translocation on concomitant deregulation of NSD2[86] and PRC2 activities provides the rationale to implement therapies targeting both epigenetic axes for the treatment of this subset of tumors. Along the same lines, global reduction in protein acetylation has identified HDAC inhibitors, and in particular those inhibiting HDAC3, as suitable anti-MM drugs.[87]

Aberrant DNA methylation is observed in several forms of B-cell NHL. In particular, inwith DLBCL, tumor B cells manifest global hypomethylation when compared with their wild-type GC B-cell counterparts, with exceptions represented by promoters of relevant tumor suppressor genes including *CDKN2A*, *CDKN1A* and *CDKN1B*.[88] Genome-wide distribution analysis of DNA methylation has confirmed a distinct cellular origin for GC- and activated B-cell-type DLBCLs.[89] Quantification of DNA methylation changes assessed genome-wide in DLBCL cells as compared with GC B cells has allowed the identification of six distinct subgroups, each characterized by aberrant methylation of a selected subset of target genes. Importantly, assignment of DLBCL to a specific DNA methylation group correlated with a particular clinical outcome.[88,90] Intersection between DNA and histone methylation patterns has revealed a close correlation between aberrant DNA methylation and EZH2-dependent H3K27me3 deposition in DLBCL.[91] These results support the cooperative action of these two epigenetic mechanisms to promote aberrant gene expression in B lymphoma cells.

MiRNAs represent another major class of epigenetic determinants that has been causally linked to B-cell transformation. Chronic lymphocytic leukemia, an indolent mature B-cell malignancy, often evolving into a lethal disease, features recurrent deletion of the 13q14 chromosomal region encompassing the miR-15a/16-1 cluster.[92] Elegant work in the mouse model has shown that miR-15a/16-1 is critical to limit age-dependent expansion of $CD5^+$ B cells that are considered the precursors of chronic lymphocytic leukemia.[93] This was achieved through negative regulation of cell-cycle regulators Ccnd2, Ccnd3, Cdk4, Cdk6 and Chk1,[93] and of the antiapoptotic protein BCL2.[94]

Deregulation of miR-155 expression is common to many forms of NHL and HD, which share a GC B-cell origin. In GC B cells, miR-155 limits the expression of AID, which is responsible for IgH-MYC t(8;14) translocations that are typical of BL. Interestingly, BL cells show low expression levels of *miR-155*, which may lead to enhanced/sustained AID mutagenic activity. Importantly, transcriptional repressor BCL6, which is expressed in BL cells, is able to silence *miR-155* expression, thereby possibly sustaining AID oncogenic function.[95] Although BL- and GC-derived DLBCL show low miR-155 levels, other lymphomas, including activated B-cell-type DLBCL, display higher levels of the same miRNA.[96] This result points to a scenario whereby the same miRNA acts either as an oncogene or tumor suppressor depending on the particular set of target mRNAs expressed by the lymphoma cell. A considerable fraction of aggressive B-cell lymphomas including DLBCL and BL display overexpression of the miR-17-92 cluster, often caused by genome amplification. Experiments based on cell-type-specific inactivation (or constitutive expression) of miR-17-92 in the mouse model have indicated an important contribution of this cluster to B-cell lymphomagenesis.[35,97] Tumor-promoting functions of miR-17-92 include downregulation of inhibitors of the PI3K (*Pten*), NF-κB (*A20, Cyld*) and the intrinsic apoptotic (*Bim*) pathways.[35,97,98] MiR-17-92 also acts within a

Table 1 List of relevant references describing the contribution of main epigenetic determinants to B-cell disorders

	Autoimmunity	*EBV-associated disorders*	*Lymphoma*
DNA methylation	31,32	48,49,50	88–91
Histone modifiers			
EZH2		48,53	15,72,73,77–85
SUZ12			83,84
EED			83,84
UTX			75,76
JMJD3		47	15,16
MLL2			64
MLL3			64
P300	33		64,65
CREBP			64,65
HDAC3			87
NSD2			86
miRNA			
Dicer	34		
Mir-155	38,39	55–58	95,96
Mir-17-92	35		97–100
Mir-15a/16-1			93,94
Mir-146a	40	59,60	

Abbreviations: EBV, Epstein–Barr virus; miR, microRNA.

MYC-centered regulatory network, which appears to be critical to sustain the transformed phenotype.[99,100]

CONCLUSION AND FUTURE PERSPECTIVES

Studies on animal models have highlighted critical contributions of different epigenetic determinants in sustaining heritable gene expression during B-cell lymphopoiesis. Epigenetic reprogramming is also critical to ensure B-cell effector functions elicited during immune responses, such as the production of neutralizing antibodies and the generation of long-lived memory B-cell pools as a result of the GC reaction.[73] Genome-wide studies addressing the distribution of specific chromatin marks in both stem and somatic cells (including B cells) have revealed close correspondence between certain histone modifications and distinct functional domains of the genome. This has allowed the assignment of specific marks to active and repressed chromatin states, as well as to other regulatory regions. The existence of such chromatin domains has been confirmed by gene knockout studies in animal models targeting essential components of different epigenetic regulatory axes. However, the biological effects associated with inactivation of epigenetic modifiers can be of difficult interpretation, as inactivation of one particular determinant could cause imbalance of functionally related (and unrelated) ones, which ultimately are responsible for the observed phenotype(s). Hence, systematic investigation of genome-wide distribution of multiple independent histone modifications (and DNA methylation) coupled to the assessment of transcriptome profiles of mutant cells, is expected to increase our understanding of the biological effects associated with functional interference of particular epigenetic factors. This information is relevant to establish the therapeutic potential of drugs directed against specific epigenetic modifiers for the treatment of disorders ranging from autoimmunity to B-cell lymphomas (Table 1). Aside from the possible epigenetic imbalances brought about as a consequence of targeting specific epigenetic components, additional issues relate to the property of epigenetic determinants to modulate targets in a cell-type- and, often, stage-specific manner. This may explain why *EZH2* acts as an oncogene in DLBCL and FL and as tumor suppressor in T-cell- and myeloid-derived malignancies. Hence, a more comprehensive understanding of the outcomes of acute and chronic perturbation of specific epigenetic axes in different organs and tissues will be needed to evaluate the therapeutic efficacy of epigenetic drugs, while minimizing their adverse effects. Although current therapies aim to target epigenetic determinants to normalize aberrant gene expression, quantitative and qualitative analyses of the proteome of diseased cells may provide relevant complementary information to help reveal the mechanisms through which epigenetic factors contribute to disease pathogenesis. Indeed, an increasing number of non-histone substrates appear to be targeted by acetyl and/or methyltransferases in both normal and transformed cells.

Finally, the integrated analysis of transcriptome and proteome data from pathological samples could lead to the identification of specific epigenetic targets whose deregulation is critical for disease pathogenesis. Potential drugs targeting the aforementioned determinants may offer higher specificity for disease treatment, limiting systemic side effects that may accompany regimens based on interference with general regulators of major epigenetic axes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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