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Epigenomic Characterization of Lymphoid Neoplasms

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Abstract

Lymphoid neoplasms represent a heterogeneous group of disease entities and subtypes with markedly different molecular and clinical features. Beyond genetic alterations, lymphoid tumors also show widespread epigenomic changes. These severely affect the levels and distribution of DNA methylation, histone modifications, chromatin accessibility, and three-dimensional genome interactions. DNA methylation stands out as a tracer of cell identity and memory, as B cell neoplasms show epigenetic imprints of their cellular origin and proliferative history, which can be quantified by an epigenetic mitotic clock. Chromatin-associated marks are informative to uncover altered regulatory regions and transcription factor networks contributing to the development of distinct lymphoid tumors. Tumor-intrinsic epigenetic and genetic aberrations cooperate and interact with microenvironmental cells to shape the transcriptome at different phases of lymphoma evolution, and intraclonal heterogeneity can now be characterized by single-cell profiling. Finally, epigenetics offers multiple clinical applications, including powerful diagnostic and prognostic biomarkers as well as therapeutic targets.

INTRODUCTION

Lymphoid neoplasms constitute a large and heterogeneous group of diseases (1, 2). Both B and T cell–derived neoplasms recapitulate morphological and phenotypic features of normal lymphoid differentiation, which makes the maturation stage one of the main principles of their classification. More than 100 entities and subtypes of lymphoid neoplasms are described on the basis of distinct clinical features and outcomes as well as phenotypic characteristics and genetic features (1, 2). The major groups of lymphoid neoplasms span the entire lymphocyte differentiation process and include precursor B and T–cell acute lymphoblastic leukemia (B-ALL and T-ALL, respectively), derived from precursor lymphocytes; mantle cell lymphoma (MCL), mostly derived from germinal center (GC)-inexperienced B cells; diffuse large B cell lymphoma (DLBCL), follicular lymphoma (FL), and Burkitt lymphoma (BL) from germinal center B cells (GCBs); chronic lymphocytic leukemia (CLL) from various stages of mature, antigen-experienced B cells; and multiple myeloma (MM) from terminally differentiated plasma cells.

The identification and characterization of genetic alterations in lymphoid neoplasms have provided essential clues into the mechanisms of lymphomagenesis and set the basis for the risk-stratification of patients as well as the development of targeted therapies (3). Despite the undisputed importance of genetic aberrations, it is evident that they cannot fully explain the whole spectrum of phenotypic manifestations and clinical evolution patterns observed in lymphoid neoplasms. In this context, the study of the epigenome represents an additional and complementary strategy to further understand the origin, pathogenic mechanisms, and clinical behavior of the patients. Initial epigenetic studies in cancer were focused mainly on gene promoters, mostly assessing the relationship between DNA hypermethylation and tumor suppressor gene silencing (reviewed elsewhere; see 4, 5). Here, we summarize a large body of recent genome-wide epigenomic profiling studies of lymphoid tumors, with particular emphasis on those derived from the B cell lineage. Instead of reviewing epigenomic features of each tumor separately, we distil epigenetic principles and features that thread across entities. We start with a succinct summary of the main epigenetic marks, to subsequently focus on the role of DNA methylation in cellular identity, tumor-specific signatures, and cell proliferative history. Next, we discuss the role of chromatin profiling to better understand pathogenetic mechanisms, and we follow that by revising the relationship between genetics and epigenetics. Finally, we summarize findings derived from single-cell transcriptomic and epigenomic data.

EPIGENOMIC MARKS, CONCEPTS, AND METHODS

Epigenetics is the science that studies how gene expression is regulated in the absence of genetic changes. More specifically, epigenetics studies how the information encoded in the genome is translated into function, is manifested as phenotype, and is inherited from parent to daughter cells (6, 7). The epigenetic language comprises various chemical modifications occurring at different scales, including the DNA sequence or chromatin-associated features (**Figure 1*a***). All these epigenetic modifications can be interrogated genome wide by a variety of methods (see the sidebar titled Main Epigenomic Profiling Methods). DNA methylation is the covalent addition of a methyl group to the fifth position of cytosines (5mC). This mark occurs mainly in the context of CpG dinucleotides and has classically been associated with gene repression when occurring at promoter regions with high CpG density (known as CpG islands). In cancer, this focal 5mC gain at gene promoters was initially proposed as a nongenetic means to inactive tumor suppressor genes (4). On the other hand, a global loss of 5mC was proposed to promote genomic instability (8). Nonetheless, genome-wide studies performed in the last 15 years have reshaped our perception of DNA methylation and suggest that its functions are broader and genomic-context dependent (9–11).

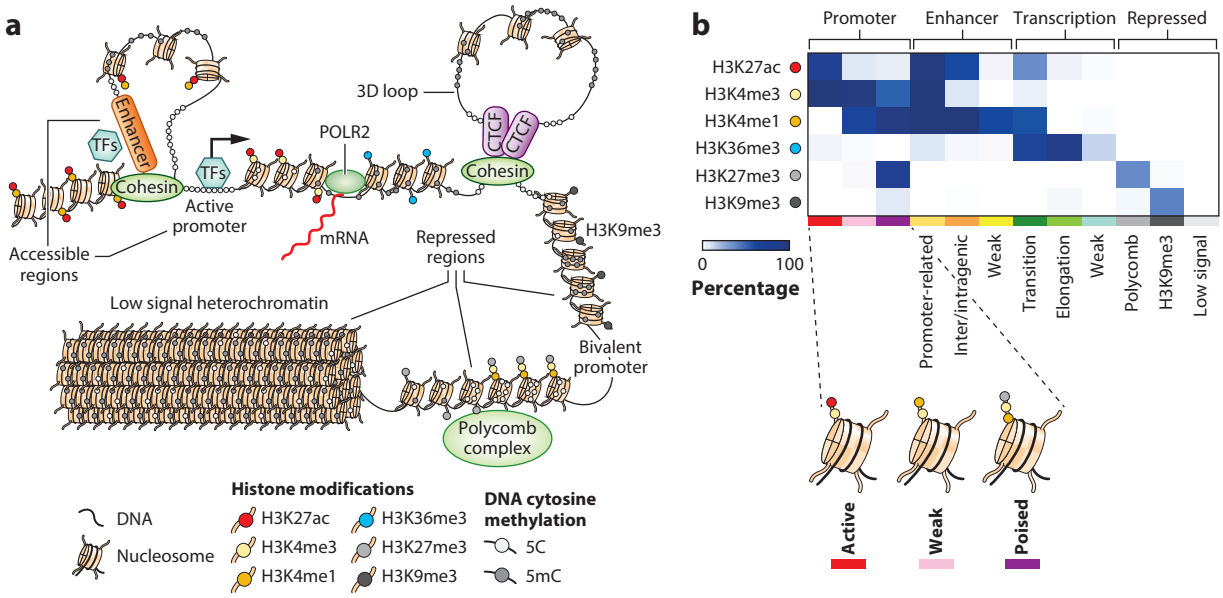

Figure 1

The epigenetic landscape of gene regulation. (*a*) Gene regulation involves a tight interconnection of various epigenetic layers, including DNA methylation, histone modifications, chromatin accessibility, and the 3D structure of the genome. Starting from the top left corner, an accessible region with the enhancer activation marks H3K27ac and H3K4me1 has come into close 3D proximity with the promoter of an actively transcribed gene. The active promoter shows unmethylated CpGs and is surrounded by the activation histone marks H3K27ac, H3K4me3, and H3K4me1. Gene-body CpG methylation, together with the presence of H3K36me3, ensures the elongation of POLR2 along the gene. CTCF and cohesin proteins configure the 3D genome architecture, creating 3D loops and topologically associating domain structures. Distinct modes of gene repression are distributed genome wide, including polycomb and H3K9me3 repression and low signal and densely packaged heterochromatin. (*b*) The canonical functions of the genome can be inferred through the assessment of six histone marks, including H3K27ac, H3K4me3, H3K4me1, H3K36me3, H3K27me3, and H3K9me3. A computationally derived combination of these six histone marks give rise to 12 chromatin states, which results in the segmentation of the genome into the chromatin functions including weak promoter, active promoter, poised promoter, weak enhancer, promoter-associated enhancer, strong enhancer, weak transcription, transcriptional elongation, transcriptional transition, polycomb-repression, H3K9me3 repression, and low signal heterochromatin. Abbreviations: CTCF, CCCTC-binding factor; POLR2, RNA polymerase II; TF, transcription factor.

Chromatin-related features indeed seem to play a more central role in gene regulation than DNA methylation (12, 13). Histone proteins not only have the structural function of packing the DNA but also undergo posttranslational modifications that produce significant changes in the chromatin properties (14). These modifications take place mainly at the amino acid residues located in histone tails, such as methylation or acetylation, among others. According to the International Human Epigenome Consortium (**https://ihec-epigenomes.org/**), six histone modifications are able to capture the canonical functions of the genome such as promoters, enhancers, transcriptional elongation, and different forms of heterochromatin (15) (**Figure 1*a***). Briefly, the monomethylation of lysine 4 of histone 3 (H3K4me1) is related to enhancer elements, whereas trimethylation of the same amino acid (H3K4me3) is linked to promoters. However, the presence of these two histone marks alone does not imply an active regulatory region. Such activation is related to the acquisition of histone acetylation such as in the lysine 27 of histone 3 (H3K27ac). Trimethylation of lysine 36 of H3 (H3K36me3), frequently found in the body of transcriptionally active genes, has been mechanistically linked to transcriptional elongation and is associated with the presence of DNA methylation (16). Finally, two additional histone modifications have been

MAIN EPIGENOMIC PROFILING METHODS

DNA Methylation

The most widely used methods are based on the sodium bisulfite treatment, which deaminates unmethylated cytosines into uracil and then into thymine after polymerase chain reaction (PCR) amplification, while methylated cytosines remain unaltered. These can be quantified by either high-density microarrays such as the Illumina EPIC array or next-generation sequencing (NGS)-based methods such as reduced-representation or whole-genome bisulfite sequencing (24). Bisulfite-free methods mainly include methylation-sensitive restriction enzyme sequencing (MRE-seq) and capture-based approaches such as methylated DNA immunoprecipitation sequencing (MeDIP-seq) or methyl-CpG-binding domain protein capture sequencing (MBP-seq). Additionally, more recent sequencing approaches can sequence the genome together with 5mC and 5-hydroxymethylation (5hmC) in a single workflow (25) or sequence long reads of native DNA to simultaneously detect changes in the nucleotide sequence and 5mC (26–28).

Histone Modifications

For many years the gold standard has been chromatin immunoprecipitation followed by NGS (ChIP-seq). However, more recent methods such as Cut&Run and Cut&Tag are increasingly preferred, as they provide high signal-to-noise ratio with less starting material (29). These methods do not require the chromatin cross-linking, as the incubation with the antibody is performed directly on permeabilized intact cells. The antibody incubation is followed by an incubation with protein A–micrococcal nuclease (pA-MNase) (Cut&Run) or pA-Tn5 (Cut&Tag) and its subsequent activation by Ca^{++} or Mg^{++}. Afterward, the DNA is extracted to prepare the library for NGS.

Chromatin Accessibility

Currently, the most widely used approach is ATAC-seq (assay for transposase-accessible chromatin with sequencing), but other approaches such as MNase-seq, NOME-seq (nucleosome occupancy and methylome sequencing), and DNase-seq (DNase I hypersensitive site sequencing) can also be applied (30, 31). The ATAC-seq protocol is robust and simple, and it requires low amounts of starting material. It uses a hyperactive Tn5 transposase to insert sequencing adaptors into accessible chromatin regions that are purified, PCR amplified, and subjected to NGS.

3D Genome Interactions

Depending on the detection of one-versus-one, one-versus-all, or all-versus-all 3D interactions, a variety of methods have been developed, which are collectively known as chromosome conformation capture techniques (32–35). In particular, HiC is used to map all-versus-all interactions. This method is based on multiple steps including chromatin cross-linking—where linearly distant (but in close 3D proximity) genomic regions are fixed—fragmentation with restriction enzymes, labeling with biotin, pull-down with streptavidin, ligation, and creation of a library of ligation products (i.e., chimeric DNA molecules), which are subsequently sequenced by NGS to generate chromatin interaction data.

Single-Cell Epigenomics

Over the last few years, various of the methods shown above have been adapted to profile the epigenome of single cells (36–39). Nowadays, single-cell ATAC-seq is the most widely used method, as it is commercially available and is also amenable to multiomic approaches (40–42).

associated with different forms of gene repression. The presence of trimethylation in the lysine 27 of histone 3 (H3K27me3) is mediated by the polycomb-repressive complex. Trimethylation in the lysine 9 of histone 3 (H3K9me3) is linked to a more stable, long-term gene silencing. Some of these marks colocalize, and computational methods have been applied to derive the so-called chromatin states (17) (**Figure 1*b***). For instance, if the H3K4me3 histone mark colocalizes with H3K27me3, it is then related to a bivalent or poised promoter. Instead, if it colocalizes with H3K27ac, it will then be an active promoter. Finally, if H3K4me3 is not combined with any of these two marks, it will remain a weak promoter (**Figure 1*b***). Different chromatin state models have been generated, although a common model comprises 12 states including weak promoter, active promoter, poised promoter, weak enhancer, promoter-associated enhancer, strong enhancer, weak transcription, transcriptional elongation, transcriptional transition, polycomb-repression, H3K9me3 repression, and low signal heterochromatin (18, 19) (**Figure 1*b***). This low-signal heterochromatin state covers a large fraction of the genome and is associated with densely packed chromatin that lacks any of the six classical histone modifications (**Figure 1*a*,*b***). In addition to histone modifications, some genomic regions lack nucleosomes, and chromatin is accessible to different cellular complexes important for gene regulation, such as transcription factors (TFs) or RNA polymerase II (POLR2) (**Figure 1*a***). Finally, another layer of epigenetic regulation is related to the chromatin interactions at the three-dimensional (3D) space (20). Chromatin is hierarchically organized into distinct 3D structures, from lower to higher resolution: chromosome territories, compartments [mainly A for active transcription and B for inactive, although other compartmentalization strategies have been proposed (21)], topologically associating domains (TADs), and loops (22). In the last structure, gene expression can be regulated by bringing together distant enhancers with promoters (**Figure 1*a***). In this context, the presence of CCCTC-binding factor (CTCF) and cohesin are involved in looping formation, and CTCF binding anticorrelates with DNA methylation (23). Collectively, all these data highlight that gene regulation is the result of a tightly regulated interaction of all the epigenetic marks.

TRACING CELL IDENTITY BY DNA METHYLATION IMPRINTS

A major principle behind the classification of lymphoid neoplasms is that each B cell tumor entity can be assigned to a particular maturation stage, under the assumption that it represents the normal cellular counterpart or cellular origin from which the tumor originated (1, 2). For instance, the phenotypic classification of B-ALL as a precursor B cell neoplasm or MM as a plasma cell–derived cancer or the subclassification of DLBCL into activated B cells (ABCs) or GCBs on the basis of transcriptional similarities to these normal cellular states (43) are clear examples. From the epigenetic perspective, normal B cell maturation is accompanied by widespread DNA methylation gains and losses that are accumulated as differentiation progresses, with prominent epigenetic programming taking place during the GC reaction (44, 45) (**Figure 2**). Additionally, each cell type is associated with a specific DNA methylation signature (46), which can be used to deconvolute a complex sample into its constituent cell types (47, 48). Accordingly, this strategy not only allows inference of the proportion of tumor cells (47, 49) but also manifests the importance of accounting for cellular composition in DNA methylation analyses (50, 51). In addition, DNA methylation represents a powerful molecular mark to determine the potential normal counterpart of tumors (52). For instance, lymphomas associated with the GC carry an epigenetic signature that can differentiate GCB-like versus ABC-like subtypes (53). BL carries hypomethylation and upregulation of dark-zone specific genes, whereas FL instead shows a hypermethylation and silencing of those genes, which supports the view that BL is mostly frozen in the dark-zone state and FL in the light-zone state (54). Additionally, hairy cell leukemia shows epigenetic imprints of

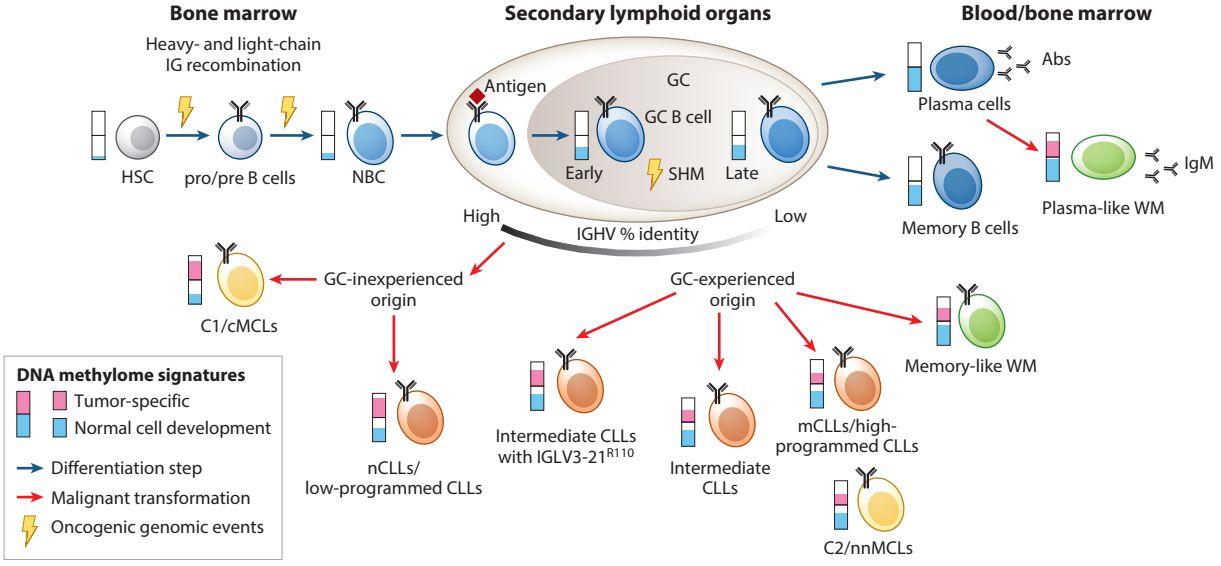

Figure 2

DNA methylation as a molecular mark to trace cell identity. Normal B cell differentiation entails widespread DNA methylation changes that are accumulated as differentiation progresses, with prominent epigenetic programming taking place during the GC reaction. Therefore, each B cell subpopulation shows a gradually accumulated specific DNA methylation signature. Upon malignant transformation, this cell type–specific methylation signature of the ancestor normal B cell is generally retained, which offers valuable insights into the cell of origin of B cell–derived tumors such as CLL, MCL, and WM. For instance, the cMCL subtype shows DNA methylation imprints of pre-GCBs, suggesting its earlier origin compared with the nnMCL subtype, which instead shows DNA methylation imprints of post-GCBs. Similarly, nCLLs show low levels of epigenetic programming, likely reflecting a GC-independent maturation. Intermediate CLLs with the presence of IGLV3-21^{R110} mutation show incremental levels or programming and moderate IGHV mutational levels, suggesting an earlier origin compared with intermediate cases lacking such mutations. Finally, mCLLs show strong epigenetic programming and high IGHV mutational load, suggestive of their later cell of origin. In the case of WM, its DNA methylome analysis showed two subtypes that align across memory and plasma cell DNA methylation signatures. Tumors with an earlier cell of origin, which are generally more aggressive, will show a greater proportion of leukemic-specific DNA methylation changes. Conversely, tumors derived from later maturation stages—which are overall more benign—will show a higher proportion of methylation changes accumulated during normal cell development. Initial genetic oncogenic events that may take place in different stages of B cell differentiation are highlighted, including IG gene rearrangements or somatic hypermutation processes. Normal B cells are color coded according to their maturation stage, whereby darker shapes correspond to more mature cells. Different subtypes of the same disease share the same color. Abbreviations: Ab, antibody; CLL, chronic lymphocytic leukemia; cMCL, conventional MCL; GC, germinal center; GCB, germinal center B cell; HSC, hematopoietic stem cell; IG, immunoglobulin; IgM, immunoglobulin M; IGHV, immunoglobulin heavy-chain variable region; MCL, mantle cell lymphoma; mCLL, memory-like CLL; NBC, naive B cell; nCLL, naive-like CLL; nnMCL, leukemic non-nodal MCL; SHM, somatic hypermutation; WM, Waldenström's macroglobulinemia.

post-GCBs (55). Other paradigmatic examples are MCL, CLL, and Waldenström's macroglobulinemia (WM), which can be classified into epigenetic subtypes or epitypes with different cellular origins (**Figure 2**), as well as biological and clinical features, and are subsequently discussed in greater detail below.

In MCL, two subtypes of patients are described on the basis of their biological and clinical features (56). Conventional MCL (cMCL) cases are generally more aggressive, lack or show low levels of somatic hypermutation (SHM) in the immunoglobulin heavy-chain variable region (IGHV) genes, and usually express the *SOX11* oncogene. Conversely, leukemic non-nodal MCL (nnMCL) cases show an overall better outcome, display higher levels of IGHV SHM, and are usually *SOX11* negative. DNA methylome analyses identified that cMCLs lack the GC epigenetic imprint and therefore are derived from GC-inexperienced cells (57) (**Figure 2**), probably from pre-GCBs, in

which *SOX11* expression prevents their entry into the GC due to negative regulation of *BCL6* (58). In contrast, nnMCL cases clearly show the epigenetic imprint of the GC reaction, which therefore suggests that the cell of origin of this MCL subtype is most likely a GC-experienced B cell (57) (**Figure 2**). These epigenetic results are concordant with a transcriptional profiling study in which cMCL was linked to naive B cells and nnMCL with memory B cells (59).

CLL is classically categorized into two subtypes distinguished by the extent of SHM in IGHV genes. Unmutated CLL (U-CLL) patients lack or have low levels of IGHV gene mutations and show an overall worse clinical outcome, whereas mutated CLL (M-CLL) patients carry a higher IGHV gene mutational load and generally have a more benign outcome (60). Analyzing the DNA methylome of M-CLL and U-CLL cases revealed that the majority of the differences are linked to CpGs whose methylation is modulated between normal GC-inexperienced versus experienced B cells, such as naive B cells and memory B cells (MBCs), respectively (61). In addition, a third CLL epitype with an intermediate DNA methylation pattern and moderate IGHV gene mutational level was identified. This third epitype group is strongly enriched with patients belonging to the stereotyped subset 2 as well as in CLLs with the IGLV3–21^{R110} and *SF3B1* mutations (62). Overall, these studies revealed that CLLs can be derived from a spectrum of maturation stages of antigen-experienced B cells, including GC-inexperienced cells (naive-like CLLs or low-programmed CLLs, mainly composed of U-CLLs); GC-experienced cells with a moderate GC DNA methylation imprint (also named intermediate CLLs, containing M-CLLs and U-CLLs with moderate IGHV SHM); and GC-experienced cells with a strong GC DNA methylation imprint (also named memory-like CLLs or high-programmed CLLs, composed mainly of M-CLLs) (reviewed in 63). Although CLLs can be clearly categorized into these three epitypes, they may reflect a continuum of epigenetic states (64) with incremental IGHV mutational levels, which may be related to distinct dynamics of the normal B cell ancestor in response to GC-dependent or -independent antigen stimulation (**Figure 2**). From the clinical perspective, these three epitypes show consistent differential clinical outcomes (61, 64–68). Nonetheless, recent evidence suggests that the initially reported intermediate prognosis of the intermediate epitype can be refined on the basis of the presence of the IGLV3-21^{R110} mutation (62). CLLs showing such mutation are clinically worse than those lacking it, which clinically resemble the memory-like CLL subtype. Therefore, a patient dichotomization into worse prognosis naive-like CLLs (mainly U-CLLs) and intermediate CLLs with the IGLV3–21^{R110} mutation and better prognosis memory-like CLLs (mainly M-CLLs) and intermediate CLLs lacking the IGLV3–21^{R110} mutation may be more appropriate in the clinical setting (62). It is worth noting that this patient dichotomization may be identified with high accuracy using a DNA methylation classifier (69).

Finally, DNA methylome analysis of WM has provided key information about its cellular origin and disease heterogeneity, revealing two subtypes based on imprints of memory B cells and plasma cells (70) (**Figure 2**). The MBC-like WM subgroup is significantly enriched in genetic features such as *CXCR4* mutations and 13q deletions as well as clinical parameters such as splenomegaly and thrombocytopenia. In contrast, the plasma-cell-like WM subgroup is enriched in 6q deletions and 6p gains, increased usage of IGHV3 genes, higher CD38 expression and plasmacytic differentiation (70).

BIOLOGICAL AND CLINICAL IMPLICATIONS OF TUMOR-SPECIFIC DNA METHYLATION PATTERNS

Beyond the DNA methylation signatures related to their cellular origin, lymphoid tumors also acquire specific de novo alterations that can be informative about their underlying pathogenic mechanisms. For instance, the binding sites of specific TFs involved in the pathogenesis of

B cell tumors become unmethylated (49), consistent with the reported DNA methylation loss in regulatory regions upon TF binding (71, 72).

In B-ALL and T-ALL, several studies identified extensive hypermethylation of polycomb-repressed regions and a comparatively minor genome-wide DNA methylation loss (73–75). Different cytogenetic subtypes of acute lymphoblastic leukemia (ALL) in turn show differential DNA methylation signatures (74). Additionally, ALLs at relapse acquire a hypermethylation signature, suggesting that DNA methylation evolves during disease progression (74).

In MCL, cMCL and nnMCL not only show differences based on their differential cellular origin but also display de novo hyper- and hypomethylation (57). The magnitude of these changes in cMCL is 10 times larger than in nnMCL, likely reflecting their more aggressive clinical behavior. Hypermethylation is related to polycomb-repressed regions, as in other B cell neoplasms. On the other hand, de novo hypomethylation in cMCLs is strongly enriched in enhancer regions, which are related to pathways involved in Notch signaling and deregulation of key oncogenes such as *SOX11* (57).

In the case of DLBCL, hypermethylation is also related to polycomb regions, and no clear differences are observed between systemic cases and those with primary involvement of the central nervous system (76, 77). Most of the studies in DLBCL analyzed the heterogeneity of its DNA methylome, as the GC is a (epi)mutagenic environment inducing strong epigenetic programming (45). Such heterogeneity seems to be mediated in part by the action of *AICDA* (78) and related to adverse outcomes and clonal selection at relapse (79, 80). Furthermore, the extent of epigenetic heterogeneity led to the identification of six DLBCL epigenetic subtypes (80), although their relationship with the recent DLBCL genetic subtypes (81, 82) remains to be explored. In FL, global hypomethylation (54) and hypermethylation in polycomb targets is also found (83). In sequential samples and transformed cases, no clear methylation changes were identified, possibly due to technical and biological limitations such as sparser arrays and varying tumor purities of samples (83). Another study comparing FL and BL identified negatively correlated differential methylation and gene expression in pathways related to inflammation and immunity in BL and to cell cycle and DNA repair in FL (54).

CLL is one of the lymphoid neoplasms with a better characterization of the DNA methylome (61, 64, 84–86). These studies identified more DNA methylation changes in U-CLL than in M-CLL, affecting genes such as *ZAP70* (87). Similar to DLBCL, increased intratumoral DNA methylation heterogeneity in CLL is associated with a worse clinical outcome (86). A recent study analyzed the DNA methylome of CLLs at diagnosis and after a high-grade transformation to a DLBCL known as Richter transformation (RT), and compared them with de novo DLBCLs. In this study, the authors identified a clear epigenetic imprint in RT samples clonally related to CLL, which was also present in apparently de novo DLBCLs with dismal prognosis (called thereafter RT-like DLBCLs) (88).

Finally, the well-defined MM cytogenetic subtypes partially show differential DNA methylation patterns, which take place in the context of a globally hypomethylated methylome (89–91). This global DNA methylation loss increases from monoclonal gammopathy of undetermined significance (MGUS) to overt MM. Hyperdiploidy and translocations involving the immunoglobulin heavy (IGH) locus seem to be associated with the highest number of DNA methylation changes, with the t(4;14) showing a specific hypermethylation signature affecting genes such as *APC*, *PAX1*, *SOC2*, or *CDKN2A*. Furthermore, the inactivation of tumor suppressor genes by promoter methylation is also present in MM, with hypermethylation of *GPX3*, *RBP1*, *SPARC*, and *TGFBI* associated with adverse outcomes (92). In addition, MM shows a remethylation of B cell–specific enhancers, which could be evocative of the typical downregulation of the B cell–specific phenotype in plasma cells. This epigenomic phenomenon is not observed in normal plasma cells,

but it is already present in the premalignant MGUS condition (89). Remarkably, a similar phenomenon was also observed in classical Hodgkin lymphoma cell lines (93) and recently in some DLBCL patients (49), suggesting that MM may not be the only lymphoid tumor with such loss of epigenetic B cell identity.

From the diagnostic perspective, several studies have developed highly accurate DNA methylation-based classifiers for B cell tumor entities and subtypes, with mean sensitivities (>90%) that have not yet been reported with other molecular alterations. For instance, cytogenetic ALL subtypes can be identified with a minimum set of 246 CpGs (94). In small B cell lymphomas, a set of only 26 CpGs can accurately classify CLL, MCL, FL, and marginal zone lymphoma (95). Finally, another study used more than 2,000 DNA methylomes of different B cell tumors to develop and validate a two-step classifier with 56 CpGs. In a first step, main B cell tumor entities could be classified (B-ALL, MCL, DLBCL, CLL, and MM); a second step was used to identify intraentity subtypes, leading to a classification of a total of 14 B cell tumor subtypes in a single assay (49). As the DNA methylome seems to be stable in circulating-free DNA (cfDNA) (96, 97), these studies could pave the way for the future use of epigenetic biomarkers in liquid biopsy for the diagnosis and monitoring of lymphoid tumors without leukemic presentation (98).

DNA METHYLATION AS A MITOTIC CLOCK WITH CLINICAL IMPACT

CpG island hypermethylation and global hypomethylation of low CpG were initially interpreted as nongenetic means to induce tumor suppressor silencing (4) and chromosomal instability in cancer (8), respectively. However, subsequent studies and the integration with other epigenetic marks reshaped our interpretation (9–11). Initial profiling of histone modifications in stem cells reported polycomb-repressed and bivalent chromatin domains (99), which were subsequently associated with genes becoming hypermethylated in cancer. This finding led to the interpretation of the presence of a stem cell–like chromatin pattern, supporting the stem cell theory of cancer (100–102). The same scenario was found in lymphomas, and therefore the link between lymphomagenesis and stem cells was also proposed (103). In these studies, polycomb-related hypermethylation was not associated with de novo gene silencing. Instead, it was suggested to reduce epigenetic plasticity in cancer cells by replacing reversible gene repression through histone modifications by permanent silencing through DNA methylation (104). In additional reports, hypermethylation in lymphomas was related to polycomb-repressed regions present already in normal B cells (93). Subsequent studies related the level of DNA hypermethylation of CpG-rich polycomb regions with cell mitosis (105), which set the basis for using these DNA methylation changes as a mitotic clock (106–108). In this context, it is not surprising then that both proliferative cancer cells and normal cells with extensive proliferative histories such as post-GCBs show hypermethylation of polycomb-repressed regions (45). Therefore, this hypermethylation seems to capture cumulative cell proliferation associated to both physiological and pathological conditions (**Figure 3*a*,*b***). In the case of hypomethylation, the initial models of chromosomal instability (8) were replaced by observations in which DNA methylation loss of late-replicating regions accumulates with cell divisions (109, 110). At least in B cells, this seems to occur in the absence of chromatin instability, as cells with high proliferative history such as healthy memory B cells or plasma cells are clearly hypomethylated and do not have genomic instability (45). This global hypomethylation at late-replicating regions take place in the form of partially methylated domains independent of gene expression changes (**Figure 3*a*,*b***). Furthermore, it has been consistently mapped in multiple cancer types and therefore proposed as a hypomethylation-based mitotic clock (111, 112).

In normal and neoplastic B cells, a dedicated mitotic clock that considers both DNA methylation gains and losses during cell division has been developed (49). This mitotic clock, termed

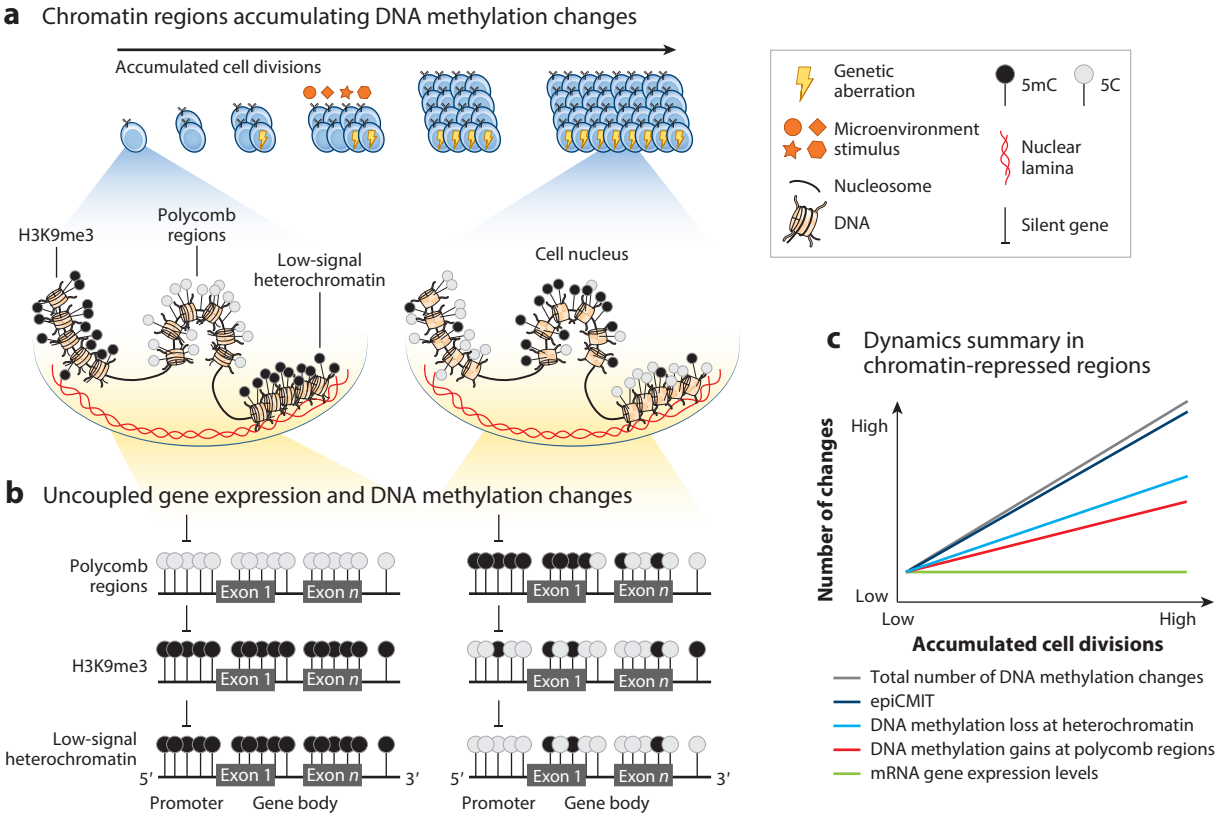

Figure 3

DNA methylation changes are accumulated in chromatin-repressed regions during cell division in the absence of gene expression changes. (*a*) Neoplastic cells proliferate in response to a variety of different factors, including intrinsic and extrinsic features such as genetic alterations and microenvironment stimuli, respectively. During normal and neoplastic cell divisions, cells accumulate hypermethylation in polycomb-repressed regions (H3K27me3) with high CpG density. Similarly, heterochromatic regions gradually accumulate DNA methylation losses during cell division. (*b*) Remarkably, the DNA methylation changes occurring at these repressive-chromatin regions during normal and neoplastic cell divisions are not related to changes in gene expression of their underlying genes, which remain silent regardless of methylation changes. (*c*) In general, DNA methylation gains at polycomb-repressed regions and losses in heterochromatin are concomitantly acquired during cell divisions. Nonetheless, some lymphoid tumors such as B-ALL and MM show differential preferences in gaining or losing DNA methylation, respectively. The epiCMIT mitotic clock considers the highest value of the two modalities and therefore strongly correlates with the total number of accumulated DNA methylation changes during cell division. Abbreviations: B-ALL, B cell acute lymphoblastic leukemia; epiCMIT, epigenetically determined cumulated mitoses; MM, multiple myeloma.

epiCMIT (epigenetically determined cumulative mitoses), was originally built using Illumina DNA methylation arrays and later adapted to sequencing data such as reduced-representation or whole-genome bisulfite sequencing (113). It was developed using both DNA methylation and chromatin immunoprecipitation sequencing (ChIP-seq) data. More specifically, CpGs whose methylation levels clearly change during B cell maturation and map to constitutive polycomb-repressed and heterochromatic regions were selected. Then, two scores were calculated, one for hypermethylation and one for hypomethylation. These two scores are highly correlated in normal B cells, MCL, CLL, and DLBCL, a finding supporting an initial report in colorectal cancer indicating that the magnitude of hyper- and hypomethylation are correlated (114). This may suggest

that DNA methylation gains at polycomb-repressed regions and losses in heterochromatin are concomitantly acquired during cell division (**Figure 3*a*,*c***). However, the authors identified that this may not be a universal phenomenon, as B-ALL seems to acquire mainly hypermethylation in polycomb-repressed regions, while MM mainly acquires hypomethylation in heterochromatic regions. The mechanisms underlying this phenomenon are currently unknown but indicate that the maturation stage of the tumor cell of origin may dictate differential tendencies in gaining or losing DNA methylation during cell division. To circumvent this issue, the authors derived a single epiCMIT score selecting the higher value of the two modalities. This mitotic clock is not a static snapshot of the current proliferation status such as the well-known Ki67 marker, but it represents a cumulative readout of all the intrinsic (e.g., genetic aberrations) and extrinsic (e.g., microenvironment stimuli) elements that promoted cell proliferation. During normal B cell development, the epiCMIT increases as maturation progresses and is consistent with known proliferation bursts at particular maturation stages. For instance, it sharply increases in GCBs and reaches the peak in terminally differentiated plasma cells, which do not proliferate but contain in their DNA methylome the entire proliferative history of the B cell linage. The epiCMIT in cancer cells contains both the proliferation history of the cellular origin and that associated with malignant transformation and progression. Thus, as B-ALL is derived from precursor B cells with low proliferative history, the epiCMIT mainly represents the cumulated leukemic proliferation, whereas in other mature lymphoid tumors such as CLL or MM, it contains a significant fraction of the cumulated cell proliferation of the normal ancestor cell. In the case of CLL, the naive-like/low-programmed CLLs show an overall lower epiCMIT than memory-like/high-programmed CLLs, which is consistent with its less mature cell or origin. Remarkably, the epiCMIT is coupled with disease evolution, as it increases progressively from precursor conditions to overt cancer such as from monoclonal B cell lymphocytosis to CLL, or from MGUS to MM, or from diagnosis to progression, to relapses, and to a high-grade transformation such as RT (49, 115). Accordingly, when fixing the cell of origin, the epiCMIT as a quantitative variable is a strong and independent prognostic factor associated with clinical outcome in B-ALL, CLL, and MCL (49, 113, 116). Thus, these findings may indicate that the past proliferative history can predict the future proliferative capacity of cancer cells and patient outcome. It is worth mentioning that the epiCMIT mitotic clock has been recently validated in multiple normal and neoplastic cell types (112), suggesting its broader applicability beyond the hematopoietic system. Given that the DNA methylome seems to be stable in cfDNA (96, 97), this may also represent a sound basis for the implementation of the epiCMIT in cfDNA in other tumors without leukemic presentation (98).

CHROMATIN PROFILING AND INTEGRATIVE EPIGENOMICS

Although DNA methylation changes may in part point to deregulated genes or pathways, a more straightforward path to identify them is to directly profile chromatin features related to regulatory regions.

In B-ALLs, there is a gain in chromatin accessibility in regions that are closed in normal B cells, which target genes associated with pathways related to cell cycle control (117). From the 3D perspective, T-ALL shows a widespread chromatin reconfiguration characterized by increased contact frequencies in TADs. In turn, this is related to increased expression of genes such as *APCDD1*, *IKZF2*, or *MYC*, the latter representing one of the main oncogenes upregulated in T-ALL downstream of *NOTCH1* signaling (118).

In MCL, an initial study analyzed the DNA methylome in the context of histone modifications related to regulatory regions (57). This study identified a specific gain of H3K4me1 and H3K27ac in a region 650 kb downstream of *SOX11*, an important oncogenic transcription factor in cMCL

(119). This gain of active enhancer-related histone marks was accompanied by a loss of DNA methylation, a gain in chromatin accessibility, and a looping between the distant enhancer and the *SOX11* gene (57). Subsequent studies analyzed in greater detail the chromatin configuration of the whole *SOX11* locus and its related enhancer (120, 121). An HiC profiling in MCLs expressing *SOX11* revealed a 6-Mb chromatin block containing *SOX11* and additional genes gaining 3D interactions, such as *ID2*, *COLEC11*, or CMPK2, which collectively may also contribute to cMCL pathogenesis. In addition, a multiomic characterization of the enhancer region looping to the *SOX11* gene identified a TF hub, with *PAX5* among the top ranked. These findings suggest that *PAX5* may cooperate in the deregulation of the *SOX11* gene in cMCL cases, although the mechanistic details still remain elusive. Supporting this role of *PAX5*, another study using a TF CRISPR-Cas9 screening integrated with ChIP-seq identified *PAX5*, *EBF1*, and *IRF4* as essential TFs for MCL pathogenesis, which were all downstream of *FOXO1*, a new promising therapeutic target for MCL (122). Other studies have found that somatic mutations in MCL tend to occur in open chromatin regions in the normal B cell counterpart (123), and chromosome 11 break points associated with the t(11;14)(q13;q32) translocation create new TAD borders and gain activation chromatin marks most likely by spreading from the active regulatory region of the IG locus (116). In addition to cell cycle deregulation, *CCND1* overexpression through the t(11;14)(q13;q32) translocation may have additional pathogenic roles. On the one hand, *CCND1* binds genome wide to regulatory regions of transcribed genes and decreases the transcript levels through an interaction with the transcriptional machinery (124). On the other hand, *CCND1* also seems to bind to specific regulatory regions activating a reduced set of genes, the expression levels of which are associated with an adverse outcome in MCL patients (125).

In GC-derived B cell lymphomas, we are not aware of genome-wide studies mapping chromatin features. However, the pathogenesis of these lymphomas has a strong connection with the chromatin structure and function, as chromatin modifier genes are frequently targeted by genetic aberrations (126). This genetics–chromatin connection is reviewed in the next section.

In CLL, several studies have focused on deciphering its chromatin landscape (19, 127–129). These studies identified hundreds of regions mostly gaining chromatin accessibility and the activation histone marks H3K27ac, H3K4me1, and H3K4me3 (**Figure 1*b***), which correlated with upregulation of multiple genes related to CLL pathogenesis. In some cases, the target genes were CLL-specific biomarkers, such as *TCF4*, *FMOD*, *CTLA4*, or *LEF1* (130). Another recent study (129) reported the presence of a typically mutually exclusive combination of the activation histone mark H3K27ac and the repressive histone mark H3K27me3 (18) (**Figure 1*b***). This finding may suggest that intratumoral epigenetic diversity could be generated by CLL subclones with diverging evolutionary paths. Remarkably, aberrant chromatin activation patterns in CLL have been associated with several TFs, such as NFAT, TCF/LEF, and FOX, among others. In the case of the high-grade transformation to RT, the chromatin seems to be reconfigured by a different set of TFs related to proliferation, such as E2F and MYC family members, and oxidative phosphorylation, such as TEAD4 (115). Interestingly, CLL cases of the stereotyped subset 8, which show a strong predisposition to undergo RT, show a similar chromatin activation pattern mediated also in part by the E2F family members (131). Fewer chromatin regions have been described to lose chromatin activation marks in CLL as compared with normal B cells, *EBF1* being a remarkable example. This gene is essential for B cell identity; however, its expression is lost in the great majority of CLLs but not in other lymphoproliferative disorders (130). Not only the activating histone marks of the *EBF1* locus are lost in CLL but also the 3D interactions of a 2-Mb region containing *EBF1* through a long-range compartment shift (132).

In MM, a genome-wide DNA methylation study revealed that B cell–specific enhancers regain DNA methylation, which in the U266 myeloma cell line is associated with the lack of H3K4me1

and H3K27ac (89). Conversely, some regions that were active in naive and memory B cells but poised in normal plasma cells undergo an enhancer reactivation in MM, the *CCND2* enhancer being a remarkable example (133). A subsequent study linking DNA methylation to histone modifications and gene expression revealed differences among the MM genetic subgroups. Some hypomethylated regions were marked by activating histone modifications, and the most common deregulated pathways are PI3K/AKT/mTOR, MAPK, Rap1, and the cell cycle (91). More detailed chromatin profiling revealed a common signature of chromatin activation in MM as a whole, which may be related to "myelomaness" beyond genetic heterogeneity (134). This unifying signature involved 1,556 de novo active regulatory elements across MM patients. These regions were enriched in binding sites of IRF, FOX, and MEF2 TF families, consistent with a previous study that in addition found ETS, E-box, and AP-1 TF families in active enhancers (135). Therefore, it seems that particular TFs are responsible for MM chromatin activation, which involves key MM-related pathways such as NOTCH, NF-kB, MTOR, and *TP53* signaling as well as osteoblast differentiation and response to oxidative stress (134). The previous studies also identified regulatory elements associated with blocks of several deregulated adjacent genes (91, 134), which are associated with a 3D structure reconfiguration of the genome involving shifts between A and B compartments (136). Finally, a more recent study of histone modification profiles in 16 MM cell lines with different drug sensitivities identified chromatin-based biomarkers of drug response (137). Similarly, another study focused on H3K27me3 identified polycomb targeting through EZH2 inhibition as a potential effective therapy in MM (138).

Collectively, these studies provide a global view of the role of chromatin features as well as corrupted TF bindings in lymphoid malignancies. Different studies and experimental approaches led to complementary findings, which altogether provide valuable insights of the magnitude and complexity of the chromatin regulatory networks that contribute to the development of lymphoid neoplasms. Although therapeutic strategies have usually focused on genes and pathways affected by genetic aberrations, it seems that genes targeted by epigenetic deregulation in lymphoid neoplasms are not frequently altered by any genetic abnormality. Therefore, these chromatin studies uncover new therapeutic opportunities for drugs inhibiting epigenetically deregulated cellular functions that are associated with malignant transformation and progression (139).

LINKING GENETIC AND EPIGENETIC FEATURES

Genetic and epigenetic aberrations cooperate in the process of disease development from multiple perspectives. For instance, the presence of germline variants and acquisition of noncoding somatic mutations in the context of particular epigenomic features is related to a higher risk of cancer development and progression. More specifically, single-nucleotide polymorphisms (SNPs) are frequently located in noncoding regions and show activating chromatin marks (140) (**Figure 4*a***). In the case of lymphoid neoplasms, risk loci related to ALL, CLL, and MM have been shown to be related to regulatory regions (141–143). In CLL, a multiomic quantitative trait locus analysis of 42 risk loci led to the refinement of the putative SNPs, genes, and TFs mediating the risk of CLL development (142). Remarkably, some risk variants increased the binding affinity of NFAT, LEF/TCF, and FOX TF families, which have been shown to be related to altered chromatin and DNA methylation patterns (19, 49, 128). Likewise, somatic noncoding mutations have also been related to altered gene regulation in lymphoid tumors (**Figure 4*b***). For instance, noncoding mutations found in CLL and DLBCL can alter the expression of genes related to their pathogenesis, such as *PAX5*, *BCL6*, *BCL2*, or *CXCR4* (144–146). These noncoding somatic mutations affect the binding affinities of transcriptional repressors of these B cell–related proto-oncogenes, such as BLIMP1 (targeting *BCL6*) or NR3C1 (targeting *BCL2* and *CXCR4*), or increase *PAX5* expression through a 3D chromatin loop.

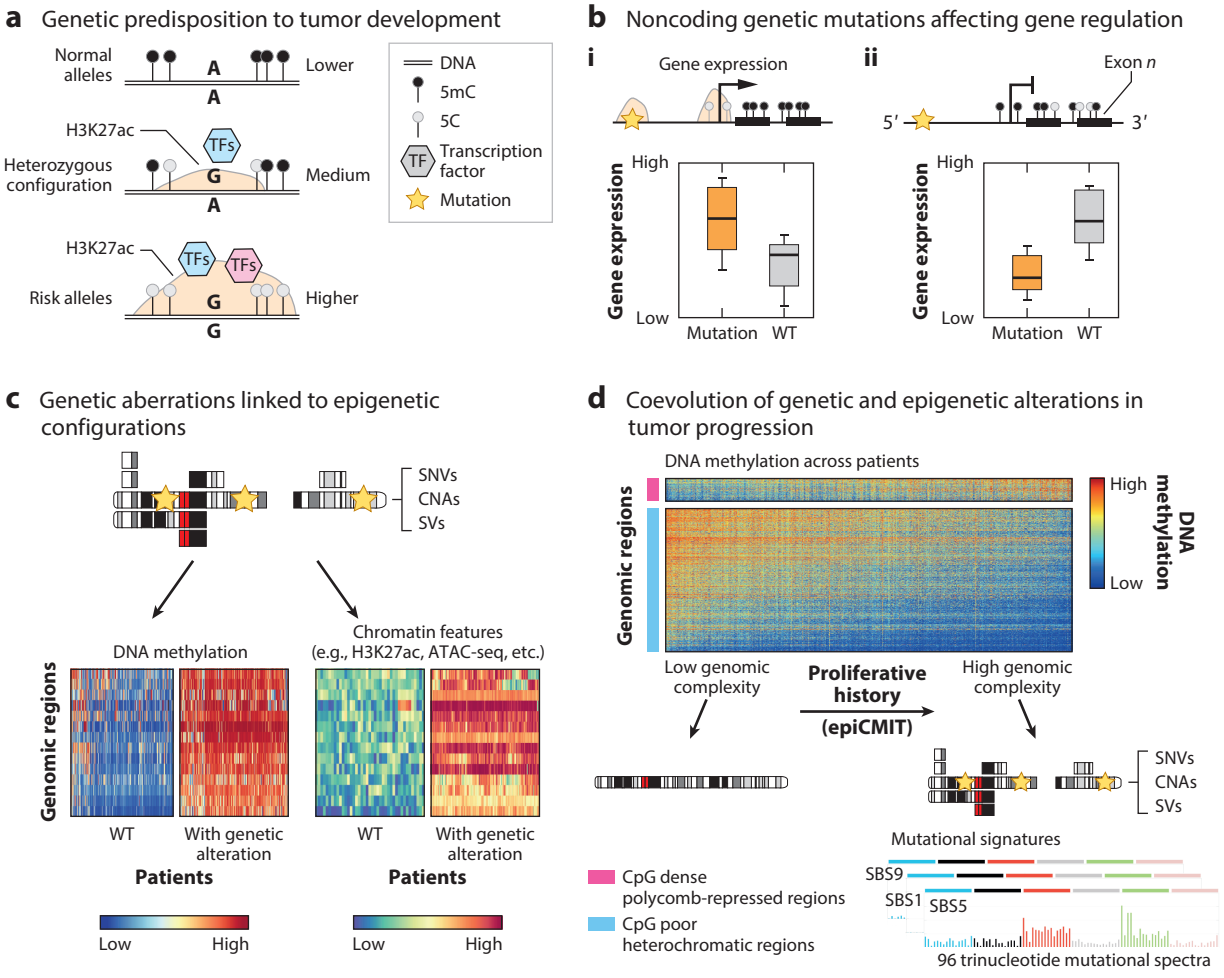

Figure 4

Genetic and epigenetic alterations cooperate in disease development. (*a*) The presence of certain germline single-nucleotide polymorphisms linked to higher risk of disease development is associated with particular epigenomic configurations. For instance, the depicted homozygous configuration of allele A is associated with a close chromatin and is not related to tumor development. The heterozygous configuration reflects a monoallelic open chromatin configuration with increasing H3K27ac levels, which may recruit some disease-associated TFs. With the homozygous configuration for the risk allele, both alleles are open and active, leading to a higher risk of promoting malignant gene expression programs. (*b*) Similar to germline noncoding variants, the epigenetic configuration of the genomic context in which somatic noncoding mutations take place can inform their potential functional impact. Some noncoding mutations may lead to de novo (over)expression of pathogenic transcriptional programs, whereas others may lead to the pathogenic silencing of required genes for normal cell function. (*c*) Particular genetic aberrations such as CNAs or genetic mutations can be tightly associated with certain epigenetic signatures. These differential epigenetic patterns can involve changes in DNA methylation levels or chromatin-related features such as H3K27ac or chromatin accessibility levels. (*d*) During cell divisions, genetic and epigenetic changes are concomitantly acquired. Particularly, DNA methylation gains at polycomb-repressed regions and losses in heterochromatin are simultaneously acquired and captured by the epiCMIT miotic clock. In turn, this epiCMIT mitotic clock correlates with mutations associated with the clock-like mutational signatures SBS1 and SBS5. In cells that have undergone the germinal center reaction, the epiCMIT is also strongly correlated with the mutational signature SBS9, which reflects genome-wide AID off-target mutations. Abbreviations: AID, activation-induced cytidine deaminase; ATAC-seq, assay for transposase-accessible chromatin with sequencing; CNA, copy number alteration; epiCMIT, epigenetically determined cumulated mitoses; SBS, single base substitution; SNV, single-nucleotide variant; SV, structural variant; TF, transcription factor; WT, wild type.

Genetic changes involving copy number alterations and driver genetic mutations have also been associated with particular chromatin and DNA methylation signatures (**Figure 4*c***). In the case of B-ALL with primary genetic alterations such as high hyperdiploidy, t(12;21), 11q23 rearrangements, t(1;19), dic(9;20), t(9;22), or iAMP21c, they show differential DNA methylation patterns (74). In addition, different ALL subtypes show specific 3D genomic configurations and chromatin accessibility patterns (117, 118). Another example is anaplastic large cell lymphoma, in which tumor cells with rearranged *DUSP22* seem to be hypomethylated compared with cells without such genomic alteration (147). In DLBCL, it is not yet clear whether recent genetic subtypes (81, 82) are related to distinct epigenetic profiles. In other lymphomas with a primary genetic alteration such as the t(8;14) in BL, t(11;14) in MCL, or t(14;18) in FL, differential intraentity epigenetic patterns based on secondary genetic changes are not clear either. In CLL cases, cells carrying the usually clonal trisomy 12 or *MYD88* genetic alterations seem to have distinct chromatin and DNA methylation patterns compared with cases lacking them (19, 148). Other genetic aberrations have not been consistently linked to clear epigenetic signatures, which may be due to the high CLL subclonal heterogeneity reflected in the significant proportion of genetic alterations with low cancer cell fractions (113). For instance, despite the previous lack of association of *NOTCH1* mutations and chromatin features, a recent in vitro study manipulating primary CLL cells mimicked the effect of *NOTCH1* mutations and identified a specific chromatin pattern (149). This aberrant chromatin pattern was associated with upregulation of NOTCH target genes and repression *HLA class II* through the transcriptional suppression of *CIITA*, possibly mediating immune escape. Finally, and as briefly mentioned before, MM cytogenetic groups also seem to have differential DNA methylation patterns, particularly the t(4;14) deregulating *NSD2* (also known as MMSET) (90, 91), a histone methyltransferase that specifically dimethylates H3K36.

Other studies reported a relationship between certain genetic aberrations such as *TP53* or *SF3B1* and higher number of DNA methylation changes (150, 151) (**Figure 4*d***). In turn, higher epigenetic burdens have been consistently associated with a worse clinical outcome (57, 84, 90, 150). Collectively, these findings may in fact reflect cases with extensive proliferative histories related to the acquisition of certain genetic driver alterations, as the total number of DNA methylation changes is strongly correlated with the epiCMIT mitotic clock (49) (**Figure 3*c***). In line with this, the epiCMIT is also correlated with genomic complexity as well as the acquisition of certain genetic drivers in CLL and MCL, such as *TP53*, *SF3B1*, or +8q24 affecting *MYC*, among others. Altogether, these findings suggest a model in which genetic driver alterations lead to a higher cell proliferation and to a concomitant acquisition of higher DNA methylation changes, which are reflected in the mitotic clock (**Figure 4*d***) (49). In addition, CLLs and MCLs show epiCMIT correlations with the clock-like single base substitution (SBS) mutational signatures SBS1 and SBS5 (49, 152), and with the noncanonical activation-induced cytidine deaminase (AID) signature SBS9 (**Figure 4*d***). This may reflect a coevolution of genome-wide off-target AID mutations and DNA methylation changes accumulated at chromatin-repressed regions, which take place during clonal expansions of the ancestor B cell before malignant transformation (49, 116).

Another example of cross talk between genetic and epigenetic alterations is the fact that epigenetic genes are frequently altered in cancer (153) and affect different layers of the epigenetic code, including multiple DNA methylation and chromatin modifiers (154). In myeloid neoplasms, mutations affecting genes related to DNA methylation such as *DNMT3A* and *TET2* are highly prevalent and involved in early clonal expansions of hematopoietic cells, but they are less frequent in lymphoid tumors. In these neoplasms, mutations in chromatin modifier genes are far more prevalent, with different genes altered depending on the maturation stage of each B cell tumor (thoroughly reviewed elsewhere; see 126, 155). Precursor B-ALLs show the *KMT2A* (MLL) rearrangement with more than 100 translocation partners and constitute a group with

dismal prognosis (156). In addition, ALL displays mutations in other chromatin remodeler genes, including *MLL1*, *CREBBP*, *NSD2*, and *SETD2* (157).

In MCL, mutations in chromatin modifier genes have also been described and take place almost exclusively in the cMCL subtype (116, 158). The involved genes can be present in approximately up to 25% of cases and include *KMT2D* (23%), *SP140* (13%), *NSD2* (12%), *SMARCA4* (9%), and *SMARCB1* (4%).

Lymphomas arising from GCBs are strongly associated with histone modifier genes essential for GC function (reviewed in 159). These mutations seem to alter epigenetic control mechanisms of the GC, which contributes to neoplastic transformation. The strongest link to chromatin modifiers is observed in FL (126), including mutations in *KMT2D* and *CREBBP* in 60–70% of the cases and *EZH2*, *EP300*, *KMT2C*, and *ARID1A* in 10–25%. In the case of GCB-like DLBCL, a similar mutational spectrum is found although at lower frequency, that is, 25% in *KMT2D*, 20% in both *CREBBP* and *EZH2*, 5–10% in *EP300*, 13% in *KMT2C*, and 11% in *ARID1A*. Remarkably, these mutations are virtually absent in ABC-like DLBCL. It is worth mentioning that mutations in chromatin modifier genes constitute important components of the recent DLBCL genetic subtypes, particularly the EZB/C3 groups (81, 82). In BL, the affected genes are in general different, with 40% of the cases showing *SMARCA4* mutations, which are mutually exclusive with mutations in *ARID1A*, described in 20–30% of patients (160).

In CLL, lower percentages are observed, with the exception of *CHD2*, which is present in approximately 5% of M-CLLs. Remarkably, genomic alterations affecting the *CHD2* gene are enriched in M-CLLs with a transcriptional profile more similar to U-CLL cases, which are also related to worse outcomes (113). In the case of MM, the t(4;14) translocation affects *NSD2* by juxtaposing the regulatory elements of the IGH locus, whereas mutations in other epigenetic modifiers are present at lower frequencies of approximately 1–2% of the patients (161).

Finally, mutations in core histone members are highly prevalent in cancer and represent a new class of genetic drivers (162). In lymphomas, mutations in the isoforms B-E of the linker histone H1 protein are highly recurrent across B cell tumors. A recent study suggests that these mutations act as disease drivers by decompacting the 3D genome structure, leading to the illegitimate expression of stem cell–related genes (163).

SINGLE-CELL MOLECULAR PROFILING: A REALITY

Despite their clonal origin, cancer cells do not form a homogeneous cell mass but rather create a cellular ecosystem with multiple phenotypic subclones. Epigenetic heterogeneity in cancer, and in lymphoid neoplasms in particular, was initially characterized by studying variations of DNA methylation patterns of individual reads in bisulfite-based NGS experiments (79, 86, 109, 164). Additionally, bulk DNA methylation data can be used to infer contemporary clonal dynamics through a recently developed mathematical model (165). This new epigenetic clock utilizes fluctuating CpGs (fCpGs), whose methylation status stochastically fluctuates between homozygous (un)methylated and heterozygous methylated states in individual diploid cells. When this fluctuation occurs on the order of decades, the distribution of methylation values of fCpGs represents a pool of methylation barcodes characterizing clonal dynamics. However, in spite of the relevance of these findings, a more precise way to unravel cellular heterogeneity in cancer is by single-cell molecular profiling. In lymphoid tumors, the field of single-cell omics is rapidly expanding (166–169). As this topic has already been the subject of dedicated reviews, in the present article we only summarize major findings from some key studies.

Most studies published so far used single-cell RNA sequencing (scRNA-seq), which is commercially available through various platforms (170). Overall, scRNA-seq reports have uncovered great transcriptional heterogeneity in lymphoid tumors (171–175), which is not always related

to subclonal genetic heterogeneity (176). A major factor leading to such variability is that cells contain functional subclusters that span more than one B cell maturation state. In cMCL, cases seem to span a window of differentiation from naive to pre-GCBs (177), whereas in FL cases span a continuum of states from proliferating GC-like to quiescent memory-like cells (178). This transcriptional heterogeneity spanning GC-like to memory-like states seem to be mediated by interactions with the tumor microenvironment (TME), mostly with follicular helper T cells. These transcriptional states occupy different niches in the affected lymph nodes, that is, GC-like cells located in the center of the follicles and memory-like cells in perifollicular areas. In CLL, individual cells from peripheral blood can be clustered into those that just exited the lymph nodes and those that are primed to home back to the lymph nodes to receive survival and proliferative signals (115, 179). In the case of high-grade transformation of CLL into RT, subclones are also heterogeneous and their presence can be traced back up to 19 years before the clinical manifestation of RT (115). These early seeds share genetic and transcriptional features of the expanded subclone at the clinical detection of RT. The transcriptome of these cells is characterized by a B cell receptor downregulation and an upregulation of the oxidative phosphorylation pathway, which represent a promising therapeutic target for RT patients. Despite having genetic alterations and a metabolic profile that should lead to a proliferative advantage, these early subclones do not lead to a clinical diagnosis of RT until many years later. The underlying mechanisms of this time lapse between early seeding and clinical manifestation of RT are of great interest but are currently unknown. Collectively, all these studies revealed a high subclonal heterogeneity related to both tumor-intrinsic features such as gene alterations and tumor-extrinsic features such as interactions with microenvironmental cells (180). This high subclonal diversity leads to a high phenotypic plasticity, which ensures resistance mechanisms and immune escape allowing tumor growth (181).

With regard to single-cell epigenetics, various technologies have been developed to measure DNA methylation, histone modifications, chromatin accessibility, and even 3D genome architecture (40, 41) (see the sidebar titled Main Epigenomic Profiling Methods). For instance, single-cell DNA (scDNA) methylation profiling using bisulfite-based methods is still costly, although other higher-throughput approaches are being developed (37, 39). Few single-cell epigenomic studies have been published in lymphomas. An initial seminal study characterized lineage histories of CLL cells by integrating scDNA methylomes with transcriptional profiles and genotyping. This article provided evidence for a rapid diversification during early clonal expansion, which may give rise to several subclones that compete to become expanded. Additionally, this study also identified that genetic subclones arising during disease development map to distinct DNA methylation branches (182). Beyond DNA methylation, most ongoing studies are analyzing chromatin accessibility using single-cell assay for transposase-accessible chromatin with sequencing (scATAC-seq). For instance, in the case of CLL, scATAC-seq was used together with mitochondrial DNA mutations to study subclonal dynamics. This study revealed epigenetic heterogeneity during disease evolution, suggesting the presence of nongenetic sources of relapse (183).

Although published epigenomic studies at single-cell resolution are still scarce, they will represent a new axis to tackle disease heterogeneity of lymphoid tumors. This will be greatly complemented by the simultaneous characterization of multiomic layers (40–42), the TME, or even several omic layers at the spatial level within tissue sections (184).

CONCLUSION

A profound analysis of the multi-layered epigenome has provided valuable insights into the cellular origin, pathogenic mechanisms, and evolutionary paths of lymphoid neoplasms. A part of increasing our understanding of the molecular complexity underlying lymphoid tumor development, epigenetics also has an immense translational potential. Epigenetic signatures and biomarkers

represent first-class diagnostic and prognostic variables that shall complement genetic assessment in the routine diagnostic setting. Epigenomic data also provide a broad range of targets for therapeutic interventions. In addition, it seems clearly manifested in this review that knowledge evolves over time hand in hand with technical developments, leading to more accurate interpretations and novel concepts. We are currently witnessing a technological revolution including single-cell technologies and long-read sequencing, multimodal, and spatial approaches. A data-driven approach coupling novel wet-lab methods and deep computational approaches shall further transform our understanding of lymphoid tumors and lead to clinical applications with unprecedented value.

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Both authors have written the manuscript and designed the figures.

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LITERATURE CITED

1. Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, de Oliveira Araujo IB, et al. 2022. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leukemia* 36(7):1720–48
2. Campo E, Jaffe ES, Cook JR, Quintanilla-Martinez L, Swerdlow SH, et al. 2022. The international consensus classification of mature lymphoid neoplasms: a report from the Clinical Advisory Committee. *Blood* 140(11):1229–53
3. Dalla-Favera R. 2012. Lymphoid malignancies: many tumor types, many altered genes, many therapeutic challenges. *J. Clin. Investig.* 122(10):3396–97
4. Esteller M. 2008. Epigenetics in cancer. *N. Engl. J. Med.* 358(11):1148–59
5. Jones PA, Baylin SB. 2002. The fundamental role of epigenetic events in cancer. *Nat. Rev. Genet.* 3(6):415–28
6. Holliday R. 2006. Epigenetics: a historical overview. *Epigenetics* 1(2):76–80
7. Deichmann U. 2016. Epigenetics: the origins and evolution of a fashionable topic. *Dev. Biol.* 416(1):249–54
8. Eden A, Gaudet F, Waghmare A, Jaenisch R. 2003. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 300(5618):455
9. Luo C, Hajkova P, Ecker JR. 2018. Dynamic DNA methylation: in the right place at the right time. *Science* 361(6409):1336–40
10. Jones PA. 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13(7):484–92

11. Mattei AL, Bailly N, Meissner A. 2022. DNA methylation: a historical perspective. *Trends Genet*. 38(7):676–707
12. Karlić R, Chung H-R, Lasserre J, Vlahovicek K, Vingron M. 2010. Histone modification levels are predictive for gene expression. *PNAS* 107(7):2926–31
13. Roadmap Epigenomics Consort., Kundaje A, Meuleman W, Ernst J, Bilenky M, et al. 2015. Integrative analysis of 111 reference human epigenomes. *Nature* 518(7539):317–30
14. Kouzarides T. 2007. Chromatin modifications and their function. *Cell* 128(4):693–705
15. Kimura H. 2013. Histone modifications for human epigenome analysis. *J. Hum. Genet.* 58(7):439–45
16. Baubec T, Colombo DF, Wirbelauer C, Schmidt J, Burger L, et al. 2015. Genomic profiling of DNA methyltransferases reveals a role for DNMT3B in genic methylation. *Nature* 520(7546):243–47
17. Ernst J, Kellis M. 2012. ChromHMM: automating chromatin-state discovery and characterization. *Nat. Methods* 9(3):215–16
18. Ernst J, Kheradpour P, Mikkelsen TS, Shoresh N, Ward LD, et al. 2011. Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature* 473(7345):43–49
19. Beekman R, Chapaprieta V, Russiñol N, Vilarrasa-Blasi R, Verdaguer-Dot N, et al. 2018. The reference epigenome and regulatory chromatin landscape of chronic lymphocytic leukemia. *Nat. Med.* 24(6):868–80
20. Dekker J, Marti-Renom MA, Mirny LA. 2013. Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data. *Nat. Rev. Genet.* 14(6):390–403
21. Rao SSP, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159(7):1665–80
22. Jerkovic I, Cavalli G. 2021. Understanding 3D genome organization by multidisciplinary methods. *Nat. Rev. Mol. Cell Biol.* 22(8):511–28
23. Dehingia B, Milewska M, Janowski M, Pękowska A. 2022. CTCF shapes chromatin structure and gene expression in health and disease. *EMBO Rep*. 23(9):e55146
24. Li S, Tollefsbol TO. 2021. DNA methylation methods: Global DNA methylation and methylomic analyses. *Methods* 187:28–43
25. Füllgrabe J, Gosal WS, Creed P, Liu S, Lumby CK, et al. 2023. Simultaneous sequencing of genetic and epigenetic bases in DNA. *Nat. Biotechnol.* **https://doi.org/10.1038/s41587-022-01652-0**
26. Yue X, Xie Z, Li M, Wang K, Li X, et al. 2022. Simultaneous profiling of histone modifications and DNA methylation via nanopore sequencing. *Nat. Commun.* 13(1):7939
27. Yang Y, Scott SA. 2017. DNA methylation profiling using long-read single molecule real-time bisulfite sequencing (SMRT-BS). *Methods Mol. Biol.* 1654:125–34
28. Liu Q, Fang L, Yu G, Wang D, Xiao C-L, Wang K. 2019. Detection of DNA base modifications by deep recurrent neural network on Oxford Nanopore sequencing data. *Nat. Commun.* 10(1):2449
29. Kaya-Okur HS, Janssens DH, Henikoff JG, Ahmad K, Henikoff S. 2020. Efficient low-cost chromatin profiling with CUT&Tag. *Nat. Protoc.* 15(10):3264–83
30. Minnoye L, Marinov GK, Krausgruber T, Pan L, Marand AP, et al. 2021. Chromatin accessibility profiling methods. *Nat. Rev. Methods Primers* 1(1):10
31. Klemm SL, Shipony Z, Greenleaf WJ. 2019. Chromatin accessibility and the regulatory epigenome. *Nat. Rev. Genet.* 20(4):207–20
32. Akgol Oksuz B, Yang L, Abraham S, Venev SV, Krietenstein N, et al. 2021. Systematic evaluation of chromosome conformation capture assays. *Nat. Methods* 18(9):1046–55
33. Bouwman BAM, Crosetto N, Bienko M. 2022. The era of 3D and spatial genomics. *Trends Genet*. 38(10):1062–75
34. Jerković I, Cavalli G. 2021. Understanding 3D genome organization by multidisciplinary methods. *Nat. Rev. Mol. Cell Biol.* 22(8):511–28
35. Kempfer R, Pombo A. 2020. Methods for mapping 3D chromosome architecture. *Nat. Rev. Genet.* 21(4):207–26
36. Smallwood SA, Lee HJ, Angermueller C, Krueger F, Saadeh H, et al. 2014. Single-cell genome-wide bisulfite sequencing for assessing epigenetic heterogeneity. *Nat. Methods* 11(8):817–20
37. Nichols RV, O'Connell BL, Mulqueen RM, Thomas J, Woodfin AR, et al. 2022. High-throughput robust single-cell DNA methylation profiling with sciMETv2. *Nat. Commun.* 13(1):7627

38. Guo H, Zhu P, Wu X, Li X, Wen L, Tang F. 2013. Single-cell methylome landscapes of mouse embryonic stem cells and early embryos analyzed using reduced representation bisulfite sequencing. *Genome Res.* 23(12):2126–35
39. Bianchi A, Scherer M, Zaurin R, Quililan K, Velten L, Beekman R. 2022. scTAM-seq enables targeted high-confidence analysis of DNA methylation in single cells. *Genome Biol.* 23(1):229
40. Vandereyken K, Sifrim A, Thienpont B, Voet T. 2023. Methods and applications for single-cell and spatial multi-omics. *Nat. Rev. Genet.* 24(8):494–515
41. Nam AS, Chaligne R, Landau DA. 2021. Integrating genetic and non-genetic determinants of cancer evolution by single-cell multi-omics. *Nat. Rev. Genet.* 22(1):3–18
42. Stuart T, Satija R. 2019. Integrative single-cell analysis. *Nat. Rev. Genet.* 20(5):257–72
43. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, et al. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403(6769):503–11
44. Martin-Subero JI, Oakes CC. 2018. Charting the dynamic epigenome during B-cell development. *Semin. Cancer Biol.* 51:139–48
45. Kulis M, Merkel A, Heath S, Queirós AC, Schuyler RP, et al. 2015. Whole-genome fingerprint of the DNA methylome during human B cell differentiation. *Nat. Genet.* 47(7):746–56
46. Loyfer N, Magenheim J, Peretz A, Cann G, Bredno J, et al. 2023. A DNA methylation atlas of normal human cell types. *Nature* 613(7943):355–64
47. Zhu T, Liu J, Beck S, Pan S, Capper D, et al. 2022. A pan-tissue DNA methylation atlas enables in silico decomposition of human tissue methylomes at cell-type resolution. *Nat. Methods* 19(3):296–306
48. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, et al. 2012. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformat.* 13:86
49. Duran-Ferrer M, Clot G, Nadeu F, Beekman R, Baumann T, et al. 2020. The proliferative history shapes the DNA methylome of B-cell tumors and predicts clinical outcome. *Nat. Cancer* 1(11):1066–81
50. Rahmani E, Schweiger R, Rhead B, Criswell LA, Barcellos LF, et al. 2019. Cell-type-specific resolution epigenetics without the need for cell sorting or single-cell biology. *Nat. Commun.* 10(1):3417
51. Jaffe AE, Irizarry RA. 2014. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol.* 15(2):R31
52. Hoadley KA, Yau C, Hinoue T, Wolf DM, Lazar AJ, et al. 2018. Cell-of-origin patterns dominate the molecular classification of 10,000 tumors from 33 types of cancer. *Cell* 173(2):291–304.e6
53. Shaknovich R, Geng H, Johnson NA, Tsikitas L, Cerchietti L, et al. 2010. DNA methylation signatures define molecular subtypes of diffuse large B-cell lymphoma. *Blood* 116(20):e81–89
54. Kretzmer H, Bernhart SH, Wang W, Haake A, Weniger MA, et al. 2015. DNA methylome analysis in Burkitt and follicular lymphomas identifies differentially methylated regions linked to somatic mutation and transcriptional control. *Nat. Genet.* 47(11):1316–25
55. Arribas AJ, Rinaldi A, Chiodin G, Kwee I, Mensah AA, et al. 2019. Genome-wide promoter methylation of hairy cell leukemia. *Blood Adv.* 3(3):384–96
56. Navarro A, Beà S, Jares P, Campo E. 2020. Molecular pathogenesis of mantle cell lymphoma. *Hematol. Oncol. Clin. North Am.* 34(5):795–807
57. Queirós AC, Beekman R, Vilarrasa-Blasi R, Duran-Ferrer M, Clot G, et al. 2016. Decoding the DNA methylome of mantle cell lymphoma in the light of the entire B cell lineage. *Cancer Cell* 30(5):806–21
58. Palomero J, Vegliante MC, Eguileor A, Rodríguez ML, Balsas P, et al. 2016. SOX11 defines two different subtypes of mantle cell lymphoma through transcriptional regulation of BCL6. *Leukemia* 30(7):1596–99
59. Navarro A, Clot G, Royo C, Jares P, Hadzidimitriou A, et al. 2012. Molecular subsets of mantle cell lymphoma defined by the *IGHV* mutational status and SOX11 expression have distinct biologic and clinical features. *Cancer Res.* 72(20):5307–16
60. Kipps TJ, Stevenson FK, Wu CJ, Croce CM, Packham G, et al. 2017. Chronic lymphocytic leukaemia. *Nat. Rev. Dis. Primers* 3:16096
61. Kulis M, Heath S, Bibikova M, Queirós AC, Navarro A, et al. 2012. Epigenomic analysis detects widespread gene-body DNA hypomethylation in chronic lymphocytic leukemia. *Nat. Genet.* 44(11):1236–42

62. Nadeu F, Royo R, Clot G, Duran-Ferrer M, Navarro A, et al. 2021. IGLV3–21R110 identifies an aggressive biological subtype of chronic lymphocytic leukemia with intermediate epigenetics. *Blood* 137(21):2935–46
63. Kulis M, Martin-Subero JI. 2023. Integrative epigenomics in chronic lymphocytic leukaemia: biological insights and clinical applications. *Br. J. Haematol.* 200(3):280–90
64. Oakes CC, Seifert M, Assenov Y, Gu L, Przekopowitz M, et al. 2016. DNA methylation dynamics during B cell maturation underlie a continuum of disease phenotypes in chronic lymphocytic leukemia. *Nat. Genet.* 48(3):253–64
65. Giacopelli B, Zhao Q, Ruppert AS, Agyeman A, Weigel C, et al. 2019. Developmental subtypes assessed by DNA methylation-iPLEX forecast the natural history of chronic lymphocytic leukemia. *Blood* 134(8):688–98
66. Queirós AC, Villamor N, Clot G, Martinez-Trillos A, Kulis M, et al. 2015. A B-cell epigenetic signature defines three biologic subgroups of chronic lymphocytic leukemia with clinical impact. *Leukemia* 29(3):598–605
67. Bhoi S, Ljungström V, Baliakas P, Mattsson M, Smedby KE, et al. 2016. Prognostic impact of epigenetic classification in chronic lymphocytic leukemia: the case of subset #2. *Epigenetics* 11(6):449–55
68. Wojdacz TK, Amarasinghe HE, Kadalayil L, Beattie A, Forster J, et al. 2019. Clinical significance of DNA methylation in chronic lymphocytic leukemia patients: results from 3 UK clinical trials. *Blood Adv.* 3(16):2474–81
69. Duran-Ferrer M, Mansouri L, Nadeu F, Clot G, Bhoi S, et al. 2022. A comprehensive DNA methylome analysis of stereotyped and non-stereotyped CLL reveals an epigenetic signature with strong clinical impact encompassing IGHV status, stereotypes and IGLV3–21R110. *Blood* 140(Suppl. 1):1800–2
70. Roos-Weil D, Giacopelli B, Armand M, Della-Valle V, Ghamlouch H, et al. 2020. Identification of 2 DNA methylation subtypes of Waldenström macroglobulinemia with plasma and memory B-cell features. *Blood* 136(5):585–95
71. Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, et al. 2011. DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* 480(7378):490–95
72. Kreibich E, Kleinendorst R, Barzaghi G, Kaspar S, Krebs AR. 2023. Single-molecule footprinting identifies context-dependent regulation of enhancers by DNA methylation. *Mol. Cell* 83(5):787–802.e9
73. Lee S-T, Muench MO, Fomin ME, Xiao J, Zhou M, et al. 2015. Epigenetic remodeling in B-cell acute lymphoblastic leukemia occurs in two tracks and employs embryonic stem cell-like signatures. *Nucleic Acids Res.* 43(5):2590–602
74. Nordlund J, Bäcklin CL, Wahlberg P, Busche S, Berglund EC, et al. 2013. Genome-wide signatures of differential DNA methylation in pediatric acute lymphoblastic leukemia. *Genome Biol.* 14(9):r105
75. Hetzel S, Mattei AL, Kretzmer H, Qu C, Chen X, et al. 2022. Acute lymphoblastic leukemia displays a distinct highly methylated genome. *Nat. Cancer* 3(6):768–82
76. Vogt J, Wagener R, Montesinos-Rongen M, Ammerpohl O, Paulus W, et al. 2019. Array-based profiling of the lymphoma cell DNA methylome does not unequivocally distinguish primary lymphomas of the central nervous system from non-CNS diffuse large B-cell lymphomas. *Genes Chromosomes Cancer* 58(1):66–69
77. Richter J, Ammerpohl O, Martín-Subero JI, Montesinos-Rongen M, Bibikova M, et al. 2009. Array-based DNA methylation profiling of primary lymphomas of the central nervous system. *BMC Cancer* 9:455
78. Teater M, Dominguez PM, Redmond D, Chen Z, Ennishi D, et al. 2018. AICDA drives epigenetic heterogeneity and accelerates germinal center-derived lymphomagenesis. *Nat. Commun.* 9(1):222
79. Pan H, Jiang Y, Boi M, Tabbò F, Redmond D, et al. 2015. Epigenomic evolution in diffuse large B-cell lymphomas. *Nat. Commun.* 6:6921
80. Chambwe N, Kormaksson M, Geng H, De S, Michor F, et al. 2014. Variability in DNA methylation defines novel epigenetic subgroups of DLBCL associated with different clinical outcomes. *Blood* 123(11):1699–708
81. Wright GW, Huang DW, Phelan JD, Coulibaly ZA, Roulland S, et al. 2020. A probabilistic classification tool for genetic subtypes of diffuse large B cell lymphoma with therapeutic implications. *Cancer Cell* 37(4):551–68.e14

82. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, et al. 2018. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat. Med.* 24(5):679–90
83. O'Riain C, O'Shea DM, Yang Y, Le Dieu R, Gribben JG, et al. 2009. Array-based DNA methylation profiling in follicular lymphoma. *Leukemia* 23(10):1858–66
84. Tsagiopoulou M, Papakonstantinou N, Moysiadis T, Mansouri L, Ljungström V, et al. 2019. DNA methylation profiles in chronic lymphocytic leukemia patients treated with chemoimmunotherapy. *Clin. Epigenet.* 11(1):177
85. Cahill N, Bergh A-C, Kanduri M, Göransson-Kultima H, Mansouri L, et al. 2013. 450K-array analysis of chronic lymphocytic leukemia cells reveals global DNA methylation to be relatively stable over time and similar in resting and proliferative compartments. *Leukemia* 27(1):150–58
86. Landau DA, Clement K, Ziller MJ, Boyle P, Fan J, et al. 2014. Locally disordered methylation forms the basis of intratumor methylome variation in chronic lymphocytic leukemia. *Cancer Cell* 26(6):813–25
87. Claus R, Lucas DM, Stilgenbauer S, Ruppert AS, Yu L, et al. 2012. Quantitative DNA methylation analysis identifies a single CpG dinucleotide important for ZAP-70 expression and predictive of prognosis in chronic lymphocytic leukemia. *J. Clin. Oncol.* 30(20):2483–91
88. Broséus J, Hergalant S, Vogt J, Tausch E, Kreuz M, et al. 2023. Molecular characterization of Richter syndrome identifies de novo diffuse large B-cell lymphomas with poor prognosis. *Nat. Commun.* 14(1):309
89. Agirre X, Castellano G, Pascual M, Heath S, Kulis M, et al. 2015. Whole-epigenome analysis in multiple myeloma reveals DNA hypermethylation of B cell-specific enhancers. *Genome Res.* 25(4):478–87
90. Walker BA, Wardell CP, Chiecchio L, Smith EM, Boyd KD, et al. 2011. Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. *Blood* 117(2):553–62
91. Choudhury SR, Ashby C, Tytarenko R, Bauer M, Wang Y, et al. 2020. The functional epigenetic landscape of aberrant gene expression in molecular subgroups of newly diagnosed multiple myeloma. *J. Hematol. Oncol.* 13(1):108
92. Kaiser MF, Johnson DC, Wu P, Walker BA, Brioli A, et al. 2013. Global methylation analysis identifies prognostically important epigenetically inactivated tumor suppressor genes in multiple myeloma. *Blood* 122(2):219–26
93. Ammerpohl O, Haake A, Pellissery S, Giefing M, Richter J, et al. 2012. Array-based DNA methylation analysis in classical Hodgkin lymphoma reveals new insights into the mechanisms underlying silencing of B cell-specific genes. *Leukemia* 26(1):185–88
94. Nordlund J, Bäcklin CL, Zachariadis V, Cavelier L, Dahlberg J, et al. 2015. DNA methylation-based subtype prediction for pediatric acute lymphoblastic leukemia. *Clin. Epigenet.* 7(1):11
95. Xia D, Leon AJ, Yan J, Silva A, Bakhtiari M, et al. 2021. DNA methylation-based classification of small B-cell lymphomas: a proof-of-principle study. *J. Mol. Diagn.* 23(12):1774–86
96. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, et al. 2018. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* 563(7732):579–83
97. Moss J, Magenheim J, Neiman D, Zemmour H, Loyfer N, et al. 2018. Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. *Nat. Commun.* 9(1):5068
98. Roschewski M, Rossi D, Kurtz DM, Alizadeh AA, Wilson WH. 2022. Circulating tumor DNA in lymphoma: principles and future directions. *Blood Cancer Discov.* 3(1):5–15
99. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, et al. 2006. Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 125(2):301–13
100. Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, et al. 2007. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. *Nat. Genet.* 39(2):232–36
101. Widschwendter M, Fiegl H, Egle D, Mueller-Holzner E, Spizzo G, et al. 2007. Epigenetic stem cell signature in cancer. *Nat. Genet.* 39(2):157–58
102. Ohm JE, McGarvey KM, Yu X, Cheng L, Schuebel KE, et al. 2007. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. *Nat. Genet.* 39(2):237–42

103. Martín-Subero JI, Kreuz M, Bibikova M, Bentink S, Ammerpohl O, et al. 2009. New insights into the biology and origin of mature aggressive B-cell lymphomas by combined epigenomic, genomic, and transcriptional profiling. *Blood* 113(11):2488–97
104. Gal-Yam EN, Egger G, Iniguez L, Holster H, Einarsson S, et al. 2008. Frequent switching of Polycomb repressive marks and DNA hypermethylation in the PC3 prostate cancer cell line. *PNAS* 105(35):12979–84
105. Beerman I, Bock C, Garrison BS, Smith ZD, Gu H, et al. 2013. Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. *Cell Stem Cell* 12(4):413–25
106. Teschendorff AE. 2020. A comparison of epigenetic mitotic-like clocks for cancer risk prediction. *Genome Med.* 12(1):56
107. Yang Z, Wong A, Kuh D, Paul DS, Rakyan VK, et al. 2016. Correlation of an epigenetic mitotic clock with cancer risk. *Genome Biol.* 17(1):205
108. Youn A, Wang S. 2018. The MiAge calculator: a DNA methylation-based mitotic age calculator of human tissue types. *Epigenetics* 13(2):192–206
109. Landan G, Cohen NM, Mukamel Z, Bar A, Molchadsky A, et al. 2012. Epigenetic polymorphism and the stochastic formation of differentially methylated regions in normal and cancerous tissues. *Nat. Genet.* 44(11):1207–14
110. Aran D, Toperoff G, Rosenberg M, Hellman A. 2011. Replication timing-related and gene body-specific methylation of active human genes. *Hum. Mol. Genet.* 20(4):670–80
111. Zhou W, Dinh HQ, Ramjan Z, Weisenberger DJ, Nicolet CM, et al. 2018. DNA methylation loss in late-replicating domains is linked to mitotic cell division. *Nat. Genet.* 50(4):591–602
112. Endicott JL, Nolte PA, Shen H, Laird PW. 2022. Cell division drives DNA methylation loss in late-replicating domains in primary human cells. *Nat. Commun.* 13(1):6659
113. Knisbacher BA, Lin Z, Hahn CK, Nadeu F, Duran-Ferrer M, et al. 2022. Molecular map of chronic lymphocytic leukemia and its impact on outcome. *Nat. Genet.* 54(11):1664–74
114. Berman BP, Weisenberger DJ, Aman JF, Hinoue T, Ramjan Z, et al. 2011. Regions of focal DNA hypermethylation and long-range hypomethylation in colorectal cancer coincide with nuclear lamina-associated domains. *Nat. Genet.* 44(1):40–46
115. Nadeu F, Royo R, Massoni-Badosa R, Playa-Albinyana H, Garcia-Torre B, et al. 2022. Detection of early seeding of Richter transformation in chronic lymphocytic leukemia. *Nat. Med.* 28(8):1662–71
116. Nadeu F, Martin-Garcia D, Clot G, Díaz-Navarro A, Duran-Ferrer M, et al. 2020. Genomic and epigenomic insights into the origin, pathogenesis, and clinical behavior of mantle cell lymphoma subtypes. *Blood* 136(12):1419–32
117. Diedrich JD, Dong Q, Ferguson DC, Bergeron BP, Autry RJ, et al. 2021. Profiling chromatin accessibility in pediatric acute lymphoblastic leukemia identifies subtype-specific chromatin landscapes and gene regulatory networks. *Leukemia* 35(11):3078–91
118. Kloetgen A, Thandapani P, Ntziachristos P, Ghebrechristos Y, Nomikou S, et al. 2020. Three-dimensional chromatin landscapes in T cell acute lymphoblastic leukemia. *Nat. Genet.* 52(4):388–400
119. Beekman R, Amador V, Campo E. 2018. SOX11, a key oncogenic factor in mantle cell lymphoma. *Curr. Opin. Hematol.* 25(4):299–306
120. Vilarrasa-Blasi R, Verdaguer-Dot N, Belver L, Soler-Vila P, Beekman R, et al. 2022. Insights into the mechanisms underlying aberrant SOX11 oncogene expression in mantle cell lymphoma. *Leukemia* 36(2):583–87
121. Vilarrasa-Blasi R, Soler-Vila P, Verdaguer-Dot N, Russiñol N, Di Stefano M, et al. 2021. Dynamics of genome architecture and chromatin function during human B cell differentiation and neoplastic transformation. *Nat. Commun.* 12(1):651
122. Jang J-Y, Hwang I, Pan H, Yao J, Alinari L, et al. 2022. A FOXO1-dependent transcription network is a targetable vulnerability of mantle cell lymphomas. *J. Clin. Investig.* 132(24):e160767
123. Zhang J, Jima D, Moffitt AB, Liu Q, Czader M, et al. 2014. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. *Blood* 123(19):2988–96
124. Albero R, Enjuanes A, Demajo S, Castellano G, Pinyol M, et al. 2018. Cyclin D1 overexpression induces global transcriptional downregulation in lymphoid neoplasms. *J. Clin. Investig.* 128(9):4132–47

125. Demajo S, Albero R, Clot G, Castellano G, Navarro A, et al. 2021. A cyclin D1-dependent transcriptional program predicts clinical outcome in mantle cell lymphoma. *Clin. Cancer Res.* 27(1):213–25
126. Lunning MA, Green MR. 2015. Mutation of chromatin modifiers; an emerging hallmark of germinal center B-cell lymphomas. *Blood Cancer J.* 5(10):e361
127. Ott CJ, Federation AJ, Schwartz LS, Kasar S, Klitgaard JL, et al. 2018. Enhancer architecture and essential core regulatory circuitry of chronic lymphocytic leukemia. *Cancer Cell*. 34(6):982–95.e7
128. Mallm J-P, Iskar M, Ishaque N, Klett LC, Kugler SJ, et al. 2019. Linking aberrant chromatin features in chronic lymphocytic leukemia to transcription factor networks. *Mol. Syst. Biol.* 15(5):e8339
129. Pastore A, Gaiti F, Lu SX, Brand RM, Kulm S, et al. 2019. Corrupted coordination of epigenetic modifications leads to diverging chromatin states and transcriptional heterogeneity in CLL. *Nat. Commun.* 10(1):1874
130. Navarro A, Clot G, Martínez-Trillos A, Pinyol M, Jares P, et al. 2017. Improved classification of leukemic B-cell lymphoproliferative disorders using a transcriptional and genetic classifier. *Haematologica* 102(9):360–63
131. Tsagiopoulou M, Chapaprieta V, Russiñol N, García-Torre B, Pechlivanis N, et al. 2023. Chromatin activation profiling of stereotyped chronic lymphocytic leukemias reveals a subset 8-specific signature. *Blood* 141(24):2955–60
132. Vilarrasa-Blasi R, Soler-Vila P, Verdaguer-Dot N, Russiñol N, Di Stefano M, et al. 2021. Dynamics of genome architecture and chromatin function during human B cell differentiation and neoplastic transformation. *Nat. Commun.* 12(1):651
133. Alvarez-Benayas J, Trasanidis N, Katsarou A, Ponnusamy K, Chaidos A, et al. 2021. Chromatin-based, in *cis* and in *trans* regulatory rewiring underpins distinct oncogenic transcriptomes in multiple myeloma. *Nat. Commun.* 12(1):5450
134. Ordoñez R, Kulis M, Russiñol N, Chapaprieta V, Carrasco-Leon A, et al. 2020. Chromatin activation as a unifying principle underlying pathogenic mechanisms in multiple myeloma. *Genome Res.* 30(9):1217–27
135. Jin Y, Chen K, De Paepe A, Hellqvist E, Krstic AD, et al. 2018. Active enhancer and chromatin accessibility landscapes chart the regulatory network of primary multiple myeloma. *Blood* 131(19):2138–50
136. Wu P, Li T, Li R, Jia L, Zhu P, et al. 2017. 3D genome of multiple myeloma reveals spatial genome disorganization associated with copy number variations. *Nat. Commun.* 8(1):1937
137. Alaterre E, Ovejero S, Herviou L, de Boussac H, Papadopoulos G, et al. 2022. Comprehensive characterization of the epigenetic landscape in multiple myeloma. *Theranostics* 12(4):1715–29
138. Agarwal P, Alzrigat M, Párraga AA, Enroth S, Singh U, et al. 2016. Genome-wide profiling of histone H3 lysine 27 and lysine 4 trimethylation in multiple myeloma reveals the importance of Polycomb gene targeting and highlights EZH2 as a potential therapeutic target. *Oncotarget* 7(6):6809–23
139. Sermer D, Pasqualucci L, Wendel H-G, Melnick A, Younes A. 2019. Emerging epigenetic-modulating therapies in lymphoma. *Nat. Rev. Clin. Oncol.* 16(8):494–507
140. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, et al. 2012. Systematic localization of common disease-associated variation in regulatory DNA. *Science* 337(6099):1190–95
141. Vijayakrishnan J, Qian M, Studd JB, Yang W, Kinnersley B, et al. 2019. Identification of four novel associations for B-cell acute lymphoblastic leukaemia risk. *Nat. Commun.* 10(1):5348
142. Speedy HE, Beekman R, Chapaprieta V, Orlando G, Law PJ, et al. 2019. Insight into genetic predisposition to chronic lymphocytic leukemia from integrative epigenomics. *Nat. Commun.* 10(1):3615
143. Went M, Sud A, Försti A, Halvarsson B-M, Weinhold N, et al. 2018. Identification of multiple risk loci and regulatory mechanisms influencing susceptibility to multiple myeloma. *Nat. Commun.* 9(1):3707
144. Robbe P, Ridout KE, Vavoulis D V, Dréau H, Kinnersley B, et al. 2022. Whole-genome sequencing of chronic lymphocytic leukemia identifies subgroups with distinct biological and clinical features. *Nat. Genet.* 54(11):1675–89
145. Bal E, Kumar R, Hadigol M, Holmes AB, Hilton LK, et al. 2022. Super-enhancer hypermutation alters oncogene expression in B cell lymphoma. *Nature* 607(7920):808–15
146. Puente XS, Beà S, Valdés-Mas R, Villamor N, Gutiérrez-Abril J, et al. 2015. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature* 526(7574):519–24

147. Luchtel RA, Dasari S, Oishi N, Pedersen MB, Hu G, et al. 2018. Molecular profiling reveals immunogenic cues in anaplastic large cell lymphomas with DUSP22 rearrangements. *Blood* 132(13):1386–98
148. Tsagiopoulou M, Chapaprieta V, Duran-Ferrer M, Moysiadis T, Psomopoulos F, et al. 2020. Chronic lymphocytic leukemias with trisomy 12 show a distinct DNA methylation profile linked to altered chromatin activation. *Haematologica* 105(12):2864–67
149. Mangolini M, Maiques-Diaz A, Charalampopoulou S, Gerhard-Hartmann E, Bloehdorn J, et al. 2022. Viral transduction of primary human lymphoma B cells reveals mechanisms of NOTCH-mediated immune escape. *Nat. Commun.* 13(1):6220
150. Enjuanes A, Albero R, Clot G, Navarro A, Beà S, et al. 2013. Genome-wide methylation analyses identify a subset of mantle cell lymphoma with a high number of methylated CpGs and aggressive clinicopathological features. *Int. J. Cancer* 133(12):2852–63
151. Oakes CC, Claus R, Gu L, Assenov Y, Hüllein J, et al. 2014. Evolution of DNA methylation is linked to genetic aberrations in chronic lymphocytic leukemia. *Cancer Discov.* 4(3):348–61
152. Alexandrov LB, Jones PH, Wedge DC, Sale JE, Campbell PJ, et al. 2015. Clock-like mutational processes in human somatic cells. *Nat. Genet.* 47(12):1402–7
153. Koschmann C, Nunez FJ, Mendez F, Brosnan-Cashman JA, Meeker AK, et al. 2017. Mutated chromatin regulatory factors as tumor drivers in cancer. *Cancer Res.* 77(2):227–33
154. Reyna MA, Haan D, Paczkowska M, Verbeke LPC, Vazquez M, et al. 2020. Pathway and network analysis of more than 2500 whole cancer genomes. *Nat. Commun.* 11(1):729
155. Jiang Y, Dominguez PM, Melnick AM. 2016. The many layers of epigenetic dysfunction in B-cell lymphomas. *Curr. Opin. Hematol.* 23(4):377–84
156. Meyer C, Hofmann J, Burmeister T, Gröger D, Park TS, et al. 2013. The MLL recombinome of acute leukemias in 2013. *Leukemia* 27(11):2165–76
157. Iacobucci I, Mullighan CG. 2017. Genetic basis of acute lymphoblastic leukemia. *J. Clin. Oncol.* 35(9):975–83
158. Pararajalingam P, Coyle KM, Arthur SE, Thomas N, Alcaide M, et al. 2020. Coding and noncoding drivers of mantle cell lymphoma identified through exome and genome sequencing. *Blood* 136(5):572–84
159. Jiang Y, Melnick A. 2015. The epigenetic basis of diffuse large B-cell lymphoma. *Semin. Hematol.* 52(2):86–96
160. López C, Burkhardt B, Chan JKC, Leoncini L, Mbulaiteye SM, et al. 2022. Burkitt lymphoma. *Nat. Rev. Dis. Primers* 8(1):78
161. Pawlyn C, Kaiser MF, Heuck C, Melchor L, Wardell CP, et al. 2016. The spectrum and clinical impact of epigenetic modifier mutations in myeloma. *Clin. Cancer Res.* 22(23):5783–94
162. Bennett RL, Bele A, Small EC, Will CM, Nabet B, et al. 2019. A mutation in histone H2B represents a new class of oncogenic driver. *Cancer Discov.* 9(10):1438–51
163. Yusufova N, Kloetgen A, Teater M, Osunsade A, Camarillo JM, et al. 2021. Histone H1 loss drives lymphoma by disrupting 3D chromatin architecture. *Nature* 589(7841):299–305
164. Dominguez PM, Teater M, Shaknovich R. 2017. The new frontier of epigenetic heterogeneity in B-cell neoplasms. *Curr. Opin. Hematol.* 24(4):402–8
165. Gabbutt C, Schenck RO, Weisenberger DJ, Kimberley C, Berner A, et al. 2022. Fluctuating methylation clocks for cell lineage tracing at high temporal resolution in human tissues. *Nat. Biotechnol.* 40(5):720–30
166. Oder B, Chatzidimitriou A, Langerak AW, Rosenquist R, Österholm C. 2023. Recent revelations and future directions using single-cell technologies in chronic lymphocytic leukemia. *Front. Oncol.* 13:1143811
167. Sturgess KHM, Wilson NK, Göttgens B. 2023. Introduction to a review series on single-cell genomics: getting ready for clinical impact in leukemia and myeloid neoplasms. *Blood* 141(4):323–25
168. Dutta AK, Alberge J-B, Sklavenitis-Pistofidis R, Lightbody ED, Getz G, Ghobrial IM. 2022. Single-cell profiling of tumour evolution in multiple myeloma—opportunities for precision medicine. *Nat. Rev. Clin. Oncol.* 19(4):223–36
169. Samur MK, Szalat R, Munshi NC. 2023. Single-cell profiling in multiple myeloma: insights, problems, and promises. *Blood* 142(4):313–24
170. Mereu E, Lafzi A, Moutinho C, Ziegenhain C, McCarthy DJ, et al. 2020. Benchmarking single-cell RNA-sequencing protocols for cell atlas projects. *Nat. Biotechnol.* 38(6):747–55

171. Ye X, Wang L, Nie M, Wang Y, Dong S, et al. 2022. A single-cell atlas of diffuse large B cell lymphoma. *Cell Rep*. 39(3):110713
172. Ysebaert L, Quillet-Mary A, Tosolini M, Pont F, Laurent C, Fournié J-J. 2021. Lymphoma heterogeneity unraveled by single-cell transcriptomics. *Front. Immunol.* 12:597651
173. Dutta AK, Alberge J-B, Sklavenitis-Pistofidis R, Lightbody ED, Getz G, Ghobrial IM. 2022. Single-cell profiling of tumour evolution in multiple myeloma—opportunities for precision medicine. *Nat. Rev. Clin. Oncol.* 19(4):223–36
174. Nagler A, Wu CJ. 2023. The end of the beginning: application of single-cell sequencing to chronic lymphocytic leukemia. *Blood* 141(4):369–79
175. Iacobucci I, Witkowski MT, Mullighan CG. 2023. Single-cell analysis of acute lymphoblastic and lineage-ambiguous leukemia: approaches and molecular insights. *Blood* 141(4):356–68
176. Wang L, Fan J, Francis JM, Georghiou G, Hergert S, et al. 2017. Integrated single-cell genetic and transcriptional analysis suggests novel drivers of chronic lymphocytic leukemia. *Genome Res*. 27(8):1300–11
177. Massoni-Badosa R, Soler-Vila P, Aguilar-Fernández S, Nieto JC, Elosua-Bayes M, et al. 2022. An atlas of cells in the human tonsil. bioRxiv 2022.06.24.497299. **https://doi.org/10.1101/2022.06.24.497299**
178. Attaf N, Dong C, Gil L, Cervera-Marzal I, Gharsalli T, et al. 2022. Functional plasticity and recurrent cell states of malignant B cells in follicular lymphoma. bioRxiv 2022.04.06.487285. **https://doi.org/10.1101/2022.04.06.487285**
179. Sun C, Chen Y-C, Martinez Zurita A, Baptista MJ, Pittaluga S, et al. 2023. The immune microenvironment shapes transcriptional and genetic heterogeneity in chronic lymphocytic leukemia. *Blood Adv*. 7(1):145–58
180. Tirier SM, Mallm J-P, Steiger S, Poos AM, Awwad MHS, et al. 2021. Subclone-specific microenvironmental impact and drug response in refractory multiple myeloma revealed by single-cell transcriptomics. *Nat. Commun.* 12(1):6960
181. Scott DW, Gascoyne RD. 2014. The tumour microenvironment in B cell lymphomas. *Nat. Rev. Cancer* 14(8):517–34
182. Gaiti F, Chaligne R, Gu H, Brand RM, Kothen-Hill S, et al. 2019. Epigenetic evolution and lineage histories of chronic lymphocytic leukaemia. *Nature* 569(7757):576–80
183. Penter L, Gohil SH, Lareau C, Ludwig LS, Parry EM, et al. 2021. longitudinal single-cell dynamics of chromatin accessibility and mitochondrial mutations in chronic lymphocytic leukemia mirror disease history. *Cancer Discov*. 11(12):3048–63
184. Zhang D, Deng Y, Kukanja P, Agirre E, Bartosovic M, et al. 2023. Spatial epigenome-transcriptome co-profiling of mammalian tissues. *Nature* 616(7955):113–22