Aberrant Epigenetic Gene Regulation in Lymphoid Malignancies

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In lymphoid malignancies, aberrant epigenetic mechanisms such as DNA methylation and histone modifications influence chromatin architecture and can result in altered gene expression. These alterations commonly affect genes that play important roles in the cell cycle, apoptosis, and DNA repair in non-Hodgkin lymphoma (NHL). The ability to identify epigenetic modifications to these important genes has increased exponentially due to advances in technology. As a result, there are well-defined, gene-specific epigenetic aberrations associated with NHL comprising follicular lymphoma (FL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), and diffuse large B-cell lymphoma (DLBCL). The identification of these genes is important because they may be used as biomarkers for prognosis, diagnosis and in developing improved treatment strategies. Also important, in the control of gene expression, is the packaging of DNA within the nucleus of a cell. This packaging can be distorted by epigenetic alterations and may alter the accessibility of certain regions of the genome in cancer cells. This review discusses the impact of known epigenetic aberration on the regulation of gene expression in NHL and provides insight into the spatial conformation of the genome (DNA packaging) in acute lymphoblastic leukemia.
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Lymphoid malignancies can generally be divided into two major groups, leukemias (acute and chronic) and lymphomas. Non-Hodgkin lymphoma (NHL) accounts for the majority of lymphoid tumors (89%) and 4% of all cancers diagnosed each year, and can be of either B- (~80%) or T-cell (~20%) subtype. Malignancies of B cells occur when the normal regulation of cell differentiation is disrupted and there is a subsequent accumulation of cells that have been blocked at a particular stage of normal B-cell differentiation (Figure 1). Small B-cell lymphomas comprise approximately 50% of the B-cell NHLs and include follicular lymphoma (FL; 40%), mantle cell lymphoma (MCL; 3%–4%), and B-cell chronic lymphocytic leukemia (B-CLL; 3%–4%). Diffuse large B-cell lymphoma (DLBCL) constitutes the majority of the remaining 50% of B-cell NHLs and can be subclassified as either germinal center (GC) B-cell–like (GCB-DLBCL) or activated B-cell–like (ABC-DLBCL). Development of lymphoid malignancies may involve a variety of mechanisms, including inactivation of tumor-suppressor genes, activation of oncogenes, and genomic instability that can result in chromosomal translocations. Multiple recurring cytogenetic abnormalities are a characteristic finding in lymphoid malignancies and likely contribute directly to malignant transformation. However, these abnormalities alone do not always result in transformation and, therefore, other mechanisms, including epigenetic alterations, are also involved.

Epigenetics can be defined as the study of heritable changes in gene expression that are the result of mechanisms other than alterations in the DNA sequence. These mechanisms include DNA methylation, post-translational histone modifications, and altered expression of non-coding RNA (ncRNA). The best characterized epigenetic mechanisms that impact the conformation of chromatin in lymphoma are DNA methylation and the post-translational modifications of histones. Progress in the field of epigenetics is expanding exponentially as the transition from small studies to large-scale studies is now occurring and ultimately may define the entire epig-

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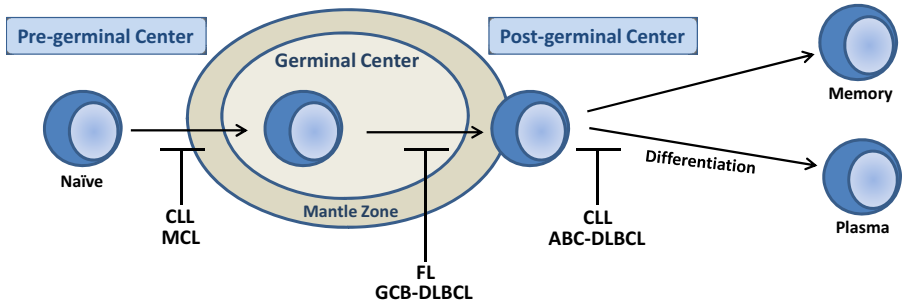

Figure 1. B-cell malignancies and their normal counterparts. Epigenetic alterations with oncogenic potential may occur at any stage of normal B-cell development and lead to an accumulation of cells at specific stages of differentiation. Abbreviations: CLL, chronic lymphocytic leukemia; MCL, mantle cell lymphoma; FL, follicular lymphoma; GCB-DLBCL, germinal center B-cell-like diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell-like diffuse large B-cell lymphoma.

enome and nuclear chromatin architecture of single cells.

In this review we will discuss the importance of aberrant epigenetic events beyond genetic alterations and how these events may alter the three-dimensional (3D) chromatin structure of cells that result in aberrant regulation of gene expression in lymphoid malignancies.

ALTERED EXPRESSION OF EPIGENETIC REGULATORY GENES

Methylation of DNA may occur at the 5′-position of a cytosine molecule typically located within a cytosine-guanine (CpG) dinucleotide. In a normal somatic cell, approximately 70%–80% of cytosines within this context are methylated. This methylation is known to play a role in gene imprinting, X-inactivation, tissue-specific gene expression, and protection against effects of retroviral sequences and genome stability. Beyond this, 5′-hydroxymethyl cytosine, which may play additional roles in epigenetics, is beginning to be investigated as well. DNA methyltransferase 1 (DNMT1) is responsible for the maintenance of DNA methylation patterns and targets hemi-methylated DNA in order to propagate DNA methylation during replication. If this mechanism is altered, regions of the genome that should be methylated, such as repetitive sequences, may lose stability as a result of the loss of methylation. This genomic instability can then lead to the development of lymphoid (and other) malignancies. In contrast, DNA methyltransferases 3A and 3B (DNMT3A, DNMT3B) are considered de novo methyltransferases and target primarily unmethylated CpGs. A significant downregulation of *DNMT3B* has been observed in CLL and there is further progression of downregulation with clinical advancement of the disease.[1] Further, Amara and colleagues have shown that *DNMT1*, *3A,* and *3B* are all overexpressed in DLBCL and that overexpression of *DNMT1* and *3B* is strongly correlated with advanced disease.[2] The acquisition of methylation in regions of the genome that normally should not be methylated and the loss of methylation in the regions that should be methylated can both lead to aberrant gene expression and result in lymphomagenesis. In general, methylation in the promoter (and other regulatory sequences) of a gene can result in gene repression, whereas methylation present within the body of a gene is frequently associated with active gene transcription.[3] In the context of malignant transformation, methylation present in the promoter of a tumor-suppressor gene is associated with an inactive chromatin conformation and may result in repression of tumor-suppressor activity. Alternately, loss of methylation in the genome can lead to the loss of imprinting, inappropriate tissue-specific gene expression, activation of retroviral DNA, and genome fragility, all hallmarks of lymphoid malignancies.

Octamers of histone proteins comprise the scaffold upon which DNA is wound to form chromatin. The histones H2A, H2B, H3, and H4 can be post-translationally modified in a number of ways such as changes in acetylation, methylation, phosphorylation, sumoylation, and ubiquitination, mainly in their N-terminal tails. These modifications result in changes to the overall chromatin structure, either inducing an open (euchromatic) or a closed (heterochromatic) state, and thus have an impact on the ability of effector molecules to bind to the DNA.[4] Particular modifications are commonly associated with transcriptionally active chromatin while others are associated with transcriptionally inactive chromatin. For example, methylation of H3 lysine 27 (H3K27) is mainly associated with inactive chromatin and gene repression. The polycomb repressor complex 2 (PRC2) is responsible for H3K27 methylation and is comprised of EZH2, SUZ12, and EED. EZH2 targets genes involved in differentiation, suppression of cell growth, and proliferation of B cells.[5] Somatic mutations in the catalytic domain of EZH2 have been identified that are associated with a gain of function of EZH2.[6,7] These mutations are seen in 7.2% of FL patients and 21.7% of GCB-DLBCL patients and are associ-