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REVIEW ARTICLE

Molecular Pathogenesis of Diffuse Large B-Cell Lymphoma

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ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous clinicopathologic entity accounting for 30% of non-Hodgkin's lymphomas. The pathogenesis of DLBCL is complex and heterogeneous. Recent studies using analysis of global gene expression with DNA microarrays and the classical molecular approaches demonstrate presence of several DLBCL subtypes characterized by different cells of origin, cytogenetic and molecular aberrations, and distinct pathogenesis. This review summarizes the progress in understanding of DLBCL biology and presents a state-of-the-art overview of DLBCL molecular pathogenesis.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a clinically and biologically heterogeneous disease. Similar to other types of cancer, the pathogenesis of DLBCL represents a multistep process that involves accumulation of multiple genetic and molecular lesions leading to the selection of a malignant clone. The differences in cellular derivation of DLBCL, distinct chromosomal translocations, aberrant somatic hypermutation mechanism and distinct transcriptional signatures, as well as aberrations common to other malignancies such as gene amplifications, deletions and mutations, all contribute to the complex process of the molecular pathogenesis of DLBCL (Fig 1). Although marked advances in our understanding of the pathobiology of this disease have been made, many pathogenetic mechanisms and biologic features of DLBCL remain largely unknown.

CELLULAR DERIVATION OF DLBCL

B-cell lymphomas arise from normal lymphocytes at different stages of B-cell differentiation. Comparison of the biologic features, surface markers and gene expression profiles of a given type of lymphoma to different maturation stages of normal lymphocytes can suggest the stage of B-cell ontogeny at which malignant transformation occurred. The presence of somatic mutations in the variable (V) region of immunoglobulin (*Ig*) genes is commonly used as a marker of germinal center (GC) transit since normal pregerminal center lymphocytes harbor unmutated *Ig* genes.[1] Furthermore, the presence of intraclonal heterogeneity in *Ig* gene mutations is regarded as a marker of ongoing somatic mutations that occur almost exclusively in the GC and thus is used as a marker of GC origin.

Analysis of DLBCL specimens reveals the presence of *Ig* gene mutations in a pattern that suggests antigen selection pressure in the majority of tumors.[2,3] Further examination of *Ig* gene intraclonal heterogeneity demonstrates the presence of two subgroups of DLBCL—one with and another without ongoing somatic mutations—thus suggesting derivation of these lymphomas from two distinct normal B-cell counterparts: GC and post-GC lymphocytes.[2] These observations corroborate the previously reported heterogeneous expression of immunohistochemical GC markers (eg, CD10 and BCL6) in DLBCL tumors.[4,5]

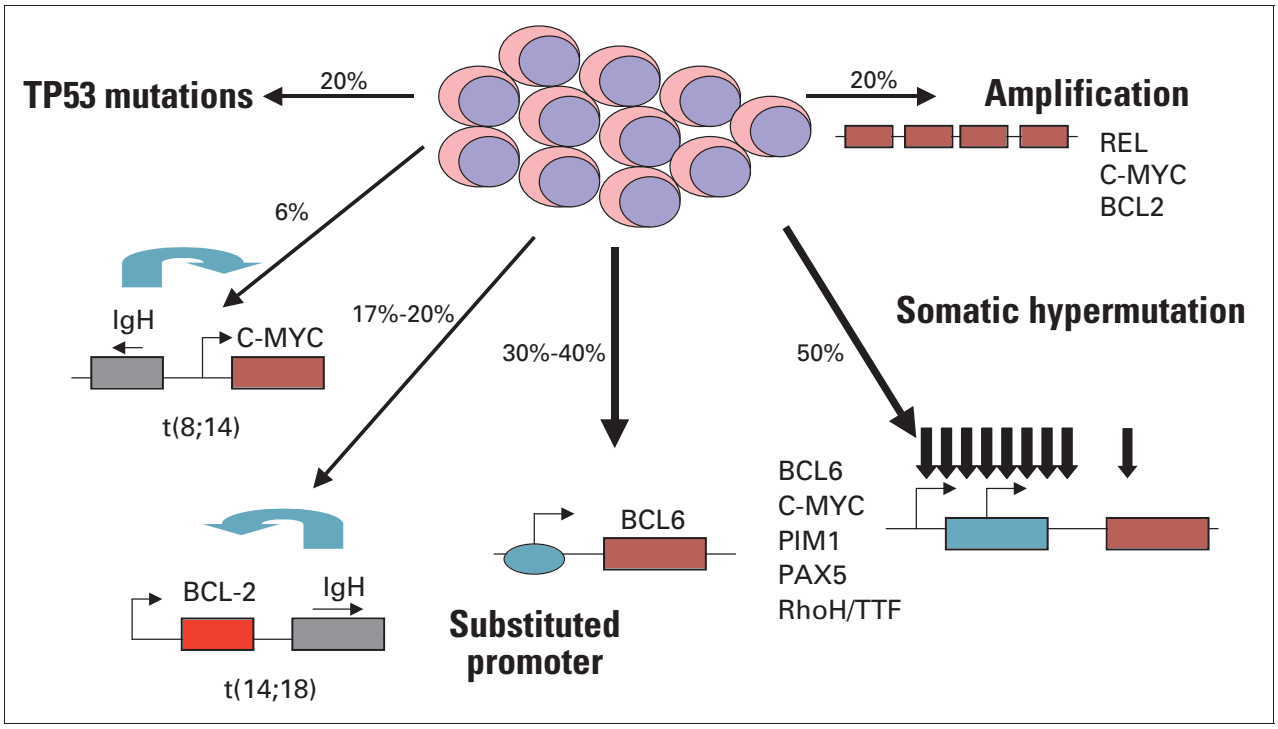

Fig 1. Model for diffuse large B-cell lymphoma (DLBCL) pathogenesis. The figure shows a schematic representation of lymphocytes and genetic lesions acquired in the process of transformation to DLBCL. Only the most common genetic lesions and their frequencies are shown.

Recent application of genome-wide gene expression profiling analysis by DNA array technology further confirmed the presence of ontogenetically distinct DLBCL subtypes: GC-like DLBCL and non–GC-like DLBCL.[6,7] GC-like DLBCL harbors the expression pattern of genes characteristic of normal GC B-cells (*GC* signature genes). Non–GC-like DLBCL consists of activated B-cell (ABC) –like DLBCL and "type 3" subtypes. The ABC-like tumors express genes characteristic of in vitro activated peripheral blood B-cells (activated B-cell signature genes) as well as some genes normally expressed by plasma cells, thus suggesting their post-GC origin. Type 3 is a heterogeneous DLBCL subtype that does not express high levels of either the *GC* or *ABC* set of genes. Notably, gene expression–defined GC-like DLBCL demonstrated the presence of *Ig* gene intraclonal heterogeneity whereas tumors exhibiting an ABC-like gene expression profile did not.[8]

Gene expression–defined DLBCL subtypes not only correspond to derivation from distinct stages of lymphocyte ontogeny but also likely represent different mechanisms of malignant transformation and distinct tumor biology (Fig 2). The t(14;18)(q32;q21) translocation involving the *BCL-2* gene and the amplification of the *c-rel* locus on chromosome 2p have been detected exclusively in GC-like DLBCL.[7,9-11] High expression of nuclear factor κ B (NF-κB) target genes has been observed in ABC-like DLBCL but not in GC-like DLBCL cell-lines.[12] NF-κB is usually retained in an inactive form in the cytoplasm, by binding to members of the IκB family of proteins. In response to signaling through diverse pathways, members of the IκB family are phosphorylated by the IκB kinase complex (IKK) and subsequently are degraded by the ubiquitin-proteasome pathway. This leads to release of NF-κB family members that translocate into the nucleus and activate transcription thus mediating proliferation, apoptosis and cell survival. ABC-like, but not GC-like, DLBCL, exhibit constitutive activity of IKK, the inhibition of which is cytotoxic to ABC-like but not to GC-like DLBCL cell lines.[12]

PDE4B is a cyclic AMP (cAMP) phosphodiesterase highly expressed in ABC-like DLBCL.[6,13] PDE4B inactivates cAMP, an intracellular second messenger that modulates several signaling pathways and induces cell cycle arrest and apoptosis of B cells. Stimulation of the cAMP pathway in GC-like DLBCL cell lines expressing low levels of PDE4B has been associated with decreased phosphorylation and activity of AKT leading to dephosphorylation of BAD, mitochondrial membrane depolarization and marked apoptosis. In contrast, stimulation of cAMP did not affect the ABC-like DLBCL cell lines expressing high levels of PDE4B.[13]

In addition, GC-like and ABC-like DLBCL cell lines exhibit distinct responsiveness and intracellular signaling in response to interleukin (IL) -4 stimulation.[14] In ABC-like DLBCL cell lines IL-4 phosphorylated AKT, did not induce a sustained increase in nuclear phosphorylated

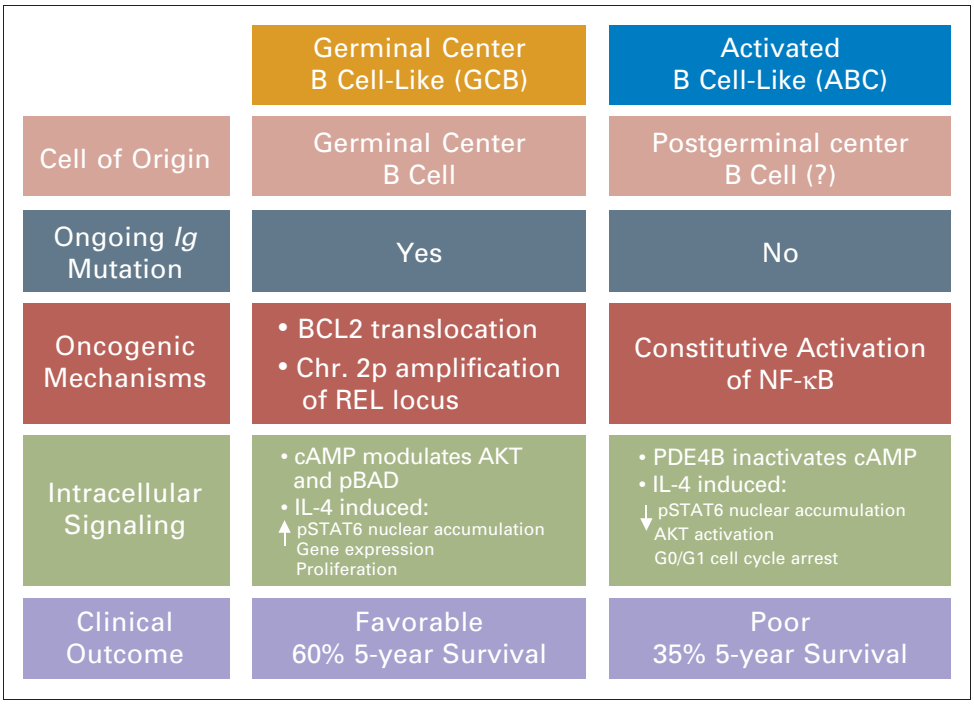

Fig 2. Molecular, pathogenetic and clinical features distinguishing germinal-center–like and activated B-cell–like diffuse large B-cell lymphoma.

STAT6 (signal transducer and activator of transcription 6), did not induce expression of IL-4 target genes, and decreased cell proliferation by cell-cycle arrest. In contrast, in GC-like DLBCL cell lines IL-4 induced expression of its known target genes, activated STAT6 intracellular signaling and mildly increased cell proliferation. Aberrant activation of the JAK (janus kinase) -STAT6 pathway in the ABC-like cell lines was attributed to increased cytoplasmic and nuclear STAT6 dephosphorylation by protein tyrosine phosphatase nonreceptor (PTPN)-1 and PTPN2, whose expression is significantly higher in primary ABC-like tumors.[14] Overall, the observed differences in GC-like and non–GC-like DLBCL biology and molecular pathogenesis might underlie the observed differences in clinical outcome of patients with distinct DLBCL subtypes after treatment with standard combination chemotherapy.

Chromosomal Translocations

Cytogenetic and molecular studies of de novo DLBCL have demonstrated the presence of recurrent chromosomal translocations, including those involving *C-MYC*,[15] *BCL2*,[9] and *BCL6*.[16-19] These translocations likely derive from illegitimate recombination following double-strand DNA breaks that are generated during variable diversity joining (VDJ) and class switch recombination and somatic hypermutation of the *Ig* genes.

The chromosomal translocations of 3q27, the locus of the *BCL6* gene, are the most characteristic and common genetic abnormalities detected in 30% to 40% of DLBCL tumors.[16,20] These translocations usually occur in a highly conserved 4.0 kb regulatory region of the *BCL6* gene (the major translocations cluster [MTC]) spanning the promoter, the first noncoding exon and the 5′ region of the first intron.[21] As a result of the translocation there is promoter substitution.[22] The 5′ regulatory region containing the *BCL6* promoter sequences is either removed or truncated, leading to the juxtaposition in the same transcriptional orientation of *BCL6* exons 2 through 10 downstream from the partner gene with its own promoter or other regulatory elements. The partners of the *BCL6* chromosomal translocations most often involve the *Ig* genes on chromosome bands 14q32, 2p12 and 22q11 but more than 20 alternate loci on other chromosomes have also been observed as partners, a phenomenon termed "promiscuous translocation."[21] The fusion partners initiated from the heterologous promoters contain intact coding exons of *BCL6*. *BCL6* gene expression is tightly regulated during B-cell differentiation, being restricted to B cells in the GC.[23] In contrast, the heterologous promoters exhibit a broader spectrum of activity in B-cell ontogenetic stages thus preventing *BCL6* downregulation in post-GC cells.

In addition to *BCL6* translocations, small deletions and somatic point mutations occurring in the *BCL6* regulatory region that overlap with the subdomain of the MTC are reported in 70% of DLBCL.[24-27] The occurrence of mutations is independent of translocation-generated rearrangements and some mutations can significantly deregulate BCL6 expression.[28]

The role of *BCL6* in the pathogenesis of DLBCL has only recently been elucidated. The *BCL6* gene encodes a 96KD sequence-specific transcriptional repressor protein with 6 C-terminal zinc finger motifs and an N-terminal

POZ/ZIN domain homologous to a family of zinc finger proteins.[19] Several lines of evidence suggest that a block in the normal downregulation of *BCL6* might favor differentiation arrest, continuous cell proliferation, survival, and genetic instability, all of which could lead ultimately to neoplastic transformation. These include the requirement for *BCL6* gene expression for GC formation,[29] an immune reaction characterized by a high proliferation rate, oligoclonal expansion of B cells and the presence of active somatic mutations, and downregulation of *BCL6* expression in cells exiting the GC microenvironment. Indeed, *BCL6* was reported to repress the expression of *PRDM1*, whose product Blimp1 is necessary for plasma cell differentiation, and also the expression of *p27kip1*, *cyclin D2* and *TP53,* which control the cell cycle, apoptosis, DNA repair and maintenance of genomic stability.[30,31] TP53 downregulation by *BCL6* may allow DLBCL cells to escape apoptosis in response to DNA damage and thus allow continued tumor cell growth.[31]

Mutations and deletions of *TP53* are reported in up to 20% of DLBCL and are associated with a more aggressive clinical course.[32,33] The presence of *TP53* mutations almost exclusively in DLBCL tumors without *BCL6* translocations and their rare detection in DLBCL cases with constitutive expression of *BCL6* due to chromosomal translocation suggests that BCL6 inactivates TP53 during DLBCL lymphomagenesis, thus bypassing the need for *TP53* mutations.[31] Furthermore, upregulation of TP53 expression following chemotherapy-induced downregulation of BCL6 expression might underlie the better prognosis of the BCL6-expressing DLBCL tumors exposed to chemotherapy.[34]

In addition to these aberrations, sites of amplification, including those containing proto-oncogenes such as *REL*, *C-MYC* and *BCL2* are reported in DLBCL.[35,36] DLBCL also displays a wide variety of other translocations, chromosomal deletions, and gains in which the target genes involved in the pathogenesis of lymphoma have not yet been identified.

Aberrant Somatic Hypermutation

In the normal GC, the process of somatic hypermutation is not restricted to *Ig* genes, but also targets 5′ regions of other genes, including *BCL6*,[24] *FAS/CD95*,[37] and the *CD79* component of the B-cell receptor.[38] Whereas the mutations of the *Ig* genes allow the affinity maturation of the antibodies to their target antigens leading to B cell selection, the purpose of somatic mutations in other genes of normal GC lymphocytes is unclear. Interestingly, a recent study demonstrated that in 50% of DLBCL, the process of somatic hypermutation may malfunction and target 5′ regions of additional genes, such as *C-MYC*, *PIM1*, *PAX5*, *RhoH/TTF* proto-oncogenes that are involved in cell proliferation, differentiation, and signal transduction.[39] In the case of the *C-MYC* and *PIM1*, the mutations target not only the untranslated regulatory regions of these genes but also their coding regions, leading to amino acid changes with potential functional consequences.

Whether the mechanism of somatic mutations in these genes relies on the action of activation-induced cytidine deaminase (AID), which mediates *Ig* gene mutations,[40] is presently unknown. However, preferential targeting of sequences in close proximity to transcription initiation sites and the observed features of the mutations suggest that they result from the same process.[39] Notably, in contrast to restricted expression of AID in normal GC-lymphocytes, in DLBCL AID expression dissociates from other GC signature gene expression, since it is expressed in both GC-like and non-GC-like subtypes.[41] This observation suggests that DLBCL tumors maintain some, but not all of the gene expression signatures of their normal B-cell counterparts. The presence of aberrantly active somatic hypermutation machinery may represent a powerful mechanism of lymphomatous transformation by targeting a multitude of genes, whose identity is still largely unknown.

Transcriptional Heterogeneity in DLBCL

In addition to differences in the cell of origin, there is a marked residual transcriptional profiling heterogeneity within these ontogenetically distinct DLBCL subtypes. Analysis of transcriptional profiles irrespective of DLBCL ontogenetic origin may disclose signaling and metabolic or immune signatures relevant to DLBCL pathogenesis. Using this approach, Monti et al[42] revealed the presence of three consensus clusters defining the DLBCL subgroups: ''oxidative phosphorylation,'' ''B-cell receptor proliferation,'' and ''host response.''

The oxidative phosphorylation cluster defined DLBCL subgroup highly expressing genes involved in the mitochondrial function and the electron transport chain, oxidative phosphorylation and apoptosis. The B-cell receptor proliferation cluster defined a DLBCL subgroup abundantly expressing cell-cycle regulatory genes, DNA repair genes and components of the B-cell–receptor signaling cascade and B-cell transcriptional factors. Interestingly, some of the genes comprising this cluster were previously reported to be differentially expressed in de novo and transformed DLBCL tumors.[43] The ''host response'' cluster defined a DLBCL subgroup with enriched expression of T cell, natural killer cell, monocyte/macrophage and dendritic cell transcripts. Many DLBCL tumors belonging to this subgroup were defined as T-cell–rich B-cell lymphomas based on morphology and immunohistochemistry. However, the ''host response'' signature might also be pathophysiologically important in classic DLBCL tumors. Infiltrating T lymphocytes isolated from B-cell lymphomas may recognize specific epitopes of the malignant clone.[44] The number of infiltrating T cells in

the initial lymphoma biopsy from DLBCL patients has been reported to be predictive of relapse-free survival.[45] Absence of major histocompatibility-encoded recognition complexes (MHC) may limit the ability of the host to mount an immune response against the tumor cells. Loss of MHC class I and class II (HLA-DP and HLA-DR) expression was reported to correlate with shortened relapse-free and overall survival.[46,47] Fewer $CD8^+$ T cells were detected in MHC class II–negative cases compared with positive cases, thus supporting the hypothesis that loss of tumor immunosurveillance might contribute to the inferior outcome of DLBCL patients.[47]

Primary Mediastinal B-Cell Lymphoma

Primary mediastinal B-cell lymphoma (PMBL) accounts for approximately 5% of aggressive lymphoma and is recognized as a specific subtype of DLBCL based on its distinctive clinical and morphologic features. Pathologically, PMBL tumors are characterized by a diffuse proliferation of large cells with clear cytoplasm and by the presence of a variable degree of sclerosis, which causes the typical compartmentalization pattern. PMBL exhibits characteristic genetic abnormalities including frequent gains of 9p and distinct high-level amplifications with a defined consensus region on 9p24, where *JAK2* genes are located.[48] In contrast, *BCL2* and *BCL6* rearrangements, frequently observed in DLBCL, appear to be rare.[49] Immunohistochemical studies confirm the B-cell origin of this lymphoma, although the neoplastic B cells express little if any surface or cytoplasmic Ig and major histocompatibility complex class I and/or class II molecules.[50] PMBLs usually express IL-4–inducible GC immunohistochemical markers—BCL6 and HGAL proteins[51]; however, the expression of CD10 is low and not uniform, thus suggesting that these tumors are most probably not of GC origin.

To better understand the pathophysiology of this lymphoma and to identify markers that can reliably distinguish PMBL from DLBCL, gene expression profile studies were performed.[52,53] These studies demonstrated a significant difference in gene expression between PMBL and DLBCL. In contrast, a striking similarity in gene expression between PMBL and nodular sclerosis Hodgkin's lymphoma (HL) was observed. Interestingly, PMBL and HL share many clinical and pathologic features, including the higher prevalence in young patients, involvement of the mediastinum, and fibrotic stromal cell reaction. These similarities, which suggest a pathogenetic overlap between the two disorders, may be caused by the activation of similar signaling pathways or transcription factors in both lymphoma types or by a common thymic origin of these tumors. Transcriptional analysis of PMBL demonstrated increased expression of the *IL-13* receptor α1 chain, *JAK2* and several IL-4/IL-13–dependent genes (*CD23, NF-IL13,* FIG1/*IL-411*, and *IL-4*–induced gene 1) suggesting constitutive activation of the IL-4/IL-13 pathway(s) in these tumors.[52-54] Indeed, PMBL tumors exhibited constitutive activation of STAT6,[55] similar to what was reported in HL.[56] Although JAK2 amplification might underlie this observation, analysis of PMBC cell lines demonstrated the presence of bi-allelic mutations of supressor of cytokine signaling (SOCS)-1, which impaired JAK2 degradation and led to sustained JAK2-STAT6 activation.[57]

CONCLUSION

Diversity in the clinical presentation, morphology and immunophenotype strongly suggests that DLBCL is a heterogeneous group of aggressive B-cell lymphomas rather than a single clinicopathologic entity. Accordingly, it is not surprising that the pathogenesis of DLBCL is complex and heterogeneous. Furthermore, tumor-specific molecular aberrations do not solely account for DLBCL pathogenesis. Distinctive cross talk between the tumor and its microenvironment might also play an important role in this process. Recent classical investigations as well as whole genome expression profiling studies markedly advanced our understanding of the molecular pathogenesis of DLBCL. However, many components of this process remain largely unknown, requiring ongoing investigational efforts to better understand the pathogenesis of DLBCL. These advances will have significant implications for the design of clinical trials, development of new therapeutic approaches, and planning of treatment during routine patient care.

Author's Disclosures of Potential Conflicts of Interest

The author indicated no potential conflicts of interest.

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