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Molecular pathogenesis of diffuse large B-cell lymphoma

Diffuse Large B-Cell Lymphomas (DLBCLs) are the most common histologic subtype within non-Hodgkin's lymphomas (NHLs), accounting for approximately 30% of them. These lymphomas are a heterogeneous clinicopathologic entity characterized by marked phenotypic and clinical differences and several data indeed suggest that under the name of DLBCL different disease entities are currently included. This vision is supported by the fact that DLBCL subtypes are characterized by different cells of origin and by different cytogenetic and molecular aberrations and, therefore, by a distinct pathogenesis.¹

Ontogenesis of DLBCL

Concerning the cellular derivation, it is well known that the presence of immunoglobulin (Ig) gene mutations in the variable (V) region suggests antigen selection pressure and may be used as a marker of germinal center (GC) transit. This feature is shared by the vast majority of DLBCLs.² However, the presence of an intraclonal heterogeneity in the pattern of the Ig gene mutations shows the occurrence of an ongoing process of somatic mutations and this process occurs almost exclusively in the GC and it can thus represent a more specific marker of GC derivation. Indeed, further analysis of DLBCL specimens reveals the presence of two subgroups of DLBCL, one with and another without ongoing somatic mutations, suggesting derivation of these lymphomas from two distinct normal B-cell counterparts: GC and post-GC lymphocytes.²

More recently, gene expression profiling analysis by microarray technology confirmed the presence of these two distinct DLBCL subtypes.^{3,4} In fact, some DLBCLs show the expression of genes characteristic of normal GC B-cells (GC signature genes) and are corresponding to the GC-derived DLBCLs, whereas others show a pattern of gene expression similar to that of *in vitro* activated peripheral blood B-cells (activated B-cell signature genes) and are corresponding to the post-GC DLBCLs. The acti-

vated B-cell (ABC) DLBCLs express also genes normally expressed by plasma cells, further confirming their post-GC origin. Finally, gene expression profiling suggests also the presence of a third group of DLBCLs (type 3) that represent a heterogeneous DLBCL subtype, not expressing a specific gene signature.

Genetic defects.

Several genetic abnormalities have been identified in subsets of DLBCLs. Recurring chromosomal translocations occur in approximately 50% of cases and the 3 most frequently deregulated genes, BCL6, BCL2, and cMYC, share a common mechanism of activation: chromosomal translocation brings the target gene under the control of an immunoglobulin gene promoter, leading to inappropriate expression.⁵

Chromosomal translocations involving the BCL6 gene on band 3q27 are the most common genetic abnormalities in DLBCL, occurring in 35% to 40% of cases. BCL6 is a zinc-finger transcription repressor normally expressed exclusively within the GC B-cells. BCL6 null animals lack the formation of GCs in response to antigenic stimulation. However, the down-regulation of BCL6 occurs in normal GC B cells in order to allow further differentiation into memory B cells or plasma cells. In DLBCL, dysregulated constitutive expression of BCL6 may lead to maturation arrest and confer a proliferative advantage. Recent studies identify a mechanism whereby BCL6 may regulate GC formation and lymphomagenesis via down-regulation of p53.⁶ Indeed, mutations and deletions of p53 are reported in up to 20% of DLBCL almost exclusively in DLBCL tumors without BCL6 translocations as constitutive expression of BCL6 due to chromosomal translocation already downmodulate p53, bypassing the need for p53 mutations and/or deletions.

An alternative mechanism by which BCL6 may contribute to lymphomagenesis is through the interaction with the transcriptional activator Miz-1 and, via Miz-1, with

CDKN1A, a cell cycle arrest gene, whose transcription is suppressed by BCL6.⁷ Through this mechanism, BCL6 may facilitate the proliferative expansion of germinal centers during the normal immune response and, when deregulated, the pathological expansion of B cell lymphomas. In addition to BCL6 translocations, small deletions and somatic point mutations occurring in the BCL6 regulatory region are reported in 70% of DLBCL. Some mutations can also significantly deregulate BCL6 expression.^{2,5}

BCL2 is a proto-oncogene that promotes B-cell survival via inhibition of apoptosis. Increased abundance of antiapoptotic BCL2 proteins limits the effects of death signals at the mitochondrial membrane, conferring a survival advantage and contributing to lymphomagenesis. BCL2 deregulation is most commonly associated with the t(14;18), present in approximately 15% of DLBCLs, but BCL2 protein overexpression can be detected in approximately 50% of DLBCLs, independent of the t(14;18).⁸ As the t(14;18) translocation is the hallmark of follicular lymphoma the reported frequencies of t(14;18) in DLBCL series reflect transformed follicular lymphomas that resemble *de novo* DLBCL.

Also cMYC, a transcription factor associated with Burkitt lymphoma, is deregulated in approximately 15% of DLBCLs and mutations of FAS(CD95), a proapoptotic protein expressed within GCs and initiating caspase-mediated apoptosis, have been reported in up to about 20% of DLBCLs, most commonly within the last exon, which encodes the death domain. Such mutations likely act in a dominant-negative manner, destabilizing trimeric FAS receptors.⁹

Transcriptional abnormalities in DLBCL

Gene expression-defined DLBCL subtypes also parallel different mechanisms of malignant transformation. The amplification of the c-rel locus on chromosome 2p have been detected exclusively in GC-like DLBCL. High expression of nuclear factor KB (NF- κ B) target genes has been observed in ABC like DLBCL but not in GC-like DLBCL cell-lines.¹⁰ PDE4B is a cyclic AMP (cAMP) phosphodiesterase also highly expressed in ABC-like DLBCL.¹¹ PDE4B inactivates cAMP, an intracellular second messenger that modulates several signaling pathways and induces cell cycle arrest and apoptosis of B cells.

BLIMP1 is a transcriptional repressor expressed in a subset of germinal center (GC) B cells and in all plasma cells, and required for terminal B cell differentiation. The BLIMP1 gene, that lies on chromosome 6q21-q22, a region frequently deleted in B cell lymphomas, is inactivated by structural alterations in approximately 25% ABC-DLBCL, but not in GC-like DLBCLs.¹² BLIMP1 alterations included gene truncations, nonsense mutations, frameshift deletions, and splice site mutations

that generate aberrant transcripts encoding truncated BLIMP1 proteins. Furthermore, most non-GC type DLBCL cases lack BLIMP1 protein expression, despite the presence of BLIMP1 mRNA.¹² As a sizable fraction of ABC-DLBCL carry an inactive BLIMP1 gene and the same gene is inactivated by epigenetic mechanisms in an additional number of cases, a role for BLIMP1 as a tumor suppressor gene, whose inactivation may contribute to lymphomagenesis by blocking post-GC differentiation of B cells toward plasma cells is easily predictable.¹²

Conclusions

Recent classical investigations as well as expression profiling studies greatly improved our understanding of the molecular pathogenesis of DLBCL. However, many components of this process still remain unknown, requiring additional efforts to better understand the pathogenesis of these disorders. These advances are important for the design of clinical trials and for the development of new therapeutic approaches.

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