Genetic lesions in diffuse large B-cell lymphomas

M. Testoni1, E. Zucca2, K. H. Young3 & F. Bertoni1,2*

1Lymphoma and Genomics Research Program, IOR Institute of Oncology Research, Bellinzona; 2Lymphoma Unit, IOSI Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; 3Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, USA

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Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in adults, accounting for 35%–40% of all cases. The combination of the anti-CD20 monoclonal antibody rituximab with anthracycline-based combination chemotherapy (R-CHOP, rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone) lead to complete remission in most and can cure more than half of patients with DLBCL. The diversity in clinical presentation, as well as the pathologic and biologic heterogeneity, suggests that DLBCL comprises several disease entities that might ultimately benefit from different therapeutic approaches. In this review, we summarize the current literature focusing on the genetic lesions identified in DLBCL.

Key words: MYD88, MYC, BCL6, NF-κB, BCL2

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma in adults, accounting for 35%–40% of all cases [1]. The combination of the anti-CD20 monoclonal antibody rituximab with anthracycline-based combination chemotherapy (R-CHOP, rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone) [2] lead to complete remission in most and can cure more than half of patients with DLBCL, representing one of the successes of modern cancer therapy. Still, a consistent portion of all DLBCL eventually relapse and this might be at least partially because DLBCL is a heterogeneous disease [2]. The diversity in clinical presentation, as well as the pathologic and biologic heterogeneity, suggests that DLBCL comprises several disease entities that might ultimately benefit from different therapeutic approaches. In this review, we summarize the current literature focusing on the genetic lesions identified in DLBCL.

Types of DLBCL

Several DLBCL variants are recognized in the 2008 World Health Organization classification of lymphomas (Table 1) [3]. DLBCL usually arises de novo but can also arise from an indolent lymphoma, such as follicular lymphoma [4–7], chronic lymphocytic leukemia/small lymphocytic lymphoma [8, 9], marginal zone lymphoma or nodular lymphocytic predominant Hodgkin lymphoma [10]. DLBCL can also occur in the setting of primary or acquired immunodeficiency, such as in recipients of solid organ transplantations or in individuals infected with human immunodeficiency virus (HIV) [11–13].

gene expression profiling

Gene expression profiling (GEP) studies have identified consistently different subsets of DLBCL.

The main contribution of GEP to the understanding of DLBCL pathogenesis is the identification of at least three main subtypes of DLBCL, all with clear underlying clinical, biologic and genetic peculiarities [14–19]: germinal center B-cell (GCB)-like DLBCL, in which genes that are expressed are hallmarks of normal GCBs; activated B-cell (ABC)-like DLBCL, which lacks expression of GCB-restricted genes and that is related to a BCR-activated B cells arrested during plasmacytic differentiation; and primary mediastinal large B-cell lymphoma (PMLBCL), in which several genes are expressed that are also characteristically expressed in Hodgkin Reed-Sternberg cells, and which is possibly derived from thymic B cells. PMLBCL is now considered a separate molecular entity of DLBCL in the World Health Organization classification [3, 20] (supplemental Material, available at Annals of Oncology online). Patients with ABC DLBCL have a worse outcome than patients with GCB DLBCL when treated with the conventional R-CHOP regimen [21]. Although ABC and GCB DLBCL have clear genetic and biologic differences (see below) [14–17, 22, 23], a limited number of antibodies cannot consistently identify individual differentially expressed genes in the two subtypes [23–32] (Table 2) and, as a result, these two subtypes are not yet recognized as distinct entities or consistently identified on diagnostic reports.
mediated immune response, the classic component pathway and host response consensus cluster is characterized by a rich host transcription factors [33]. The BCR consensus cluster is characterized by the expression of genes involved in B-cell receptor signaling and increased expression of cell cycle regulatory genes, DNA repair genes and B-cell-specific transcription factors [33]. The host response consensus cluster is characterized by a rich host inflammatory response, enriched by genes involved in T-cell-mediated immune response, the classic component pathway and inflammatory response [33]. The consensus cluster classification has no prognostic relevance but might have therapeutic implications [33, 34].

Both somatic mutations and gross genomic alterations, such as chromosomal translocations and copy number changes of specific chromosomal regions, contribute to the pathogenesis of DLBCL, which presents a high degree of genomic complexity (Figures 1 and 2). Whole-genome sequencing studies have identified an average somatic frequency of 4–5 mutations/Megabase (Mb), which is higher than what seen in chronic lymphocytic leukemia or acute leukemia (<1 mutations/Mb) but much less than in melanoma or lung cancers (>10 mutations/Mb) [36–38]. This huge number of mutations across the whole-genome results in a high number of nonsilent mutations (affecting the coding DNA sequences) ranging from 20 to 400 per lymphoma [38–40]. There is a nonrandom distribution of most genetic alterations across the various DLBCL subtypes, with some lesions occurring only in a specific subtype (Table 3).

### GCB subtype lesions

#### BCL2 chromosomal translocation

The antiapoptotic oncogene BCL2 (18q21) is very often deregulated in DLBCL. The chromosomal translocation t(14;18)(q32; q21) juxtaposes the BCL2 gene with the immunoglobulin heavy chain (IGHV) gene enhancer (14q32), resulting in deregulated expression of BCL2 [41–45]. The t(14;18) is the most common translocation in GCB DLBCL, detected in 30%–40% of cases [43], but it is not specific to DLBCL; it is also present in more than 90% of follicular lymphomas, and some GCB DLBCL might represent transformation from clinically silent follicular lymphoma. The translocation is virtually absent in cases of GCB DLBCL diagnosed in patients younger than 18 years [46], and it is less common in cases localised at the Waldeyer’s ring [47]. Although the BCL2 gene is only very rarely translocated in ABC

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**Table 1.** Diffuse large B-cell lymphoma (DLBCL) variants, subgroups and subtypes/entities

<table>
<thead>
<tr>
<th>DLBCL variants</th>
<th>Common morphologic variants</th>
<th>Molecular subgroups</th>
<th>CD5-positive DLBCL</th>
<th>GCB</th>
<th>Non-GCB</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell/histiocyte-rich large B-cell lymphoma</td>
<td>Centroblastic</td>
<td>Germinal center B-cell-like (GCB)</td>
<td></td>
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<tr>
<td>Primary DLBCL of the central nervous system</td>
<td>Immunoblastic</td>
<td>Activated B-cell-like (ABC)</td>
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<td></td>
</tr>
<tr>
<td>Primary cutaneous DLBCL, leg type</td>
<td>Anaplastic</td>
<td>Immunohistochemical subgroups</td>
<td></td>
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<tr>
<td>Epstein–Barr virus-positive DLBCL of the elderly</td>
<td>CD5-negative DLBCL</td>
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<tr>
<td>DLBCL subtypes</td>
<td>Primary mediastinal (thymic) large B-cell lymphoma</td>
<td>DLBLCL associated with chronic inflammation</td>
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<tr>
<td>Other lymphomas of large B cells</td>
<td>Large B-cell lymphoma</td>
<td>Germinal lymphoma</td>
<td></td>
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<tr>
<td>DLBCL associated with chronic inflammation</td>
<td>Immunoblastic</td>
<td>Plasmablastic lymphoma</td>
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<tr>
<td>Lymphomatoid granulomatosis</td>
<td>Anaplastic lymphoma kinase-positive DLBCL</td>
<td>Large B-cell lymphoma arising in human herpesvirus-8-associated multicentric Castleman disease</td>
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<tr>
<td>Plasmablastic lymphoma</td>
<td>Primary effusion lymphoma</td>
<td>Primary effusion lymphoma</td>
<td></td>
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<tr>
<td>Borderline cases</td>
<td>Borderline cases</td>
<td>B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma</td>
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<td></td>
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<tr>
<td></td>
<td>B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic Hodgkin lymphoma</td>
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</tbody>
</table>

A gene expression-based survival predictor model consisting of three gene expression signatures, ‘GCB’, ‘stromal-1’ and ‘stromal-2’, can divide DLBCL patients into prognostically different groups [21]. The GCB signature mirrors the distinction between GCB and ABC DLBCL, whereas the stromal-1 and stromal-2 signatures reflect the composition of the tumor microenvironment [21]. The prognostically favorable stromal-1 signature reflects extracellular matrix deposition and infiltration of the tumor with macrophages. Conversely, the prognostically unfavorable stromal-2 signature reflects a high density of blood vessels [21].

In another study, three DLBCL subgroups (consensus clusters) were identified that do not overlap with the ABC and GCB subtypes [33]. The OxPhos consensus cluster is characterized by the expression of genes involved in oxidative phosphorylation and mitochondrial metabolism [33]. The BCR consensus cluster is identified by genes involved in B-cell receptor signaling and increased expression of cell cycle regulatory genes, DNA repair genes and B-cell-specific transcription factors [33]. The host response consensus cluster is characterized by a rich host inflammatory response, enriched by genes involved in T-cell-mediated immune response, the classic component pathway and inflammatory response [33]. The consensus cluster classification has no prognostic relevance but might have therapeutic implications [33, 34].

Both somatic mutations and gross genomic alterations, such as chromosomal translocations and copy number changes of specific chromosomal regions, contribute to the pathogenesis of DLBCL, which presents a high degree of genomic complexity (Figures 1 and 2). Whole-genome sequencing studies have identified an average somatic frequency of 4–5 mutations/Megabase (Mb), which is higher than what seen in chronic lymphocytic leukemia or acute leukemia (<1 mutations/Mb) but much less than in melanoma or lung cancers (>10 mutations/Mb) [36–38]. This huge number of mutations across the whole-genome results in a high number of nonsilent mutations (affecting the coding DNA sequences) ranging from 20 to 400 per lymphoma [38–40]. There is a nonrandom distribution of most genetic alterations across the various DLBCL subtypes, with some lesions occurring only in a specific subtype (Table 3).

**Table 2.** Markers used in various researchers’ algorithms to predict the cell of origin in diffuse large B-cell lymphoma

<table>
<thead>
<tr>
<th>Researcher algorithm</th>
<th>Markers used</th>
<th>GCB cell-like DLBCL</th>
<th>Activated B-cell-like DLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hans et al. [24]</td>
<td>CD10 &gt; 30%</td>
<td>BCL6 &gt; 30%</td>
<td>MUM1 &gt; 30%</td>
</tr>
<tr>
<td>Muris et al. [30]</td>
<td>CD10 &gt; 30%</td>
<td>MUM1 &gt; 30%</td>
<td>BCL2 &gt; 50%</td>
</tr>
<tr>
<td>Nyman et al. [31]</td>
<td>CD10 &gt; 30%</td>
<td>MUM1 &gt; 30%</td>
<td>FOXP1 &gt; 80%</td>
</tr>
<tr>
<td>Natkunam et al. [29]</td>
<td>LMO2 &gt; 30%</td>
<td>MUM &gt; 80%</td>
<td>FOXP1 &gt; 80%</td>
</tr>
<tr>
<td>Choi et al. [26]</td>
<td>GCET1 &gt; 80%</td>
<td>CD10 &gt; 10%</td>
<td>BCL6 &gt; 30%</td>
</tr>
<tr>
<td>Meyer et al. [27]</td>
<td>GCET1 &gt; 80%</td>
<td>CD10 &gt; 10%</td>
<td>LMO2 &gt; 30%</td>
</tr>
<tr>
<td>Visco et al. [23]</td>
<td>CD10 &gt; 30%</td>
<td>BCL6 &gt; 30%</td>
<td>FOXP1 &gt; 60%</td>
</tr>
</tbody>
</table>
DLBCL, it is much more frequently gained or amplified in this DLBCL subtype (30%–40% of the cases) than in GCB DLBCL (15%) [19, 44, 48].

The BCL2 gene and its promoter region are also very commonly mutated in GCB DLBCL [40, 49–51]. Likely due to aberrant somatic hypermutation, mutations in BCL2 gene are observed almost exclusively in GCB DLBCL and are associated with the presence of t(14;18). Mutations targeting the promoter regions cause loss of MIZ1-mediated BCL6 suppression of BCL2 [49], mutations affecting the coding part of BCL2 gene have been hypothesized to alter the interaction of its protein with other molecules, such as TP53 protein [50].

The clinical relevance of t(14;18) and expression of the BCL2 protein have always been controversial [44, 45, 52–55]. Although the prognostic impact likely depends on the type of treatment received by the patient (e.g. R-CHOP versus CHOP), technical biases might also impede efforts to draw any strong conclusions [48, 56, 57]. Two different large collaborative groups have recently analyzed the prognostic impact of t(14;18) and BCL2 protein expression in patients treated with R-CHOP; only one of the studies found that t(14;18) was associated with poor outcomes in patients with GCB DLBCL [45]. However, both studies identified BCL2 protein as a marker of poor prognosis in GCB DLBCL but not in ABC DLBCL [44, 45]. In contrast with a previous report showing that BCL2 expression was a marker of poor prognosis in ABC-DLBCL only [58].

**EZH2**

The use of deep sequencing combined with DNA profiling has identified recurrent somatic mutations in genes encoding chromatin-modifying enzymes, including histone methyltransferases and histone acetyltransferases [39, 40, 51, 59, 60].

One of the most commonly mutated epigenetic modifiers is EZH2, whose gene presents gain-of-function mutations in 6%–14% of DLBCL [39, 51, 61]. It is detected altered almost exclusively in GCB DLBCL [39, 40, 59, 61] especially in GCB DLBCL bearing BCL2 translocations [62]. EZH2 encodes a histone methyltransferase, a member of polycomb complex PRC2 that is responsible for methylation of histone 3 on lysine 27 (H3K27), leading to transcriptional silencing [63]. EZH2 mutations tend to occur in association with the presence of t(14;18) (in ~20% of cases of GCB DLBCL), and they virtually never occur in ABC DLBCL [61, 62]. EZH2 gain-of-function mutations affect the catalytic suppressor of variegation 3–9, enhancer of zeste and trithorax (SET) domain of EZH2, in particular affect the tyrosine Y641 that corresponds to a key residue in the active site of the protein. The mutations lead to a change in the enzyme
affinity for its substrate: wild-type EZH2 has a high affinity for unmethylated H3K27 (H3K27m0), a lower affinity for dimethylated H3K27 (H3K27me2) and an even lower affinity for trimethylated histone H3 on lysine 27 (H3K27me3), whereas the mutant protein has the highest affinity for H3K27me2, leading to hypertrimethylation of H3K27 [62, 64–66]. Promising preclinical data have been obtained with the use of EZH2 inhibitors [66–69], and early clinical trials are ongoing.

Among chromatin modifiers also other genes are found altered by somatic mutations in DLBCL but, both due to a relatively low mutations rate and to differences in the studied series, it is difficult to assess the association with any specific subtype based on the cell of origin. These genes are: MLL2 (KMT2D) (22%–32% DLBCL), CREBBP (18%–20% DLBCL), EP300 (5%–10%) and MLL3 (KMT2C) (15%) [39, 40, 51, 59–61]. These genes will be discussed later.

other lesions
A locus on chromosome 2p (2p12-p16) is frequently amplified in GCB DLBCL and PMLBCL, and only rarely in ABC DLBCL, but its functional meaning is still unknown. There are no clear data linking the presence of the genomic lesion with expression levels or activity of the two putative target genes, REL, coding an NF-κB transcription factor subunit, and BCL11A, coding a zinc-finger protein that can interact with BCL6 [22, 70–72].

Amplifications in the MIRHG1 locus (13q31.3) have been identified in around 12% of GCB-DLBCL cases causing an overexpression of the mir-17–92 microRNA polycistronic cluster. This event is documented in different B-cell lymphomas and is correlated with MYC rearrangements or amplifications. MIRHG1 positively regulates MYC and vice-versa: indeed, cases with 13q31.3 amplification express MYC and MYC target genes at significant higher levels than cases without the abnormality [19, 73–79].

Approximately 10% of GCB-DLBCL cases carry deletion the chromosome 10q23 locus containing PTEN gene [19], and up to 55% of GCB DLBCL and 14% of ABC DLBCL present PTEN inactivation [80]. The latter appears inversely correlated with activation of the oncogenic (PI3K/AKT) pathway [80].

Gains on chromosome 12, possibly causing up-regulation of the MDM2 gene encoding a negative regulator of the tumor suppressor TP53, are much more common in GCB DLBCL than in ABC DLBCL. Similarly, gains affecting chromosome 7 (especially gains affecting its integrity) are more common in the GCB subtype. The pathogenetic effect of chromosome 7 gains is still unclear, but might involve overexpression of a series of miRNAs mapped on the chromosome, especially miR-96, miR-182, miR-589 and miR-25 [81]. The presence of 7q gains seems to predict a better outcome and a low probability of bone marrow involvement [81, 82].

ABC subtype lesions

B-cell differentiation

Deregulation of the normal process of GCB development represents one of the main mechanisms underlying the pathogenesis of the lymphoma.
of DLBCL. Genetic lesions directly affect this process mainly by deregulating BCL6, the master regulator of GCB differentiation, and BLIMP1, the master regulator of plasma cell differentiation. BCL6 controls different cellular functions, including DNA damage responses, cell cycle progression and signal transduction [17, 49, 83–90]. BCL6 (3q27) is frequently affected by chromosomal translocations, occurring more frequently in ABC DLBCL than in GCB DLBCL and leading to the deregulation of BCL6 expression by preventing BCL6 silencing at the conclusion of the germinal center response. BCL6 also works by repressing miRNAs such as miR-155 that negatively affect the expression levels of important germinal center genes (e.g. AID, SPI1, IRF8 and MYB) [90]. BCL6 expression has often been associated with improved outcomes, reflecting the GCB signature [86, 91–94]. BCL6 translocations do not seem to have any relevant prognostic significance [95], although in some studies the presence of a BCL6 translocation was associated with an inferior outcome, re-

**Table 3. Overview of genetic lesions in germinal center B-cell-like (GCB) and activated B-cell-like (ABC) subtypes of diffuse large B-cell lymphoma (DLBCL)**

<table>
<thead>
<tr>
<th>DLBCL subtype</th>
<th>Recurrent translocations</th>
<th>Most common genomic aberrations</th>
<th>Most common somatic mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCB</td>
<td>t(14;18)(q32;q21) IGHV–BCL2, 20%–45%; 8q24 rearrangements involving MYC, 20%; 3q27 rearrangement involving BCL6, 10%</td>
<td>+1q, +2p16 (REL), +7q, +12q (DM2M), +13q31 (MIRHG1), −1p36 (TNFRSF14), −10q23 (PTEN), −13q34 (ING1), −17p (TP53)</td>
<td>Chromatin remodeling (EZH2, MLL2, MEF2B, EP300, CRBPP1, TP53, BCL6 regulatory region and other aberrant somatic hypermutation targets</td>
</tr>
<tr>
<td>ABC</td>
<td>3q27 rearrangements involving BCL6, 25%; 8q24 rearrangements involving MYC, 5%</td>
<td>Trisomy 3 (FOXP1, NFkB1), +18q21 (BCL2, NFATC1), +19q13 (SPI1), −6q21 (PRDM1), −6q23 (TNFAIP3), −9p21 (CDKN2A), −17p (TP53)</td>
<td>Chromatin remodeling (MLL2, EP300, CREBBP), BCR signaling and NF-κB pathway (TNFAIP3, CARD11, CD79B, MYD88, TRAF2, TRAF3, MAP3K7, TNFRSF11A, ITKPB), PRDM1, BCL6 regulatory region and other aberrant somatic hypermutation targets, TP53</td>
</tr>
<tr>
<td>PMLBCL</td>
<td>3q27 rearrangements involving BCL6, 30%; 16p13 rearrangements disrupting CIITA and SOCS1, 40%–45%</td>
<td>SOCS1, TNFAIP3, STAT6, BCL6 regulatory region and other ASHM targets</td>
<td>+2p16 (REL), +9p21 (JAK2, JMD2C), −6q23 (TNFAIP3), −16p13 (SOCS1)</td>
</tr>
</tbody>
</table>

Deletions frequently found in DLBCL affect various portions of the long arm of chromosome 6, mainly 6q23 and 6q21 [39, 62, 89, 98–102]. The 6q21 region contains PRDM1 (PR domain containing 1, with ZNF domain), which encodes for BLIMP1 protein, a transcriptional repressor essential for the terminal differentiation of B cells into plasma cells. Structural BLIMP1 alterations frequently occur almost exclusively in ABC DLBCL, as do somatic mutations, both of which cause an impairment of PRDM1 transrepression activity. Importantly, BCL6 is one of the main direct repressors of BLIMP1, and BCL6 alterations represent an alternative mechanism of BLIMP1 deregulation. Notably, genomic events targeting BCL6 (translocations or mutations in the exon 1 binding site) are never found concomitantly with BLIMP1 mutations or deletions [39, 86, 99]. These observations suggest that translocations or mutations in the regulatory regions of BCL6 represent alternative pathogenetic mechanisms contributing to the development of ABC DLBCL by blocking post-GCB differentiation, and these events are likely to cooperate with constitutive NF-κB activation (see below) [89, 102].

The SPIB locus on chromosome 19 (19q13.3–q13.4) is the target of both chromosomal translocations and genomic gains in ABC DLBCL [19, 103]. SPIB is an Ets family transcription factor that is required for full B-cell receptor signaling and T-cell-dependent antibody responses [104]. SPIB is critical for germinal center reaction because SPIB-deficient mice show immunologic disorders that include smaller germinal centers, a shorter duration of response after immunization, and more apoptotic cells than immunologic disorders observed in animals with wild-type SPIB [104]. SPIB is also a direct target of BLIMP1 [105] and shows significantly higher expression in ABC-DLBCL clinical samples than in GCB-DLBCL clinical samples [22]. Accordingly, silencing of SPIB expression is detrimental for ABC-DLBCL cell lines but has little or no toxicity in GCB-DLBCL and PMLBCL cell lines [19]. Additional genetic lesions affect other important transcription factors involved in B-cell differentiation, including PAX5, IRF4, ETS1 [39, 106, 107].

**BCR and NF-κB signaling**

NF-κB constitutive activation is the most important alteration found in ABC DLBCL. Constitutive activation of the NF-κB pathway occurs via genetic lesions of a variety of genes, reflecting the different mechanisms that lead to NF-κB activation in normal GCBs. Although some genes are inactivated by somatic mutations and deletions such as TNFAIP3 (30% of cases), others, such as CARD11 (10% of cases), CD79B and CD79A (~20% of cases), TRAF2 (3% of cases), TRAF5 (5% of cases), RANK (8% of cases) and MYD88 (30% of cases) are activated mostly by somatic mutations [38, 39, 51, 60, 100, 108–110]. TNFAIP3 is one of the most commonly altered: deletions or losses occur in 20%–30% of all cases of DLBCL, although most occur in ABC DLBCL. TNFAIP3 codes for a negative regulator of NF-κB and is also commonly inactivated in other lymphomas [63, 93, 100, 101, 106]. TNFAIP3 might be negatively regulated...
also via high levels of the microRNAs miR-125a and miR-125b [111], which target PRDM1 as well [112]. CARD11 mutations induce NF-κB constitutively in the absence of antigen receptor signals (such as CD40-CD40L) that confer a gain-of-function phenotype, which is characterized by constitutive activity of IKK (IκB kinase complex) that is absent in GCB-DLBCL cell lines. CD79A and CD79B are components of BCR complex and are mutated in ~20% of ABC-DLBCL cases. These mutations affect critical tyrosine residues in the ITAM motif of CD79B and CD79A leading to an increment in surface BCR expression and attenuated the effect of the BCR inhibitor Lyn kinase [109]. The gene coding for MYD88, an adaptor protein that mediates toll and interleukin-1 receptor signaling [108], is mutated in up to 30% of cases of ABC DLBCL [110]. MYD88 mutations, commonly represented by a L256P mutation, promote not only NF-κB activation but also activation of the JAK-STAT3 pathway [113, 114].

A characteristic lesion of ABC DLBCL is a gain of chromosome 3, particularly of its long arm. The fact that these lesions are usually very large suggests that more than one gene might contribute to lymphomagenesis. BCL6, which is mapped at 3q27, and FOXP1, which is mapped at 3p14, are genes known to be deregulated in ABC DLBCL. Another candidate gene contributing to lymphomagenesis is NFKBIZ, which encodes an IκB-like protein and is involved in both the NF-κB pathway and STAT3 signaling, both of which are activated in DLBCL [115].

cell cycle

Another genetic lesion more common in ABC DLBCL than in GCB DLBCL is deletion of the INK4/ARF locus at 9p21 (24%–30% of cases) [19, 116, 117]. The INK4/ARF locus encodes three tumor suppressors (CDKN2B, ARF and CDKN2A) and is among the most frequently inactivated loci in human cancers. CDKN2A, together with CDKN2B, CDKN2C and CDKN2D, selectively inhibits the cyclin D-associated kinases, leading to a block in the G1-S phase. ARF is a protein that stabilizes TP53, preventing MDM2-mediated proteasome degradation [118]. Cases of ABC DLBCL with del(9p21) show a specific gene expression signature characterized by deregulation of the RB/E2F pathway [116, 117], activation of cellular metabolism and decreased immune and inflammatory responses [116]. INK4/ARF locus deletion is associated with a poor outcome in ABC DLBCL, defining a subgroup with a particularly unfavorable outcome [19, 116]. Moreover, the lesion contributes to the histologic transformation from indolent lymphoproliferations to DLBCL [6, 7, 9, 119]. del(9p21) is the most common lesion occurring at the time of histologic transformation from chronic lymphocytic leukemia to DLBCL (Richter syndrome), in which del(9p21) is associated with TP53 inactivation [9, 119]. Similarly, INK4/ARF locus inactivation takes place at the transformation from follicular lymphoma to DLBCL [7].

lesions occurring across subtypes

chromatin remodeling and transcription regulation

As already mentioned, mutations affecting genes coding for proteins involved in chromatin remodeling are recurrent in DLBCL (CREBBP, EP300, MLL2 and MLL3). None of these mutations is disease- or subtype-specific, being detected in other lymphomas as well, and occurring in both GCB and ABC DLBCL, although at different frequencies.

MLL2 codes for a histone trimethyltransferase, promoting the expression of its target genes modifying histone 3 on lysine 4 (H3K4me3) thanks to the SET domain. Inactivating mutations that occur in MLL2 generate a truncated protein lacking the SET domain and therefore unable to methylate chromatin [40, 59].

The acetyltransferase gene CREBBP is inactivated by somatic mutations or DNA losses in ~15% of cases of DLBCL, and the acetyltransferase gene EP300 is inactivated in about 5% of cases [40, 59]. CREBBP and EP300 belong to the KAT3 family of histone/protein lysine acetyltransferases [120, 121], regulating important cellular proteins. In particular, because acetylation of BCL6 by EP300 causes loss of protein transcriptional repressing activity and TP53 acetylation is required for TP53 activation, CREBBP and EP300 mutations contribute to BCL6 activation and TP53 inactivation in DLBCL [59].

In 10%–18% of DLBCL [39, 40, 51, 122], recurrent mutations occur in the gene coding the transcription factor MEF2B, recently shown activate BCL6 transcription in normal GC B cells [122]. Most mutations, mainly affecting the N-terminal domain of the protein with an impaired interaction with its co-repressor Cabin1 or affecting the C-terminal part rendering MEF2B insensitive to inhibitory phosphorylation and sumoylation mechanisms, deregulate BCL6 expression [122].

immune surveillance

Escape from immune surveillance is a necessary step for tumor development [123]. DLBCL cells are known to exhibit downregulation of both major histocompatibility complex (MHC) I and MHC II on the cell surface and both genetic and epigenetic mechanisms can contribute to this process [124–129]. B2M (β2-microglobulin) forms MHC I with the human leukocyte antigen (HLA) heavy chain. The B2M gene is genetically inactivated in one-third of cases of DLBCL, with similar frequency in the GCB and ABC subtypes [39, 51, 130], and in a much larger fraction of cases of DLBCL (75%), the protein is not expressed or has an aberrant pattern of expression [130].

Genetic deletions of the genes coding for MHC II are common in cases of DLBCL arising in immune-privileged anatomical sites such as the testis and central nervous system, but less common among other cases of DLBCL [125, 127, 128]. Downregulation of the MHC class II transactivator CIITA, the loci coding for HLA I and HLA II, an upstream event, seems to be an important mechanism contributing to MHC II silencing [127, 128, 131]. Genetic disruption of the CIITA gene by chromosomal translocations is common in PMLBCL (38% of cases exhibit translocations inactivating the gene) but rare in DLBCL (~5%) [131]. DNA losses are also not common in DLBCL [39, 132]. Although mutations have been reported, their biologic consequences have not yet been demonstrated [39].

Although no CIITA promoter methylation was reported in one study [133], epigenetic mechanisms might play a relevant role in DLBCL, and pharmacologic induction of MHC II re-expression is also possible. Despite the lack of frequent losses of CIITA in DLBCL, both mapped at 6p, are targets of loss of heterozygosity [134], namely of copy neutral loss of heterozygosity [135]. Copy
neutral loss of heterozygosity is much less common in DLBCL arising in individuals with an acquired immunodeficiency, such as in those with HIV infection or in recipients of solid organ transplantation [136, 137], a setting in which T-cell-mediated immune surveillance is already reduced.

Various other genetic events have been reported as possibly contributing to immune escape. CD58 is the receptor of the CD2 molecule expressed by T cells and natural killer cells and expression of CD58 is necessary for T-cell- and natural killer cell-mediated cytotoxicity [130]. The CD58 gene is genetically inactivated in 21% of cases of DLBCL, more frequently in ABC DLBCL (68%) than in GCB DLBCL (32%), but CD58 protein expression is deregulated in 67% of all cases of DLBCL, with no differences between ABC and GCB subtypes (68% versus 65%) [39, 130].

The above-mentioned rearrangements inactivating the CIITA gene usually also cause overexpression of the ligands of receptor molecule programmed cell death 1 (CD274/PDL1 and CD273/PDL2) [131]. Similar rearrangements or DNA amplifications also target the 2 genes in ~20% of cases of DLBCL [39], suggesting that disrupting the PD-1/PD-L1 pathway could be a potential therapeutic strategy [138].

The TNFSF9 gene has been reported in 12% of cases of DLBCL and might also affect the interaction of lymphoma cells with T-cell-mediated immune surveillance [39]. TNFRSF14, also involved in CD8+ T-cells, has been reported to be inactivated in 20% of cases of DLBCL [51]. TNFRSF14 inactivation is also observed in ~20% of cases of follicular lymphoma, in which TNFRSF14 inactivation is associated with poor outcomes and might be involved in the histologic transformation to DLBCL [139].

FAS (TNFRSF6/CDF95) can lead to cell death via the Fas-associated death domain (FADD) protein signaling complex. The FAS gene is inactivated by mutations or deletions (including homozygous losses) in 5%–20% of cases of DLBCL [40, 140, 141], but the frequency seems to be higher in cases arising in extranodal sites or associated with autoimmune disorders [141, 142] and in cases belonging to the ‘oxidative phosphorylation’ cluster and bearing BCL2 chromosomal rearrangements [143]. Similarly, genes coding for TRAIL receptors, TNFRSF10A (TRAIL-R1) and TNFRSF10B (TRAIL-R2), are also targets of DNA losses in DLBCL [135, 144].

As in copy neutral loss of heterozygosity at 6p, possible hints that specific lesions play a role in immune escape might come from the comparison of DLBCL in immunocompetent patients with DLBCL in immunocompromised patients. Deletion at 13q14.3, mainly affecting miR-15A and miR-16, but often also encompassing the tumor suppressor gene RAI, is almost never detected in DLBCL in immunocompromised patients [136, 137], indicating that del(13q14.3) might contribute to avoidance of immune system control in lymphoma cells, although the exact mechanism is still unknown and possibly involves FAS [140]. The very low frequency of otherwise commonly observed genomic lesions such as 3q gain in DLBCL patients who have undergone organ transplantation [137] or 18q gain in DLBCL patients with HIV infection [136] also indicates that genes mapped in these regions might provide specific advantages to lymphoma cells that allow the cells to avoid different types of immune surveillance, because the immunodeficiency observed in the HIV or posttransplantation settings is different than immunodeficiency observed in other settings.

Importantly, inactivation of molecules involved in immune surveillance, such as FAS or the TRAIL receptors, protects lymphoma cells not only from immune system cells, but also from the apoptotic response following chemotherapy, and this might explain why some of these genomic defects have been associated with poor treatment outcomes [135, 145].

**aberrant somatic hypermutations**

Aberrant somatic hypermutations are the result of aberrant activity of the physiologic somatic hypermutation process, which is necessary for the creation of antibodies with a high affinity for specific antigens [86, 146, 147]. More than 50% of cases of DLBCL include mutations, affecting a region of DNA up to 2 kb from the start of the transcription site, which can disrupt the regulation of genes or modify proteins. Although aberrant somatic hypermutations are detected in both GCB and ABC DLBCL, the pattern of affected genes might differ between the two subtypes (e.g. BCL2 mutations and mutations affecting the 5′ untranslated exon of BCL6 are more common in GCB DLBCL [86, 146]).

**FOXO1 mutations**

Somatic mutations in the gene coding for the transcription factor FOXO1, involved in the PI3K signaling downstream to BCR activation GC development, have been reported in 8.6% of cases of DLBCL, associated with an inferior outcome but with no differences between GCB and ABC subtypes, and their functional significance are still to defined [148, 149].

**MYC deregulation and double-hit/triple-hit lymphomas**

Chromosomal translocations deregulating MYC (8q24) occur in 5%–15% of cases of DLBCL [55], more commonly in GCB DLBCL. The t(8;14)(q24;q32), which deregulates MYC by juxtaposing it with the IGHV enhancer [150], is a characteristic cytogenetic event of Burkitt lymphoma, but the MYC gene can also be translocated to other genomic loci. An immunohistochemistry protocol has been reported to have the ability to predict cases of DLBCL bearing a MYC gene translocation [151]; however, although immunohistochemistry scores have been presented [93, 152, 153], they still need validation in larger studies. The translocation, but not necessarily the presence of an extra copy of the gene, is associated with a very poor outcome [23, 154–168], and a subtype of DLBCL is characterized by the presence of multiple chromosomal translocations, mostly affecting MYC, BCL2, BCL6 or CCND1 (Cyclin D1). These so-called double-hit and triple-hit lymphomas occur in a low percentage of cases of DLBCL (up to 5%) but have a very poor prognosis, whether treated with standard or high-dose chemotherapy, despite usually having a GCB phenotype [154–159, 162–166, 169, 170]. These lymphomas usually have DLBCL morphology at the histologic examination and only the genetic analyses (FISH) are able to separate these entities. A series of 311 double-hit and triple-hit lymphomas has been recently described [171]. The histology was DLBCL in 50% and 48% had a diagnosis of B-cell lymphoma unclassifiable with features intermediate between DLBCL and Burkitt lymphoma [171]. MYC translocations were present with rearrangements of BCL2 in 87% of the cases, of
BCL6 in 5%, of both BCL6 and BCL2 in 7%, and 93% of cases expressed the GCB marker CD10 [171]. While the majority of patients faced early treatment failure and death, a 2-year OS of 91% was estimated for a small group of patients (7%), with normal LDH, stage I–II, nor leukocytosis nor central nervous system involvement [171]. This large retrospective series also suggested a possible advantage for high-dose chemotherapy followed by stem-cell transplantation in comparison with standard R-CHOP regimens [171].

Analogous to the algorithms defining GCB and ABC DLBCL, the concept of the double-hit lymphomas has been extended to include the pattern of protein expression [153, 172, 173], but there is only a partial overlap between the genetic and the immunophenotype results. In a series of 164 cases, 28 lymphoma specimens (17%) expressed both MYC and BCL2 proteins, and this was associated with inferior overall survival outcomes [153]. In a different series of 466 cases, 34% of the specimens expressed both MYC and BCL2 proteins, and these patients had a poorer outcome than the remaining patients [172]. In a series of 106 cases, 44% were positive for both BCL2 and MYC protein expression, which was associated with inferior outcome [173]. The co-expression of MYC and BCL2 is more common in the ABC subtype than the GCB subtype, although it is an independent prognostic marker predictive of inferior outcome in both GCB and ABC DLBCL [153, 172]. Importantly, co-expression of the MYC and BCL2 proteins, independent from the presence of chromosomal translocations, is much more common than the concomitant presence of the translocations deregulating the MYC and BCL2 genes.

**conclusion**

As our review of the literature demonstrates, DLBCL is a very heterogeneous group of disorders. Their characterization is improving in terms of the underlying genetic lesions and, more importantly, the relevant pathways that can be pharmacologically targeted, as summarized in Figure 3. However, further work is needed to translate the recent discoveries to the clinical setting, and to design trials and therapeutic plans based on the underlying tumor genetics. Current guidelines [174, 175] recognize the prognostic relevance of the DLBCL cell of origin (GCB versus non-GCB) and of the presence of MYC rearrangements, but they still recommend R-CHOP, or comparable anthracycline-based regimens, for all DLBCL patients, irrespectively of the cell of origin or MYC status. Although there are over 30 phase II trials with targeted compounds in DLBCL registered at ClinicalTrials.gov (November 2014), there are only two phase III trials (NCT01855750, NCT02285062) specifically dedicated to non-GCB DLBCL and one (NCT01324596) addressing whether different treatment may have a different impact on the outcome of the GCB and non-GCB GEP-defined subtypes.

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