Chapter

# B Cell Lymphomagenesis

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## Abstract

Lymphoid neoplasms are a heterogeneous group of malignancies whose diagnosis depends on a very good analysis of hematopathology and morphology, immunophenotype, cytogenetic, molecular, and clinical characteristics. B-cell lymphomas begin from different developmental stages of B cells in germinal centers of secondary lymphoid tissue. The evolution of B-cell lymphomagenesis depends on different numbers of signal pathways. Proteins that play key point of signaling networks are changed by aberrant chromosomal expression, translocation, and/or accumulation, and those events determine the fate of the affected B cells. Many chemokines and cytokines have been implicated in providing the line for the cellular surviving and interaction in lymphoid organogenesis. Specific chromosomal alterations were associated with significant changes in gene-expression signatures that reflect various aspects of lymphoma cell biology as well as the host response to the lymphoma. The goal of this study was to find out a correlation between tumor markers and survival in patients with subgroups of DLBCL. The goal is to find out chronic autoimmune or pathogen-induced immune reactions resulting in lymphoid neogenesis. So we address (i) chemokines and adhesion molecules involved in lymphoid neogenesis, (ii) the autoimmune diseases and pathogens which are associated with the development of B-cell lymphomas, and (iii) the molecular mechanisms involved in the initiation and progression of DLBCL.

**Keywords:** DLBCL, lymphomagenesis, GCB, ABC

## 1. Germinal center B-cell dynamics

Characteristic chromosome translocation is associated with certain entities of the disease that is known to play a key role in the development of lymphoma. Chromosomal translocation alone, however, is not sufficient for the appearance of a tumor. Various factors, including microenvironment, epigenetic, and genetic changes, play a role in the development of lymphomas. Follicular dendritic cells (FDC) produce high-affinity antibody needed for the development of B-cell memory. These cells can be detected in B-cell non-Hodgkin lymphomas (NHL). Gene-expression profiling revealed that the FDC network could affect the clinical outcome in DLBCL [1–3].

Transgenic mouse model with different genes linked to chromosomal translocations has shown that chromosomal translocation alone is not sufficient for the development of lymphoma [1]. Transgenic mice with the c-*MYC* (myelomatosis viral oncogene homolog) genome develop lymphoma after a long time. The *c-MYC* gene is located at 8q24 and is associated with the translocation t (8; 14) (q24; q32). This was the first recurring chromosome abnormality ever reported in lymphoproliferative disorders. *C-MYC* in place next to immunoglobulin heavy locus (IGH) results in the expression of c-Myc protein, which is a key transcriptional factor that promotes cell cycle and

tumor proliferation. 8q24/*c-MYC* restenosis is found in 7–15% of the activated B-cell-like (ABC) subgroup of diffuse large B-cell lymphoma (DLBCL), and the reports are predominantly associated with extranodal localization [4–8]. B-cell lymphomas include follicular lymphoma (FL), mantle cell lymphoma (MCL), and marginal zone lymphoma (MZL). Tumor cells show a strict topographical and functional relationship with FDCs, included with reactive lymphoid and stromal cells [3, 9]. The FDC patterns, described for FL and MCL, are reminiscent of the distribution pattern of FCD meshwork seen in the GC or the mantle zone of the secondary lymphoid follicle, respectively [3, 9]. T cells migrate to B-cell-enriched follicles and germinal centers within secondary lymphoid organs to provide help to B cells. Correlation T and B cells within germinal centers are essential for B-cell differentiation into germinal center B cells and selection of mutated cells into memory B cells or memory plasma cells. Different stages of maturation lead to B cell becoming a helper T cells and B follicular helper T ($T_{FH}$) cells [10].

*C-MYC* translocation is characteristic of Burkitt's lymphoma, where it is found in 80–90% of cases. In B-cell lymphoma 2 (*BCL2*) transgenic mice, it has been shown that polyclonal B-cell proliferation causes the growth of lymphoid follicles, but only 20% of mice develop monoclonal diffuse lymphomas after long latency. Long-term exposure to oncogene, progression from polyclonal to monoclonal disease, and appearance of the *BCL2* transgenic mouse model suggests that lymphoma development depends on the secondary genetic events [8].

*C-MYC* is a strong transcription factor and promotes cell proliferation and growth, DNA replication, and protein biosynthesis [11]. Dysregulation of c-MYC is essential in the pathogenesis of a number of B-cell lymphomas but is rarely reported in T-cell lymphomas. *C-MYC* rearrangement is found in multiple B-cell lymphomas including Burkitt lymphoma, diffuse large B-cell lymphoma (DLBCL), and B-cell lymphoma unclassifiable, with features intermedia between DLBCL and Burkitt lymphoma (BCLU). In normal cells *c-MYC* is a highly regulated transcription factor that is involved in cell cycle regulation, cell metabolism, mitochondrial biogenesis, nucleic acid synthesis, and apoptosis [12]. *C-MYC* expression is detected in a much higher proportion of DLBCL (30–50%) and is associated with concomitant expression of *BCL2* in 20 to 35% of cases [8]. Most of these tumors do not carry *c-MYC/BCL2* chromosomal alterations and have been named "double-hit lymphoma." Most studies use a cutoff of 40% *c-MYC*-expressing cells to define these cases; the cutoff for *BCL2* expression has varied considerably in the literature, but a figure of 50% is recommended [8, 13–16].

Germinal centers (GCs) form in the center of the B-cell follicles of secondary lymphoid organs, interspersed within a network of stromal cells known as follicular dendritic cells (FDCs) [17, 18]. In follicles that do not contain GCs (primary follicles), FDCs play an important role, helping B cells to cluster into compact, well-defined follicle [18–20]. FDC has a long-term retention of antigen within complement immune complexes in a form that can support affinity-dependent "testing" of immunoglobulin somatic hypermutation (SHM)-modified B-cell Ag receptors (BCRs) that occurs during GC selection [21, 22]. Adaptive immunity involves the development of antibodies in GCs through SHM in GC dark zones (DZs) and clonal selection in light zones (LZs) [22, 23].

The spleen, lymph nodes, and mucosal-associated lymphoid tissues constitute secondary lymphoid tissues that are located strategically to efficiently trap foreign antigens entering peripheral tissues and mucosal sites. In secondary lymphoid tissues, activation of B cells and T cells by native and processed antigenic determinants represents the beginning of the GC reaction [24]. The B cells bind membrane-bound immunoglobulins (B-cell receptors (BCR)) and express Ag-derived peptides in major histocompatibility complex (MHC-II) molecules on their surface [6]. Secondary lymphoid tissues facilitate interactions between antigen-bearing dendritic cells (DCs), B cells, and T cells to initiate adaptive immune responses. B cells express

B-cell receptors (BCRs) on their cell membrane, and BCRs allow the cell to bind to a specific antigen and initiate an antibody response [18, 23, 25]. Despite these common features, differences in routes of antigen transport, lymphocyte trafficking, and unique cell populations determine the role of a specific secondary lymphoid tissue in immune responses to various foreign antigens. In the GCs, naive B cells can have clonal expansion, somatic hypermutation, and maturation [26, 27].

Germinal centers (GCs) are organized into two major zones: dark and light zones. The dark zone contains large centroblasts that are rapidly proliferating and undergoing somatic mutation. These cells grow to small nonproliferating centrocytes in the light zone, and binding antigen on follicular dendritic cells (FDCs) and development further depend on receiving signals from helper T cells [18, 22, 23, 28].

The activated B cells have two possibilities: (1) turnover into extrafollicular areas followed by proliferation and terminal differentiation into short-lived plasma cells that secrete antibody and (2) turnover into B-cell follicles followed by proliferation of GCs [27]. The mechanisms responsible for this remain poorly understood, although various studies suggest that the affinity of the BCR for the foreign antigen, the amount of antigen-receptor engagement, and the costimulatory signals received from T cells may all be involved. These observations suggest that the affinity maturation of the antibody response occurs in GCs, through the processes of clonal proliferation, somatic hypermutation, and selection [27].

T cells play a key role to induce B-cell proliferation and somatic hypermutation and to limit GC reactions. T cells in the germinal center activate helper T cells (CD4+CD57+CD25−) migrating in the germinal center to activate the B-cell zone-homing C-X-C chemokine receptor type 5 (CXCR5) [29, 30]. B cells primarily excrete interleukin 4 (IL-4). They begin in the paracortical T-cell area of IgD follicle expression, but T-cell maturation results in a characteristic follicular CD10+ immunophenotype that has been acquired. Although the germinal center formation is intact in the presence of the FDC network, FDC plays a key role in the formation of memory B cell, and interaction is dependent on the expression of the tumor necrosis factor (TNF) family. The germinal center is not only created from different stages of B-cell maturation but also through the division of immunophenotypically different and functionally specialized T cells, dendritic cells, and subpopulations of stromal cells and their interactions. The antigen-stimulated B cells with the germinal center are highly mobile within the zone as well as between the dark and light zone [18, 23, 31].

In general, naive B cells in the central lymphatic system mature through the transformation and proliferation of B cells toward plasma cells and B cells that produce antibodies. Malignant diseases may develop during such processes, and B-cellular neoplasms tend to imitate different stages of normal B-cell differentiation [1].

In the primary phase of differentiation, the progenitor of the B cell passes through renaming, and further development is followed by immunoglobulin genes (Ig) and occurs at naive B cells in peripheral lymphoid tissue or passes through apoptosis. Naive B cells can enter the primary follicle, forming a germinal center (GC), where mature CD10- and B-cell lymphoma 6 (*BCL6)* centroblasts expression mature [2]. Centroblasts continue maturing in centrocytes, which later co-express *BCL2 gene*. Naive B cells can be directly developed in plasma cells. The germinal center is partially monitored by Bcl6 protein, which in humans is encoded by the *BCL6* [32] and IRF4/MUM1 proteins, which is essential for further lymphocyte differentiation into plasma cells or memory B cell. On the other hand, antigen stimulation of lymphocyte can go toward apoptosis [2]. MUM1 is a lymphocyte-specific transcriptional factor of the interferon regulatory factor (IRF) family, included in the regulation of gene expression in response to interferon and other cytokines. MUM1 provides immunoglobulin gene expression in the final step of B-cell differentiation within germinal center light zones. At this stage Bcl-6 is downregulated [2].

Secondary follicles are important for B-cell differentiation and maturation. B-cell differentiation begins via gene resection for the DJ region of the IgH gene. Each immunoglobulin molecule consists of two identical heavy chains and two identical light chains. This region represents the organization of heavy-chain locus. The locus includes variable (V), diversity (D), joining (J), and constant (C) segments. Within B-cell development, DNA recombination level joins a single D segment with a J segment; the fused DJ segment of a DNA molecule containing information coding for a protein or peptide sequence of this partially rearranged DJ region is then joined to the V segment. The introduction of region V into DJ results in VDJ coupling, and then the conversion successfully generates IGH protein chain. After the successful generation of the IgH gene, Ig light chains (IgL) are formed. The removal of IgL starts with light chain kappa. When the IgH and IgL genes are productive, B cells are called naive B cells because they are not exposed to the appropriate antigens and reside in the marginal zone of the secondary follicle [4]. The cells that have not been exposed to antigens are known as naïve or virgin B cells and remain at the mantle zone until they are exposed to antigens [22]. According to the B-cell differentiation stage, B-cell precursor includes B lymphoblastic leukemia/lymphoma, while pre-GC neoplasms include cell lymphoma. GC B-cell tumors are follicular lymphoma, Burkitt's lymphoma, Hodgkin's lymphoma, and diffuse large B-cell lymphoma (DLBCL). In the last stage of B-cell differentiation, post-GC neoplasms, including marginal zone lymphoma, mucosa-associated lymphoma tissue (MALT), lymphoplasmic lymphoma, chronic lymphocytic leukemia (CLL), plasma cell myeloma, and some cases of DLBCL, are developed [1, 22] (**Figure 1**).

The majority of B-cell lymphomas appear from germinal center (GC) B cells, but it is unclear to what extent B cells in lymphoma keep the functional dynamics of GC B cells or are blocked at a special stage of the GC reaction [20].

The germinal center is probably the source of many lymphomas. The germinal center reaction begins when antigens, with T-cell signals, activate centroblasts that migrate from the dark zone to the light zone and begin to express their antibodies

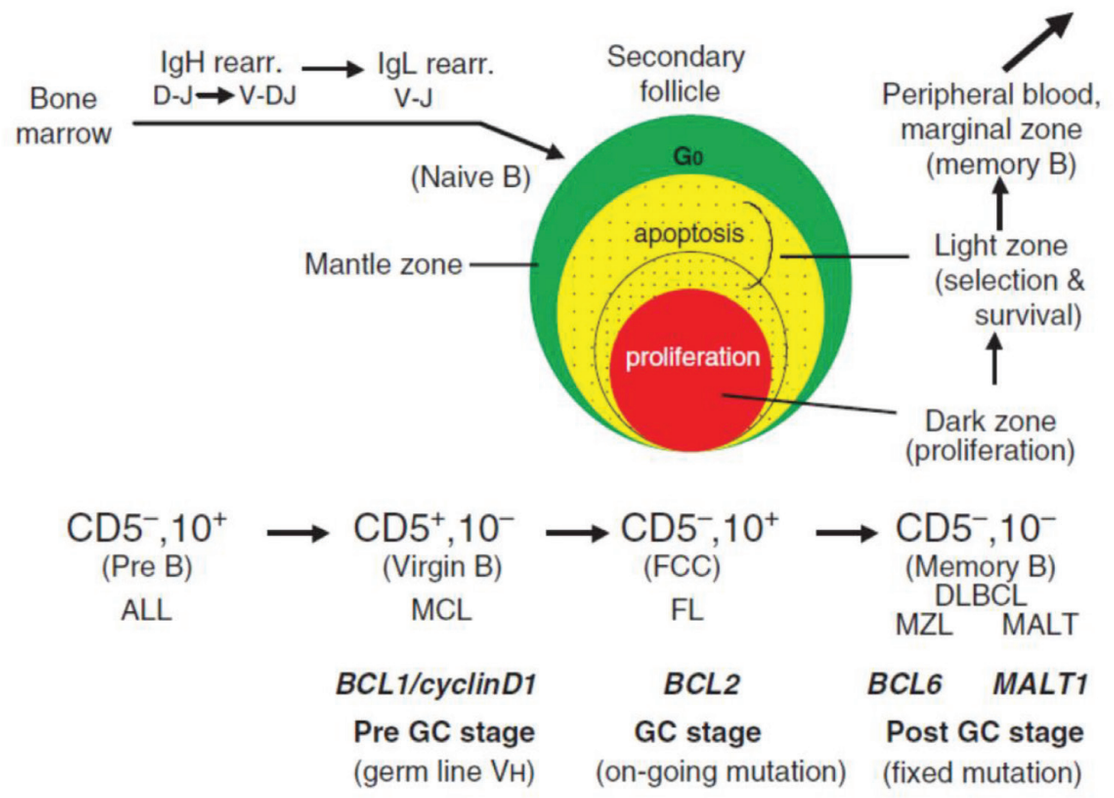


**Figure 1.**
*B-cell differentiation and association of B-cell neoplasms [1].*

on the cell surface known as centrocytes. Then apoptosis begins and competes for survival signals derived from FDCs and $T_{FH}$ cells. Germinal center selection is dependent on the tendency of their surface antibody to the antigen and can enter into the light zone of germinal centers, which are morphologically centrocytes and which are indivisible B cells with a notched core. The action or process of reentering into the dark zone as centroblasts gives a chance for nonselected B- cell mutants to gain more mutations in order to improve affinity toward antigen. Interactions with T cells are to prevent the generation of autoreactive germinal center B cells [18, 23, 33].

During the germinal center reaction, two different modifications of the B-cell DNA change B-cell receptor: somatic hypermutation and switch-off recombination. Both require activation-induced cytidine deaminase (AID). IgM, IgA, or IgE recurrence of IgM, IgG heavy-chain recombination changes occur, while somatic hypermutations imply mutations in the immunoglobulin-variable region, region that produces a population of B cells with an increase (or decrease) affinity for a particular antigen. These genetic modifications are essential for normal immune response but also a source of DNA damage that can become pathological with lymphoma [8].

Mature B-cell neoplasms involve about 30 different entities, while DLBCL is the most common type of non-Hodgkin's lymphoma (NHL), which accounts for about 40% of all non-Hodgkin's lymphomas (NHLs). B-cell lymphomas are identified by a combination of morphological features and recognizable immunophenotype [20]. A list of transcription factors that modulate the B-cell germinal center phenotype and where cell selectively express BCL6, and includes interferon-regulatory factor 4 (IRF4). IRF4 co-bound with the transcription factors PU.1 (plays a critical role in the development of white cells), and B-cell-activating transcription factor (BATF) to Ets or activator protein 1 (AP-1) composite motifs, united with genes involved in B cell activation and the GC response [18, 20, 23].

CD5 is a transmembrane glycoprotein with expression on the surface of T cells and a subset of B cells. The absence of CD5 performs thymocytes hyperresponsive to stimulation through the T-cell antigen receptor (TCR) in vitro. CD5 can influence the developing thymocytes by acting as a negative regulator of TCR-mediated signal transduction [34]. The CD5 signature included downregulated extracellular matrix genes such as *POSTN*, *SPARC*, *COL1A1*, *COL3A1*, *CTSK*, *MMP9*, and *LAMB3* and comprised upregulated genes including *TRPM4* [35–38] (**Figure 2**).

Bcl6 protein suppresses many genes involved in plasma cell differentiation, cell cycle progression, and responses to DNA damage and cell death. One of these genes is the transcription factor Blimp-1, the main regulator of plasma cell differentiation that reduces gene expression of mature B cells. The transcription factors Blimp-1 and IRF4 are essential for plasma cell generation. However, their function in mature plasma cells has remained elusive. B cells germinate center begins to differentiate plasma cells, monitored by interferon regulatory factor 4 (IRF4)/MUM1, whose transcription factor is needed for conversion to plasma cells. IRF4 increases the expression of Blimp-1 [39], and this growth is suppressed by *BCL6*, so differentiation takes place in favor of plasma cells [25, 39].

The transcriptional repressor Blimp-1 originally cloned as a silencer of type I interferon (IFN)-β gene expression controls cell fate decisions in multiple tissue contexts. Blimp-1 functional loss in 3D cultures of mammary epithelial cells (MECs) results in the accumulation of dsRNA and expression of type III IFN-λ [39] (**Figure 3**).

Chromosomal translocation in B-cell neoplasms is divided into three functional categories: proliferation, inhibition of differentiation, and antiapoptotic activity. These three categories are essential for understanding the mechanism of lymphomagenesis. For DLBCL with *BCL6* gene translocation, Bcl6 protein expression is a side for post-GC staging cells, but it is not yet clear what signal is needed. It is clear that the antiapoptotic function must be present for *BCL2*, which is expressed at the post-GC

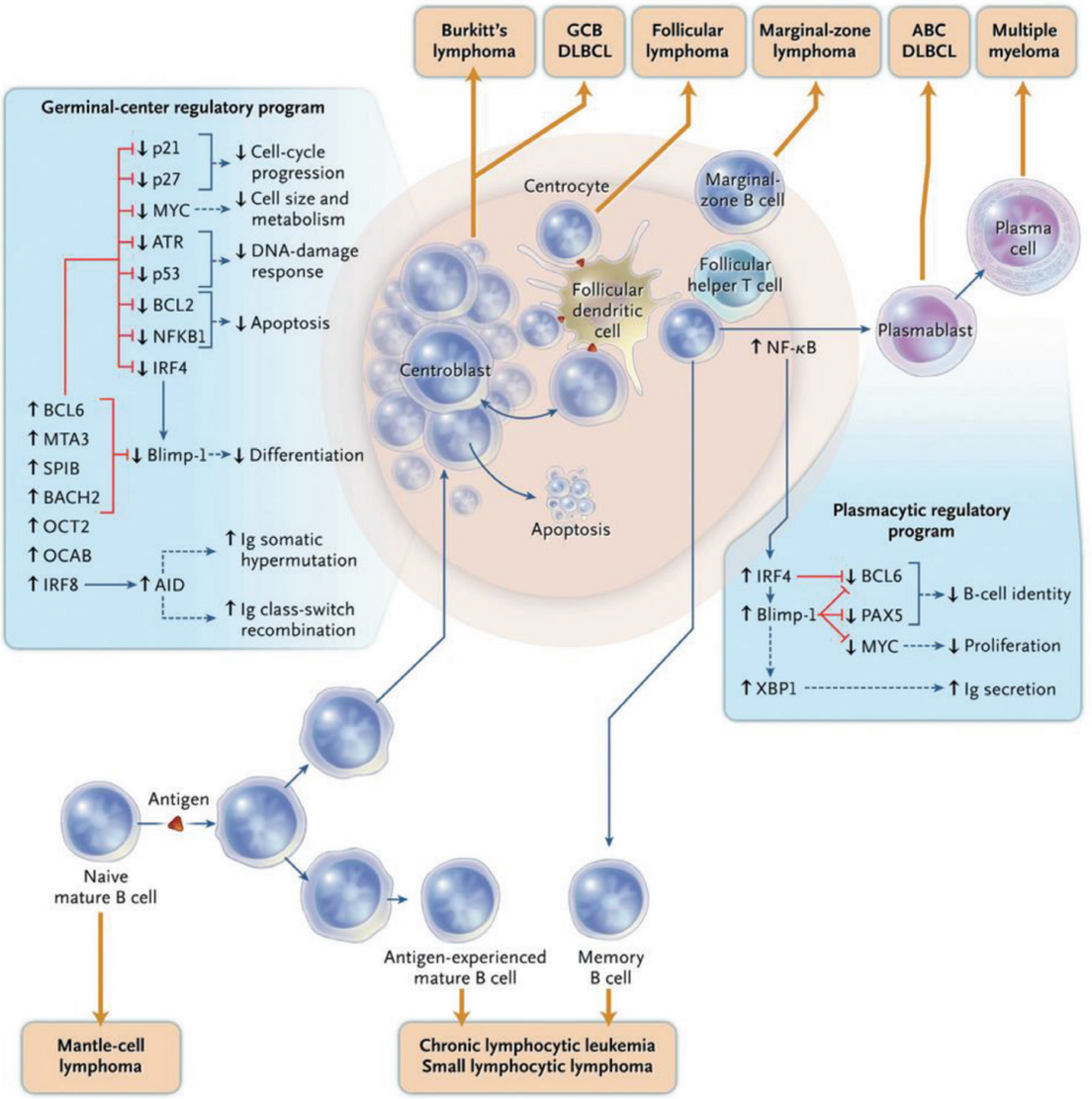


**Figure 2.**
*B-cellular differentiation and lymphomagenesis ([25], p. 1418).*

centers. There are indications that *BCL6* has the function of protecting cells from terminal differentiation and shows epigenetic changes that are conserved from human hematopoietic stem/progenitor cells (HSPCs) to mature B cells. These results suggest that *BCL6* may function in a "hit-and-run" role in lymphomagenesis [8].

Lymphomagenesis is a complex process that takes place in many steps, in which lymphocyte signal distortion, transcriptional regulation, and apoptosis lead to transformation. Experiments suggest that distorted expression of casein kinase II subunit alpha (CK2alpha) creates predispositions for lymphoma development. Further experiments suggest that CK2alpha lymphomas monitor the panel of lymphocyte transcription factors such as, for example, activator protein 1 (AP-1) and nuclear factor-kappaB (NF-κB) [40, 41]. NF-κB represents a group of related homodimeric and heterodimeric transcription factors that can activate different target genes involved in proliferation, differentiation, immune response, cell growth, and survival. Among the molecules induced by NF-κB are the cytokines, chemokines, and effectors of the immune response. NF-κB participates in response to inflammatory signals through T receptors, TNF receptors, and IL-1 receptors [40–42]. CREB is one of the best understood phosphorylation-dependent transcription factors (CREB) [16]. In effector T and B cells, immune receptor signals induce within minutes a rise of intracellular Ca++, the activation of the phosphatase calcineurin, and the

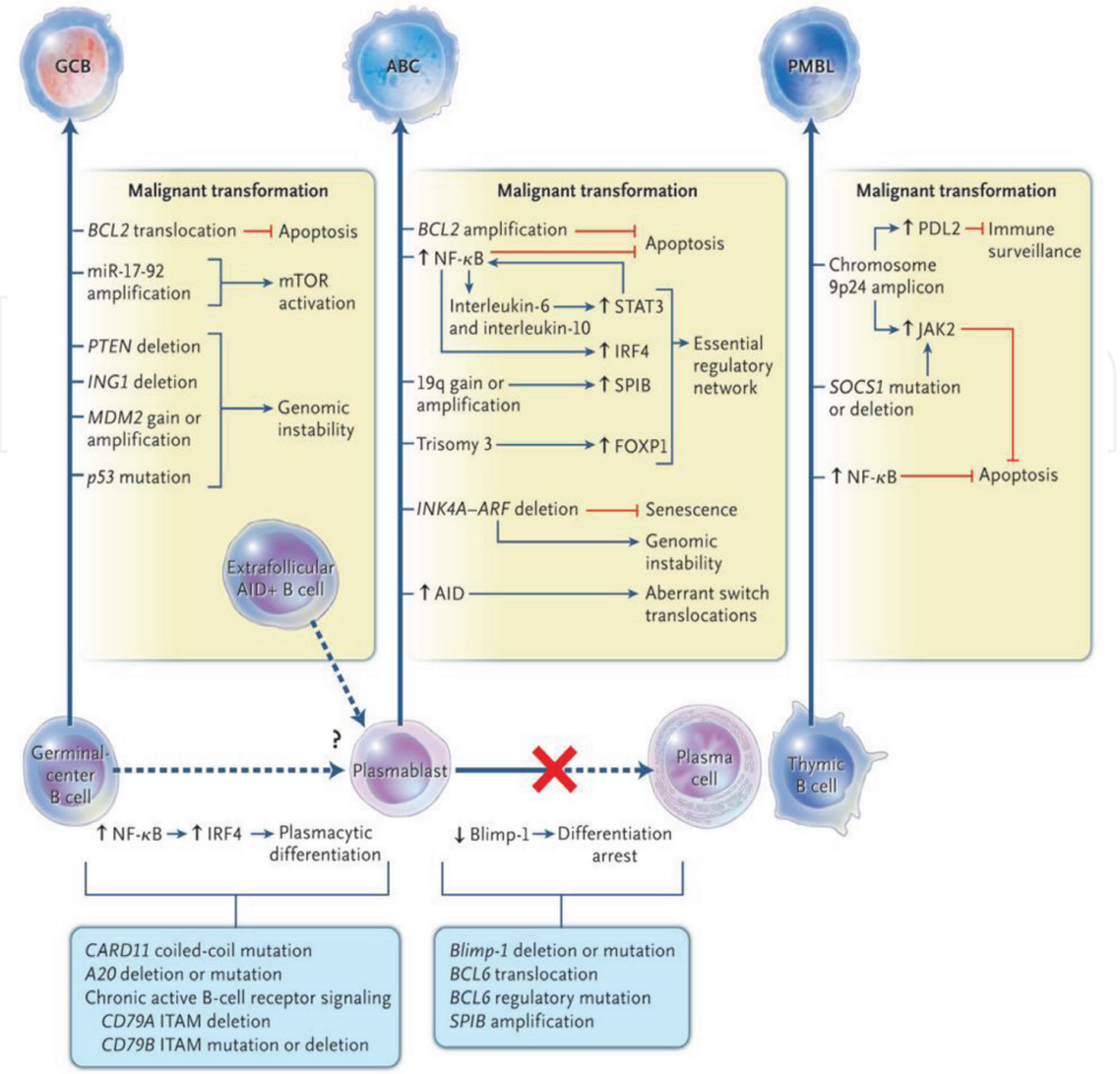


**Figure 3.**
*Oncogene path for three subtypes of DLBCL ([25], p. 1421).*

translocation of NFAT transcription factors from cytosol to nucleus; the induction of NFATc1/αA takes place during the G1 phase of cell cycle [43, 44]. The NFAT signaling pathway oversees different aspects of cellular functions; NFAT has a function like calcium sensor, including calcium signaling with other pathways involved in development and growth, immune response, and inflammatory response. The NFAT family of transcription factors regulates different cellular functions such as cell survival, proliferation, migration, invasion, and angiogenesis [44, 45].

TCF/LEFs are multifunctional proteins that use their sequence-specific DNA-binding and context-dependent interactions to specify which genes will be regulated by Wnts. The TCF/LEF families have been shown to regulate the expression of specific genes, such as c-Myc and Cyclin D1 [46]. These transcription factors are interdependent and closely linked with each other to form a network in the above signaling pathways, which regulate the entire process of osteoblast proliferation and differentiation [14]. Normally, these factors appear to be involved in the physiological activation response of lymphocytes before controlling the growth of malignant cells [47, 48].

Proliferation, differentiation, and apoptosis are associated with different types of lymphoma and chromosomal translocations. Each lymphoma has a separate proliferation of the site, which is most clearly described in the follicular lymphoma that proliferates in the follicle. Special chromosomal translocations are associated with

each type of lymphoma. *BCL1* translocation results in disturbed cell cycle regulation. *BCL2* and API2-MALT1 have an antiapoptotic function [49]. API2-apoptosis inhibitor 2-MALT1 (mucosa-associated lymphoid tissue lymphoma translocation gene 1)-induced NF-kappaB activation may contribute to antiapoptotic action probably through NF-kappaB-mediated upregulation of apoptotic inhibitor genes [49].

## 2. Classification and pathogenesis of DLBCL

Non-Hodgkin's lymphoma (NHL) classification is based on pathohistological findings. The history of classification of lymphoid neoplasms begins in the 1940s. Based on the advancement in knowledge, the work classification and Kiel have replaced the revised European-American Classification of Lymphomas, and it is a step ahead of the classification given by the WHO [8].

Classifications are an essential part of modern medicine, offering a consensus on the terminology and disease definitions to be used interdisciplinary both in research and clinical practice. Evolution of lymphoma classification includes numerous attempts from descriptive schemes, relying on morphology to strictly clinically oriented stratifications proposed by hematologists, usually without significant international acceptance. The classification of the WHO (Swerdlow et al. 2008) differs from the previous classifications in defining the real entities of the disease through multiple divisions. The new entities defined in this release include extended immunophenotypes and some gene information relevant to the classification. Panel antibody was selected based on morphological differential diagnosis and tumor location. DLBCL is a diffuse proliferation of large neoplastic B lymphoid cells of the same size or up to two times than the normal lymphocyte or extremely large as in macrophages. The origin of these cells are B cells of the germinating center and postgerminant cells [8]. DLBCL can occur in the form of nodal and extranodal diseases. Approximately 40% of patients are present with extranodal disease. Any tissue organ can be the primary site of DLBCL, but the gastrointestinal tract is the most common site [33].

Tumor cells are B large transformed lymphocytes, but the morphological picture of DLBCL is varied, and the predominant morphological cell type can distinguish six subtypes of DLBCL: centroblastic, immunoblastic, T lymphocyte/histiocyte rich, anaplastic, plasmablastic, and DLBCL with cells similar to Reed-Sternberg cells and expression of anaplastic lymphoma kinase (ALK) protein. Tumor cells are positive, as defined by the disease, on pan-B markers CD19, CD20, CD22, and CD79a, and the positivity of intracytoplasmic or surface immunoglobulin (Ig) markers is distinctly expressed as CD5, CD10, CD30, and CD45 markers. Numerous chromosomal abnormalities have been detected with DLBCL, but no specific diagnostic changes have been isolated. The most frequent changes are breaks of regions 14q32, 3p21, 3q27, 22q12, 1q25, and 18q21 and additional chromosomes 7,9,12 and 4q31-35,13p13-14 and 17p11-13,8,9. In 11–23% of cases, DLBCL with advanced clinical stage of the disease revealed nine regions of chromosomal amplification and isolated amplified genes with a potential role in the formation of lymphoma. The clinical significance of each individual change has not been established, but some changes are associated with advanced clinical stage or poorer response to therapy. The abovementioned chromosomal abnormalities are generally not found as individual changes. The accumulation of a greater number of abnormalities indicates the progression of genetic changes, which play an important role in the pathogenesis of DLBCL [8, 33].

## 3. The WHO classification which was revised in 2016

See **Table 1**.

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|---|
| Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS) |
| Germinal center B cell-like (GCB) |
| Activated B-cell-like (ACB) |
| T-cell/histiocyte-rich large B-cell lymphoma |
| Primary DLBCL of the central nervous system |
| Primary cutaneous DLBCL, leg type |
| Epstein–Barr virus (EBV) positive DLBCL, not otherwise specified (NOS) |
| EBV mucocutataneous ulcer |
| DLBCL associated with chronic inflammation |
| Lymphomatoid granulomatosis |
| Primary mediastinal (thymic) large B-cell lymphoma |
| Intravascular large B-cell lymphoma |
| ALK+ large B-cell lymphoma |
| Plasmablastic lymphoma |
| Primary effusion lymphoma |
| Human herpesvirus 8 (HHV8)+ DLBCL, not otherwise specified (NOS) |
| Burkitt lymphoma |
| Burkitt-like lymphoma with 11q aberration |
| High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 translocations |
| High-grade B-cell lymphoma, NOS |
| B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical |
| Hodgkin lymphoma |

**Table 1.**
*The 2016 update of WHO classification of DLBCL: subtypes and related entities [8].*

## 4. Prognostic markers in DLBCL

### 4.1 Gene expression profile (GEP)

Clinical and morphological differences in DLBCL suggest the biological significance of subtypes, which may help in adapting the therapy to be defined. The development of DNA microarray techniques provided the ability to identify gene expression of the tumor with a new molecular entity and a molecular predictor essential for survival. DNA microarray can analyze thousands of sites of previously identified genes. DLBCL has been identified by several tumor markers associated with unfavorable outcome after therapy and which can be linked to tumor cells and normal B lymphocytes. Phenotypic cells resemble normal B lymphocytes, but the clinical course is completely different [50–52].

Gene expression profile has shown that DLBCL can be divided into two molecular entities that differ in the gene expression profile. Hans et al. have identified two subgroups of DLBCL [34, 52]. One subgroup was called germinal center B-cell-like (GCB) and has a characteristic gene expression of normal GC B cells, in about 50% of cases, and the second group was called activated B-cell-like (ABC). Type-3 group is defined as nonclassified cases. According to the GEP classification, the observed prognosis distinguishes two major subtypes after the chemotherapy. Patients with GCB lymphoma have a more favorable outcome in 5-year survival than patients in the ABC group (68% vs. 24%) [52]. The distinction between GCB and ABC

subgroups and their effect on survival are independent of any clinical features of the disease, and these findings are further confirmed in other retrospective series with patients treated with chemotherapy [34, 42, 45–49].

In DLBCL, the ratio of *BCL2*-positive cases is highly variable, ranging from 24 to 80% among the study population in the previous studies that used immunohistochemistry (IHC); therefore, its prognostic relevance is controversial [16, 53–55].

Overexpression of the *BCL2* is associated with poor prognosis [55]. ABC-DLBCL subgroup is characterized by transcription expression of the *BCL2* gene [55], trisomy 3, and 18q21-q22 and loss of 6q21-q22 [56]. Activation of the antiapoptotic NF-κB signal pathway occurs at ABC but not at GCB DLBCL [56].

GCB DLBCL is characterized by frequent *REL* amplifications, *BCL2* translocations, and ongoing somatic hypermutation of the immunoglobulin genes [56].

Immunohistochemistry studies have a significant role, although complexity in the expression and performance of the interpretation of findings is very complex. They are used in the prognosis of DLBCL and in the therapeutic advantage of using rituximab [52].

DLBCL is a common type of aggressive non-Hodgkin's lymphoma with clear biological and clinical characteristics. Many studies have focused on the significance of internal tumor characteristics, such as the presence of proteins or the presence of gene expression and chromosomal translocation. Significant component of cell biology is inherited from non-transformed stem cells. Each of the B-cellular lymphoma inherits the traces of B-cell differentiation, although the extent to which this malignancy is maintained and the maintenance of molecular and physiological properties of normal B cells are not yet clear. Redistribution of immunoglobulin genes in DLBCL and other lymphomas carries mutations having somatic hypermutation characteristics, and the diversity of antibodies normally occurs only with the germinal center of the secondary lymphoid organ. This data suggest that DLBCL is generated from each of the germinal centers of the B cell or from the B cell at a later stage of differentiation [1, 25, 50, 52, 56].

### *4.1.1 Germinal center B-cell-like (GCB)*

CB DLBCL appears to be generated from the normal germinal B cell, the translocation t (14, 18) involving *BCL2* [54], and the amplification of *c-REL* on the chromosome 2p was detected in 45% GCB DLBCL, whereas it was never detected at ABC subtype [56]. This finding suggests that different subgroups represent different diseases and the consequence is a different mechanism of malignant transformation and significantly different survival after chemotherapy. CD10 and *BCL-6* are considered as germinal center markers, while IRF4/MUM1 is expressed on plasma cells in atypical light zones of the germinal center. By morphological characteristics, the center is uniform form monomorphic. Expressions CD10, BCL-6, and MUM1 are used to classify GCB and ABC lymphoma subtype [1, 25, 46, 50, 56].

Many factors of B-cell differentiation in the germinal center affect the expression of transcription factors. The key B-cell transcription factor is PAX5 (PAX5 is a nuclear protein that has the ability to bind to wild-type PAX5 target loci) [53] and is required for regulating many B-cell-specific transcription factors and for B-cellular development from the B stage to mature B cells. The transcription factor PU.1 is an E26 transformation-specific family transcription factor that is required for the development of the immune system. PU.1 functions at both early and late stages of lymphoid and myeloid differentiation [57], and the IRF involved with Blim1/PRDM1 was isolated from the family in further regulation of the transcriptional factor *BCL6*, which is essential for the formation and maintenance of the germinal center. Translocation t (3q27) is included in *BCL6* gene and is found in 20–40% diffuse lymphomas. IRF4/MUM1 expression is required for activation-induced cytidine deaminase (AID) expression, which is important for two significant functions

of the germinal center, namely, somatic hypermutation (SHM) and AID-mediated class switch recombination (CSR) [58]. They depend on hierarchical expression and the common regulation of transcription factors. For example, *BCL6* and IRF4/MUM1 are expressed mutually exclusively in germinal center cells, but with DLBCL *BCL6* and IRF4 / MUM1, they have co-expression [8, 59].

The Epstein–Barr virus (EBV) is the second factor associated with disturbed GCB regulation. EBV is associated with numerous lymphoid neoplasms including Burkitt's lymphoma and classical Hodgkin's lymphoma and posttransplant lymphoproliferative disease. The transformation that leads through EBV is capable of major negative selection in the germinal center. EBV/LMP2A induces escape of the defective B cells of the germinal center of the apoptosis and does not only offer an intriguing model of lymphomagenesis but also confirms the significant role of exogenous factors such as viruses involved in forming the response of the germinal center [60]. EBV latent membrane protein 2A (LMP2A) is expressed on the membranes of B lymphocytes and blocks B-cell receptor (BCR) signaling in EBV-transformed B lymphocytes in vitro. In vivo studies indicate that LMP2A allows B-cell survival in the absence of normal BCR signals [60].

CD10 expression is associated with a more favorable prognosis, and MUM1 is normally found on plasma cells and in a small part of the atypical spot zone in the germinal center and represents a marker for a non-germinal center [61].

Patients in this group have significantly better 5-year overall survival and survival without signs of disease activity after chemotherapy with combined drugs (68% for GCB vs. 24.7% for non-GCB with use of gene-expression profiling) [8, 52, 56].

#### *4.1.2 Activated B-cell-like (ABC)*

ABC-DLBCL is generated from the postgerminal center of B cells during plasmacytic differentiation. ABC is characterized by frequent changes in chromosome arm 3p but not 3q [62]. ABC lymphoma has a subtype gene expression that has the properties of normal B cells that are activated over B-cell receptors.

Trisomy 3 is a common chromosomal aberration and is found in 26% of respondents. The forkhead transcription factor (FOXP1) is the third most potentially monitored gene in trisomy 3. High FOXP1 expression can contribute to B-cell lymphomagenesis by promoting B-cell survival inhibiting plasma cell differentiation. The small FOXP1 isoform (FOXP1-iso) is predominantly expressed in ABC-DLBCL, and studying its oncogenic potential and transcriptional activity is directly compared to FOXP1-FL in DLBCL cell lines and primary human B cells [61–64].

ABC lymphomas have expression of the plasma cell genome, including transcriptional factor XBP1, which is the regulator of immunoglobulin secretion. XPB1 is a protein which in humans is encoded by the *XPB1* gene located on chromosome 22. The XBP1 protein is a transcription factor that regulates the expression of genes important of the immune system and in the cellular stress response. *C-MYC* is necessary and sufficient for activation of the IRE1/XBP1 pathway [65, 66].

The constituent part of the NF-κB activation causes ABC lymphoma with expressing the IRF4 transcription factor, and this may push them toward differentiation in the plasma cells [42].

ABC lymphomas can obtain genetic changes that interfere with Blimp-1, thereby blocking full differentiation into plasma cells. The block in differentiation represents an early step in the pathogenesis of ABC subtypes, but the nature of the cell precursor is unclear. These lymphomas contain high activation-induced cytidine deaminase (AID) values, and their IgH genes have severe mutations. However, most ABC lymphomas did not undergo a recombinant trigger, and they have IgM expression, unlike most of the normal B cells of the germinal center and other GCB lymphomas [39].

NF-κB is present as a latent, inactive, IκB-bound complex in the cytoplasm. When a cell accepts any of a large number of extracellular signals, NF-κB quickly enters the nucleus and activates gene expression. Key step for controlling NF-κB activity is the regulation of the I kappa B kinase (IKK) [42, 55, 62].

Various genetic abnormalities activate CARD11 in ABC subtype lymphoma. In normal B cells, antigen-receptor-induced NF-kB activation requires CARD11, a cytoplasmic scaffolding protein. To determine whether CARD11 contributes to tumorigenesis, we sequenced the CARD11 gene in human DLBCL tumors [67]. In about 10% of patients, CARD11 is an oncogene with acquired mutations that activate NF-κB and can prolong cell survival. Mutant CARD11 generates large protein aggregates in the cytoplasm, which represent possible NF-κB activation sites. The survival of lymphoma ABC with the wild-type CARD11 depends on B-cell receptors and kinases [67].

ABC lymphomas have expression of the plasma cell genome, including transcriptional factor XBP1, which is the main regulator of immunoglobulin secretion. ABC lymphoma has presented expression of genes characteristic of mitogen-activated B cells in the blood and is associated with a worse prognosis [66]. ABC-DLBCL is characterized by gain of 3q, 18q, and 19q and loss of 6q and 9p21, and GCB DLBCL is characterized by gain of 1q, 2p, 7q, and 12q; the genomic imbalances characteristic of the CD5(+) and CD5(−)CD10(+) groups were similar to those of the ABC and GCB types [68].

Lymphoma ABC does not have one expression of the germinal center gene, but has gene expression that is normally present in B cells after B-cell receptor stimulation. MUM1 is found on plasma cells at later stage B-cellular differentiation and is associated with the gene profile of activated B-cellular DLBCL. The presence of *BCL2* and Cyklin D2 is adverse prognostic parameters. Polymorphic centroblasts and immunoblasts are more commonly present in activated B-cell DLBCL. This group is characterized by a group of genes that are characteristic of plasma cells, in part, added to the endoplasmic reticulum and Golgi's proteins involved in secretion. There is a significantly higher percentage of patients in this group [69].

## 5. Conclusion

The contribution of GC selection to phenomena such as immunodominance and original antigenic guilt has also not been established and is likely to be of considerable interest. Answering these questions will be crucial to our quest to produce more broad and new medications and will undoubtedly bring novel insights into the general functioning of GC, as well as of the immune system in general.

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