

Biology of Germinal Center B Cells Relating to Lymphomagenesis

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Abstract

The germinal center (GC) reaction is a key feature of adaptive humoral immunity. GCs represent the site where mature B cells refine their B-cell receptor (BCR) and are selected based on the newly acquired affinity for the antigen. In the GC, B cells undergo multiple cycles of proliferation, BCR remodeling by immunoglobulin somatic hypermutation (SHM), and affinity-based selection before emerging as effector memory B cells or antibody-secreting plasma cells. At least 2 histologically and functionally distinct compartments are identified in the GC: the dark zone (DZ) and the light zone (LZ). The proliferative burst and immunoglobulin remodeling by SHM occur prevalently in the DZ compartment. In the LZ, GC B cells undergo an affinity-based selection process that requires the interaction with the antigen and accessory cells. GC B cells are also targeted by class switch recombination, an additional mechanism of immunoglobulin remodeling that ensures the expression of diverse isotype classes. These processes are regulated by a complex network of transcription factors, epigenetic modifiers, and signaling pathways that act in concert with mechanisms of intra-GC B-cell trafficking. The same mechanisms underlying the unique ability of GC B cells to generate high affinity antibodies and ensure immunological memory are hijacked during lymphomagenesis and become powerful weapons for malignant transformation. This review will summarize the main processes and transcriptional networks that drive GC B-cell development and are relevant for human B-cell lymphomagenesis.

The germinal center reaction

Germinal centers (GCs) are histological structures that develop upon engagement of the B-cell receptor (BCR) on naive B cells by a cognate antigen. Activated naive B cells migrate into the T cell zones of the secondary lymphoid organs where they are stimulated to proliferate and to acquire features of GC B cells. The GC reaction aims to generate B cells with high affinity BCRs and to foster the production of effector memory B cells and antibody-secreting plasma cells.^{1,2} The process of BCR affinity maturation relies on the introduction of somatic mutations in the variable region of the immunoglobulin genes by the somatic hypermutation (SHM) machinery followed by affinity-based selection.

The GC comprises at least 2 compartments that are histologically and functionally distinct: the dark zone (DZ) and the light zone (LZ).³ The DZ is the site of active proliferation and where SHM occurs. The LZ is the site of affinity-based selection, which drives the DZ re-entry, commitment to post-GC differentiation, or elimination by apoptosis^{2,3} (Figure 1). GC B cells undergo class switch recombination (CSR) in order to acquire the ability to express different antibody isotype classes with diverse effector functions.⁴ Although, CSR was considered a mechanism associated with the LZ compartment, recent data suggest that this

process may start early in naive B cells primed to become GC B cells.⁵ In addition, CSR is not an obligatory consequence of transit through the GC: post-GC cells may exit as not switched lymphocytes, especially if they reside in the GC for a short time.^{6,7}

The simplified view of a bi-compartmental GC structure has been expanded by recent data showing that a more granular assessment of GC B cells by single-cell transcriptomic analyses identifies a much broader spectrum of subpopulations, including an additional “intermediate zone” between the DZ and the LZ.⁸⁻¹⁰ These data suggest that GC B cells are likely to move along a continuum of states rather than between 2 defined and uniform populations.

Immunoglobulin remodeling

The BCR is formed by the immunoglobulins associated with co-factors (CD79A and CD79B) that participate in signal transduction. Antibodies are composed of 4 polypeptides: 2 identical heavy chains (immunoglobulin heavy locus [IGH]) and 2 identical light chains (immunoglobulin lambda locus [IGL] or immunoglobulin kappa locus [IGK]). Each polypeptide consists of an amino terminal variable segment (V) and a constant carboxy-terminal region (C). Each B cell acquires the ability to express a specific BCR on the surface during the early stages of maturation occurring in the bone marrow. The production of a functional and unique BCR occurs through V(D)J recombination, a process that leads to rearrangements in the IGH locus of the genes encoding for the variable (V), diversity (D), and joining (J) regions, followed by the rearrangements in one of the light chain loci (IGL or IGK) of the genes encoding for the V and J regions (Figure 2A). As a result of productive V(D)J recombination in the IGH and IGL or IGK loci, immature B cells express BCRs with the immunoglobulin heavy constant mu (IGHM) isotype and are ready to undergo counter selection for autoreactivity.¹¹

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Received: 15 March 2021 / Accepted: 15 April 2021

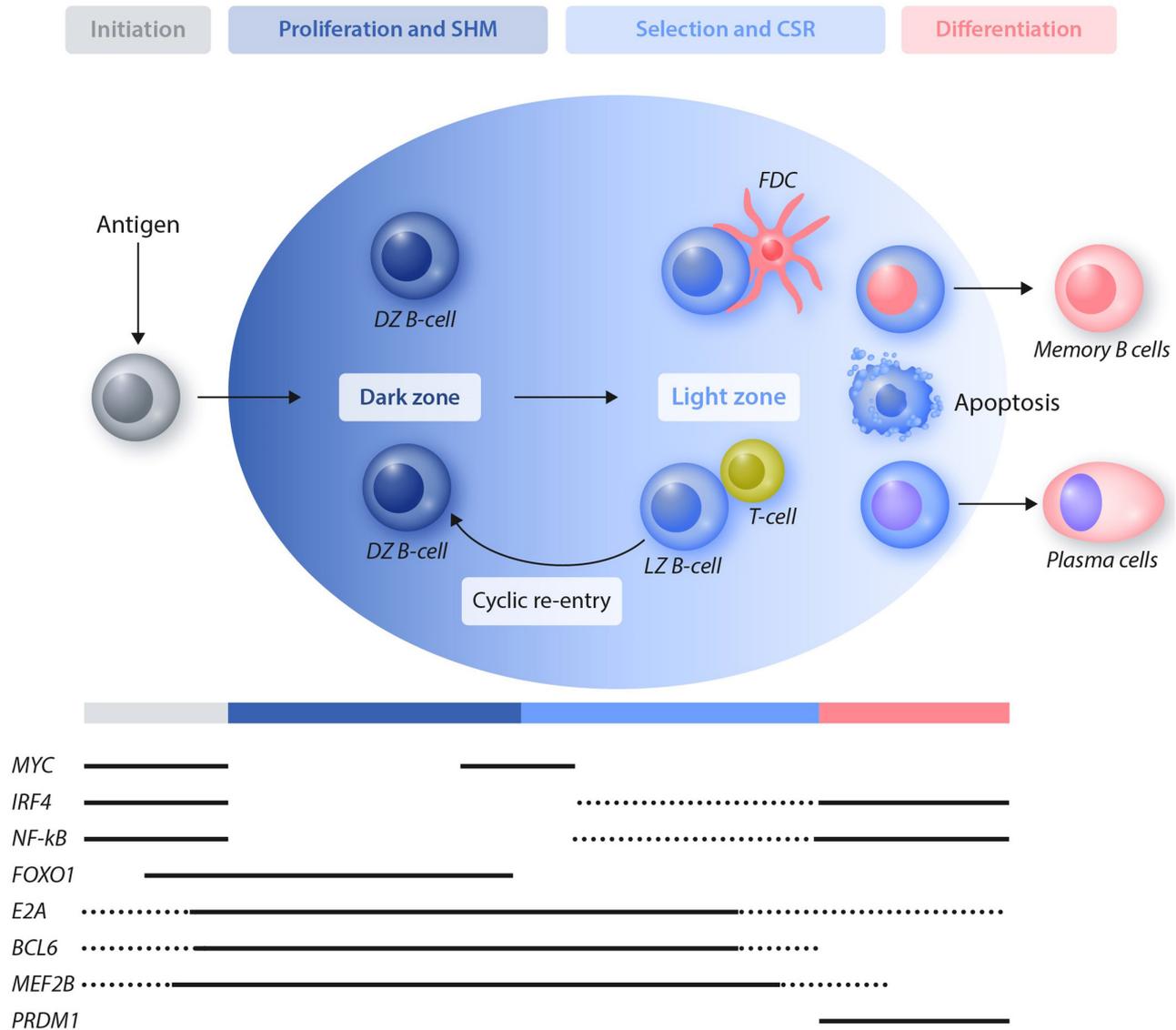


Figure 1. The GC reaction and the expression pattern of key transcription factors. Antigen-activated B cells differentiate into DZ GC B cells that are characterized by a high rate of proliferation and are subject to SHM. B cells then move into the LZ compartment and undergo activation, affinity-based selection and CSR. GC B cells will undergo multiple cycles of re-entry from the LZ to the DZ before terminally differentiating into memory B cells or plasma cells. Cells that have not acquired a favorable B-cell receptor will be negatively selected and eliminated by apoptosis. Multiple transcription factors are involved in regulating the GC initiation, expansion, and exit toward terminal differentiation. The pattern of expression of selected transcription factors that are relevant for the GC reaction and are also hijacked during lymphomagenesis is displayed. Dotted lines refer to expression in a subset of the cells. BCL6 = BCL6 transcription repressor; CSR = class switch recombination; DZ = dark zone; E2A = transcription factor E2-alpha; FDC = follicular dendritic cell; FOXO1 = forkhead box O1; GC = germinal center; IRF4 = interferon regulatory factor 4; LZ = light zone; MEF2B = myocyte enhancer factor 2B; MYC = MYC proto-oncogene, bHLH transcription factor; NF-kB = nuclear factor kappa B; PRDM1 = PR/SET domain 1; SHM = somatic hypermutation.

Positively selected cells become mature naive B cells that co-express IGHM and immunoglobulin heavy constant delta isotypes and leave the bone marrow to circulate in the peripheral blood and in the secondary lymphoid organs.

The encounter with cognate antigens triggers naive B cells to proceed into the differentiation process and become GC B cells. In order to expand BCR diversity and therefore increase the probability to generate high affinity antibodies, GC B cells undergo SHM: a process that introduces point mutations, small deletions, insertions, and duplications in the rearranged variable genes of the *IGH* and *IGL* or *IGK* loci¹² (Figure 2B). It affects a defined region of 1–2 Kb downstream the V gene promoters and it depends on the enzyme activation-induced cytidine deaminase (AICDA) that converts cytidine into uracil triggering an error-prone DNA repair mechanism.¹² Several nonimmunoglobulin

loci are also targeted by SHM, although with a much lower mutational rate.¹³

Most GC B cells undergo an additional step of *IGH* remodeling by CSR. During CSR, the C region of the *IGH* chain can be replaced by a mechanism of DNA recombination depending on AICDA and DNA double-strand breaks in specialized switch regions upstream of the C genes⁴ (Figure 2C).

Role of SHM and CSR in GC lymphomagenesis

GC B cells are the cell-of-origin for the majority of mature B-cell lymphomas. The most common GC-derived B-cell lymphomas include Burkitt lymphoma (BL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL).¹⁴ The latter

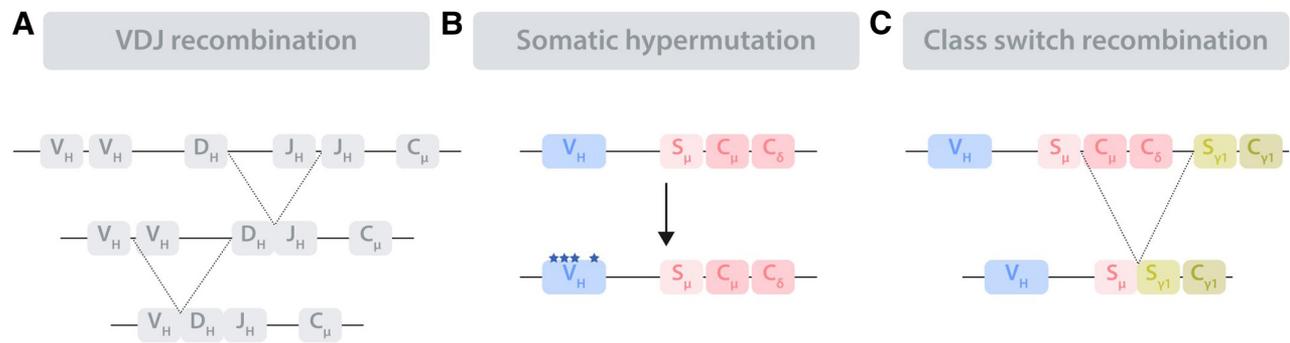


Figure 2. Schematic representation of the immunoglobulin remodeling processes. The immunoglobulin loci undergo diverse remodeling processes in order to encode a functional, high-affinity BCR. (A), Immunoglobulin remodeling starts in bone marrow precursor B cells by V(D)J recombination, a process that assembles 3 (V, D, and J) or 2 (V and J) genes in the immunoglobulin loci encoding for the immunoglobulin heavy locus (IGH) and light chain (not shown), respectively. (B), Somatic hypermutation takes place in the GC DZ compartment and introduces point mutations, deletions, and duplications in the rearranged V regions of the immunoglobulin loci. (C), Class switch recombination targets the constant (C) region of the IGH, leading to the replacement of the original C μ and C δ with one of several other C regions (in the scheme C γ 1), each resulting in an antibody with different effector functions. The exchange occurs at the switch (S) regions. BCR = B-cell receptor; D = diversity; DZ = dark zone; GC = germinal center; J = joining; V = variable.

comprises at least 2 distinct subtypes: the GC B-cell like (GCB) and the activated B-cell like (ABC) DLBCL.¹⁵ The footprints of their GC transit are detectable in the immunoglobulin loci of these malignant cells that carry evidence of SHM.

The physiologic mechanisms of recombination and SHM targeting the immunoglobulin loci are also involved in the generation of genetic alterations identified in B-cell lymphomas. A hallmark of many B-cell lymphomas are chromosomal translocations involving an immunoglobulin locus and a proto-oncogene. These translocations have features consistent with the involvement of V(D)J recombination, CSR, and SHM, supporting the notion that the DNA breaks associated with each of these processes carry a risk of generating translocations.¹⁶ The t(14;18) translocation involving BCL2 apoptosis regulator (BCL2) and IGH displays structural features related to V(D)J recombination, thus occurring in bone marrow precursors, although representing an hallmark of FL that is a GC-derived B-cell malignancy. Therefore, V(D)J recombination is involved in the generation of translocations associated with lymphomas that originate at later time in the differentiation process. This observation suggests that seeding oncogenic events can happen early in the hematopoietic development, but require further differentiation to take place before additional occurrences lead to malignant transformation.

The process of SHM introduces nucleotide exchange, but also a significant fraction of insertion and deletions that are associated with DNA breaks.¹² Along this line, SHM has been involved in the generation of a subset of translocations targeting MYC proto-oncogene, bHLH transcription factor (MYC) and BCL6 transcription repressor (BCL6) and displaying breakpoints in the V and J regions of the immunoglobulin loci.¹⁶ In addition, DLBCL display features of an aberrant SHM mechanism affecting genes that are not physiologic targets of SHM in GC B cells.¹⁷

The footprint of CSR is evident in a number of translocations involving the IGH switch regions and various oncogenes, including: MYC, resulting in the t(8;14) translocation, a hallmark of sporadic BL; BCL6, leading to the t(3;14) translocation often detected in DLBCL; MAF bZIP transcription factor in the t(14;16) and interferon regulatory factor 4 (IRF4) in the t(6;14) associated with multiple myeloma. These translocations lead to heterologous promoters and/or enhancers being juxtaposed to an oncogene, leading to dysregulated or ectopic expression of the oncogene.¹⁶

In conclusion, the physiologic mechanisms that empower the generation of antibody diversity come with the risk of promoting genetic alterations.

BCR signaling in normal and malignant GC B cells

A key step in the early differentiation of B cells is the acquisition of a functional and nonautoreactive BCR that, during the GC reaction, is further refined to improve affinity (Figure 2). BCR expression is essential for B-cell survival^{18,19} and GC B cells are no exception.¹¹

BCR signaling can occur independently of antigen engagement. This antigen-independent or “tonic” BCR signaling depends on phosphatidylinositol 3-kinase (PI3K)-mediated activation of the AKT serine/threonine kinase (AKT), which affects several downstream survival pathways.²⁰ Tonic BCR signaling does not engage nuclear factor kappa B (NF- κ B) activation, which requires antigen-dependent or “active” BCR signaling. DZ B cells do not display evidence of AKT or NF- κ B activation,^{21,22} while LZ B cells that express high affinity BCR experience active BCR signaling and NF- κ B activation as essential mechanisms toward cell fate determination.

Most GC-derived lymphomas retain expression of the BCR, and their need for BCR-driven survival signals is supported by the fact that translocations into the immunoglobulin loci are virtually always found on the nonproductively rearranged alleles.²³ Specific subtypes of lymphomas appear to rely on different BCR mode of action. BL are dependent on tonic BCR signaling that is promoted by multiple genetic alterations, including disruption of the transcription factor E2-alpha (E2A)-inhibitor of DNA binding 3, HLH protein (ID3) module.^{24,25} Consistently, the constitutive activation of the PI3K signaling pathway, together with ectopic MYC expression, is required for development of BL in mice.²⁶ Conversely, the ABC subtype of DLBCL depends on a chronic form of active BCR signaling that is associated with self-reactive BCRs and is sustained by numerous oncogenic aberrations mostly targeting proximal members of the pathway.^{27–29}

GC transcriptional networks hijacked by lymphomagenesis

The transcriptional networks wiring the GC reaction rely on multiple transcription factors and epigenetic modifiers. While transcription factors are key players in establishing defined transcriptional programs, epigenetic chromatin remodeling ensures coordinated and rapid switching between discrete transcriptional programs. Recent genomic analyses have shown that a

large fraction of these molecules are targeted by genetic alterations in lymphoma. This section focuses on selected proteins that are involved in driving the GC reaction and are recurrently targeted by genetic lesions in GC-derived lymphomas.

MYC

MYC is a pleiotropic protein that functions as a transcription factor regulating an extremely large network of targets and a broad range of cellular programs,³⁰ but also acts as a modulator of DNA replication through mechanisms that are independent of its transcriptional activity.³¹

The very limited expression of MYC in the GC has remained a puzzling observation that has been addressed only in the recent years.^{32,33} MYC is required at the initial stages of the GC formation, but it is then downregulated by mechanisms that include repression by BCL6.^{34,35} In GC B cells, expression of MYC is restricted to a subset of LZ B cells that are primed to re-enter the DZ^{34,35} (Figure 1).

The ectopic expression of MYC is a common feature of GC-derived lymphoma. In particular, translocations juxtaposing MYC to the immunoglobulin loci represent the hallmark of BL. The contribution of MYC to malignant transformation in GC B cells is most likely related to its constitutive and ectopic expression that disrupts the tightly regulated bimodal pattern of expression observed in normal GC B cells.

FOXO1

Forkhead box O1 (FOXO1) is a transcription factor that acts at different stages of B-cell differentiation.³⁶ In the GC, FOXO1 is expressed in the DZ compartment consistent with the low activity of PI3K-AKT signaling that promotes cytoplasmic translocation and degradation of FOXO1.²² FOXO1 transcriptional network acts to sustain the DZ program while repressing LZ features such as activation and differentiation.²² Consistently, GC-specific deletion of *Foxo1* in mice leads to the development of GC containing only LZ cells.^{22,37}

In GC-derived lymphomas, FOXO1 nuclear expression is detected in the vast majority of BL and a subset of DLBCL, regardless of PI3K-AKT activity in these tumors. In a fraction of cases, the fact that FOXO1 is retained in the nucleus in the presence of PI3K signaling has been linked to mutations that prevent phosphorylation and negative regulation of FOXO1.^{38,39}

BCL6

BCL6 acts as a master regulator of the GC reaction and it is indeed required for GC formation and maintenance.^{40,41} BCL6 is a transcriptional repressor that controls a wide set of targets aiming to restrain the DNA damage response, modulate apoptosis, and hamper the premature activation and differentiation of GC B cells.⁴² BCL6 repression activity is dependent on its ability to bind specific DNA motifs and to recruit class I and class II histone deacetylase complexes either directly or through interaction with multiple co-repressors.⁴³⁻⁴⁵ BCL6 expression in mature B cells is restricted to the GC stage (Figure 1). The signals inducing the expression of BCL6 at the initiation of the GC remain elusive, although several transcription factors including interferon regulatory factor 8,⁴⁶ IRF4,⁴⁷ and myocyte enhancer factor 2B (MEF2B)⁴⁸ have been shown to contribute to BCL6 expression. Expression of BCL6 in GC B cells is finely tuned by an auto-regulatory mechanism through which BCL6 binds to its own promoter to negatively regulate its transcription.⁴⁹

In the LZ, BCL6 expression and activity are negatively modulated by multiple signaling pathways. Indeed, post-GC differentiation requires BCL6 silencing, thus multiple pathways

coordinately contribute to negatively modulate BCL6 at different levels.⁴² IRF4-mediated transcriptional repression of *BCL6* occurs upon NF- κ B activation following BCR, CD40, or Toll-like receptor signaling.⁵⁰ In addition, CD40 signaling triggers cytoplasmic translocation of the BCL6 co-repressor nuclear receptor corepressor 2, leading to a rapid inactivation of BCL6 transcriptional activity on a subset of targets.⁵¹ Interaction with co-repressors is also impaired by BCL6 acetylation, which provides an additional relatively fast and reversible mechanism to inactivate BCL6 prior to its transcriptional and post-transcriptional down-regulation.⁵² BCR signaling, induced by high-affinity interactions with the antigen, leads to mitogen-activated protein kinase-mediated BCL6 phosphorylation and degradation.⁵³ BCL6 protein degradation is mediated by F-box protein 11 (FBXO11), a specific adaptor protein that recruits BCL6 into the S-phase kinase associated protein 1-Cullin 1 ubiquitin ligase complex and targets it for ubiquitylation and proteasome-mediated degradation.⁵⁴

BCL6 dysregulated expression and activity is a common feature of GC-derived lymphomas and contributes to lymphomagenesis by enforcing a proliferative phenotype, suppressing DNA damage responses and blocking differentiation. The oncogenic potential of BCL6 dysregulated constitutive expression has been confirmed in mouse models.⁵⁵

An expanding number of mechanisms converge to dysregulate BCL6 expression and activity in lymphoma. *BCL6* was first identified for its involvement in translocations that replace its regulatory region with heterologous promoters including the IGH locus.⁵⁶⁻⁵⁸ Mutations targeting the auto-regulatory circuit⁴⁹ or the IRF4 binding sites⁵⁰ in *BCL6* promoter interfere with basal or CD40-induced negative modulation. In addition, several indirect mechanisms affect BCL6 during lymphomagenesis, including gain-of-function mutations in *MEF2B*, a positive modulator of BCL6; inactivation of *FBXO11*, involved in BCL6 protein degradation; and loss-of-function alterations in the acetyl transferases CREB binding protein (CREBBP) and E1A binding protein p300 (EP300), which are involved in acetylation-mediated inactivation of BCL6.¹⁴

MEF2B

MEF2B is a member of the MEF2 family of transcription factors, which includes 3 additional members (MEF2A, MEF2C, and MEF2D) originally identified as regulators of myocyte differentiation.⁵⁹ Similarly to BCL6, MEF2B expression in mature B cells is restricted to the GC stage. However, MEF2B induction, upon T cell-dependent antigen activation, occurs slightly earlier than BCL6⁴⁸ (Figure 1). This observation is consistent with BCL6 transactivation following MEF2B binding in regulatory regions of the BCL6 locus.^{48,60,61} In mice, GC-specific loss of *Mef2b* leads to a partial GC impairment, most likely due to compensatory mechanisms through other Mef2 family members.⁶² Mef2c, which is expressed at all stages of mature B-cell differentiation, modulates B-cell proliferation by acting downstream of BCR signaling and its deficiency impacts GC development.⁶³ Although Mef2b and Mef2c appear to have different functions in GC B cells, they could partially compensate for each other, as shown by the fact that only their co-deletion fully abrogates GC formation *in vivo*.^{62,63}

MEF2B binds to a wide range of regulatory regions in the genome of GC B cells and contributes to the modulation of a large network of genes involved in cell cycle, DNA replication and repair, apoptosis, GC confinement, differentiation, and chromatin remodeling.⁶² This transcriptional network is consistent with a role in promoting the GC, and in particular the DZ, program. Similarly to BCL6, MEF2B expression is negatively regulated in the LZ compartment in order to allow differentiation (Figure 1). However, the mechanisms leading to MEF2B silencing at the end of the GC reaction remain to be identified.

In FL and DLBCL, *MEF2B* is targeted by genetic alterations, which comprise mostly of recurrent missense mutations targeting the N-terminus, and scattered missense, nonsense, and frame-shift mutations affecting the C-terminus of the protein. Most N-terminus mutants abrogate the interaction with co-repressors leading to an enhanced and de-regulated *MEF2B* activity, which has been shown to promote malignant transformation in vivo.^{48,62} Mutations in the C-terminus appear to function through different mechanisms converging to provide escape from inactivation mediated by post-translational modifications.⁴⁸ Overall, lymphoma-associated genetic alterations of *MEF2B* appear to interfere with diverse layers of negative modulation leading to a de-regulated activity of this protein.

E2A (*TCF3*)

The *TCF3* gene encodes for the E2A transcription factor that is highly expressed in the DZ compartment⁶⁴ (Figure 1) and activates expression of cyclin D3 (*CCND3*), a key player in the proliferative expansion of DZ GC B cells.^{65–67} In addition, E2A regulates the expression of BCR components (immunoglobulin heavy- and light-chains) and of protein tyrosine phosphatase non-receptor type 6, a negative regulator of BCR signaling.²⁵ E2A activity is negatively modulated by heterodimerization with ID3, which impairs E2A binding to DNA.⁶⁸ The E2A-ID3 axis appears of relevance in GC B cells, as shown by the requirement of *Tcf3* for GC formation in mice.⁶⁹

In BL, *TCF3* and *ID3* genes are frequently targeted by genetic alterations, which impair interaction of E2A with ID3 or lead to ID3 inactivation, respectively.^{24,25} Impaired negative modulation of E2A by ID3 contributes to sustain the survival of lymphoma cells by promoting tonic (antigen-independent) BCR signaling and transactivation of *CCND3*.²⁵ In addition, *CCND3* is often stabilized at protein level due to mutations that impair its ubiquitin-mediated degradation, suggesting that expression of *CCND3* is relevant for BL cell proliferation.^{24,25}

NF-κB

NF-κB transcriptional activity is critical for the generation of normal mature B cells and for antigen-dependent B-cell activation.⁷⁰ The NF-κB transcription factor transduces the expression of a broad network of genes sustaining many cellular processes, including cell survival and proliferation.⁷⁰ The nuclear translocation of NF-κB is induced at the early stages of the GC initiation, but it is efficiently blocked in the DZ compartment and re-induced later in a subset of LZ GC B cells (Figure 1).^{3,21,33} During affinity-based selection, B cells that display high-affinity BCR are sustained by the engagement of additional receptors including CD40, TNF receptor superfamily member 13C, and Toll-like receptors. These interactions trigger numerous signaling pathways and converge on the activation of NF-κB. Different NF-κB subunits are associated with distinct functions in GC B cells: REL proto-oncogene, NF-κB subunit is required for GC initiation and maintenance, while activation of the RELA proto-oncogene, NF-κB subunit becomes indispensable at later stages for the generation of plasma cells.⁷¹ Consistently, GC-specific deletion of *Rela* in mice impairs plasma cell differentiation,⁷¹ while constitutive activation of NF-κB leads to plasma-cell accumulation.^{72,73} A key target of NF-κB in LZ GC B cells is represented by IRF4, the induction of which represents an early event toward plasma cell differentiation. IRF4 down-regulates *BCL6*,⁵⁰ thus favoring termination of the GC program and expression of the plasma cell master regulator B lymphocyte-induced maturation protein 1 (*BLIMP1*).⁷⁴

Constitutive NF-κB activation is a feature of the ABC subtype of DLBCL that is required for lymphoma cell survival and sustained by a variety of genetic lesions.⁷⁵ These lesions act on

multiple levels: by promoting chronic, active BCR signaling (through activating mutations in *CD79A* and *CD79B*), which feeds into NF-κB activation^{27,28}; by mutating the MYD88 adaptor molecule, which is part of a signaling hub for NF-κB activation^{76–78}; and by removing negative modulators of the NF-κB signaling pathway, such as TNF alpha induced protein 3.^{79,80}

IRF4

IRF4 is a member of the interferon-regulatory factor family of transcription factors that can function both as repressor or activator due to its alternative interactions with diverse partners. In GC B cells, IRF4 displays a bimodal pattern of expression, being induced at the initiation stage when it contributes to *BCL6* induction,^{47,81} repressed in the DZ, and re-expressed in a subset of LZ B cells that are committed to plasma cell differentiation⁸² (Figure 1). IRF4 is required for plasma cell differentiation as shown by the fact that its deletion in mice leads to lack of plasma cells.^{83,84} In the LZ, IRF4 downregulates *BCL6*⁵⁰: the molecular mechanisms by which IRF4 acts to negatively regulate *BCL6* in the LZ, while it induces its expression in early GC B cells, are not known and may involve the recruitment of distinct co-transcriptional complexes. IRF4 promotes plasma-cell differentiation by activating PR/SET domain 1 (*PRDM1*) directly,^{84,85} as well as by releasing the *BCL6*-mediated repression on the *PRDM1* promoter.⁸⁶

IRF4 was originally identified as a proto-oncogene involved in chromosomal translocations in multiple myeloma,⁸⁷ but it is targeted by genetic alterations also in DLBCL, and the ABC subtype depends on IRF4 expression.⁸⁸

BLIMP1 (*PRDM1*)

The transcriptional repressor BLIMP1, encoded by the *PRDM1* gene, is a master regulator of plasma cell differentiation, a process that requires termination of the GC program.^{74,89} BLIMP1 contributes to extinguish the GC B-cell identity,^{90,91} while it implements a plasma cell-specific transcriptional program,⁹² which includes repression of cell proliferation and the acquisition of an antibody secretory phenotype dependent upon the expression of X-box binding protein 1.^{93,94} In the GC, *PRDM1* is induced in LZ cells that have committed to plasma cell differentiation (Figure 1).

The inability to terminally differentiate is a common process in GC lymphomagenesis. In ABC-DLBCL it appears to depend on 2 mechanisms that are largely mutually exclusive and converge on the negative regulation of BLIMP1. *PRDM1* is targeted either by deletions that are often bi-allelic^{95–97} or it is constitutively repressed by *BCL6* dysregulation.⁸⁶ In addition, a subset of ABC DLBCL display gain-of-function alterations of SPIB,^{29,98} a transcription factor that directly represses *PRDM1* transcription.^{88,99,100} Mouse models, carrying *Prdm1* genetic inactivation in GC B cells develop ABC-DLBCL that display constitutive NF-κB activation, demonstrating the cooperation of these pathways in malignant transformation.^{95,101}

Enhancer of zeste homolog 2

The enhancer of zeste homolog 2 (*EZH2*) methyl transferase is a component of the polycomb repressive complex-2 that negative regulates gene transcription by inducing trimethylation of the lysine 27 of histone H3.^{102–104} In mature B cells, *EZH2* expression is restricted to the GC stage^{105,106} and it is required for GC development.^{107,108} *EZH2* promotes GC B-cell proliferation, through epigenetic silencing of cyclin dependent kinase inhibitor 1A, and release of E2F transcription factor 1, and it prevents terminal differentiation by affecting IRF4 and

PRDM1 expression.^{109,110} Overall, EZH2 acts to maintain key features associated with GC B cells and in particular, with the DZ compartment.

The *EZH2* gene is targeted by gain-of-function mutations in GCB DLBCL.¹¹¹ Mice engineered to express the *Ezh2* mutant develop GC hyperplasia that, in the presence of dysregulated *Bcl2* expression, evolves into lymphomas, consistent with the co-occurrence of these genetic lesions in GCB DLBCL.^{107,108}

CREBBP and EP300

CREBBP and EP300 are acetyl transferases that are ubiquitously expressed and catalyze lysine acetylation of both histone and non-histone proteins.¹¹² In the GC, CREBBP and EP300 display partially overlapping functions, as shown by the fact that only their co-deletion abrogates GC formation in mice.¹¹³ Although CREBBP and EP300 appear to compensate for each other during GC formation, they also affect distinct transcriptional programs. CREBBP appears to be more involved in the modulation of pathways related to the LZ, while EP300 affects preferentially DZ-associated genes.¹¹³ In particular, CREBBP appears to contrast the *BCL6* program by promoting acetylation, and thus transcriptional activation, at the promoters and enhancers of virtually all *BCL6* targets.^{114,115} CREBBP-mediated *BCL6* acetylation and inactivation provides an additional layer of negative regulation on the GC program.⁵²

CREBBP and, less frequently, EP300, are genetically inactivated in FL and DLBCL. The genetic alterations usually target only 1 allele of either *CREBBP* or *EP300* in a mutually exclusive fashion.^{116,117} Although GC-specific loss of *Crebbp* in mice is insufficient to induce lymphomagenesis, it leads to lymphoma development when combined with *Bcl2* dysregulated expression, consistent with the co-occurrence of these genetic lesions in the human malignancies.^{114,115}

KMTs2D

Lysine methyltransferase 2D (KMT2D) is a histone methyltransferase that catalyzes mono- and di-methylation of lysine at position 4 of histone H3.¹¹⁸ These modifications characterize competent enhancers and transcriptionally active chromatin. In GC B cells, KMT2D is involved in modulating the expression of genes related to apoptosis, proliferation, CD40 signaling, and cell migration.^{119,120}

Inactivation of KMT2D is a highly recurrent genetic lesion in FL (about 80% of cases) and DLBCL (about 30% of cases).^{116,121} Interestingly, KMT2D inactivation appears to be a very early event in FL pathogenesis, suggesting that prior changes in the epigenome, perhaps in combination with the dysregulated expression of *BCL2*, may facilitate malignant transformation in the GC.^{122,123} Consistently, conditional loss of *Kmt2d* by expression of cre recombinase driven by CD19, but not by the GC-specific *Cγ1* promoter, leads to expansion of the GC compartment in mice.¹²⁰

Lymphoma cell-of-origin relating to GC B cells

The attempts to associate lymphoma cells to certain stages of normal B-cell development were initially based on the analysis of morphological and immune phenotypic features and were later complemented by BCR characterization. Based on these observations, most types of B-cell lymphoma have been shown to originate from either GC or post-GC B cells, and they consistently carry somatically hypermutated BCRs. Further advances in the characterization of the transcriptome, genome, and epigenome of B-cell lymphomas have led to a more detailed

characterization of lymphoma cell-of-origin, expanding the number of lymphoma subtypes that are identified.

BL, FL, and DLBCL are the most common B-cell lymphomas, the normal counterpart of which is found in the GC. BL originates from the malignant transformation of DZ GC B cells, of which they retain the fast proliferative rate, the highly mutated BCR, and a defined transcriptional signature⁶⁴ (Figure 3).

The GC origin of FL is indicated by several features including: its appearance as a follicular-like structure, at least in the early stages; the expression of specific GC markers, such as CD10 and *BCL6*; the presence of mutated BCR with evidence of ongoing SHM activity; and the presence of a transcriptional signature related to LZ GB B cells⁶⁴ (Figure 3). Although FL clearly arise from the malignant transformation of GCB cells, it is recognized that the transformation process starts at earlier stages of differentiation and requires multiple events before the final step of clonal expansion occurring in the GC. The t(14;18) translocation, representing the hallmark of FL, is a byproduct of V(D)J recombination occurring in bone marrow B-cell precursors that then move through the differentiation process and enter the GC reaction. In the GC, they display a survival advantage due to the ectopic *BCL2* expression and are selected as memory B cells that can undergo multiple GC transits and be exposed to the risk of accumulating additional genetic alterations ultimately driving malignant transformation.¹²⁴

DLBCL are the most heterogeneous group of GC-derived lymphomas that are characterized by diverse phenotypic, molecular, and clinical features. Based on transcriptional signatures, DLBCL have been classified in at least 2 subgroups: the GCB- and the ABC-DLBCL that appear to originate from either cells at the LZ stage or from GC B cells committed to post-GC differentiation, respectively (Figure 3).^{8,15,64} The GCB and ABC subtypes are also associated with distinct genetic lesions,¹²⁵⁻¹²⁷ suggesting that they rely on different mechanisms of malignant transformation. Indeed, common pathways altered in GCB DLBCL aim to enhance the GC program, to restrain apoptosis, and to interfere with GC confinement, while ABC DLBCL rely on promoting activation of NF-κB and impairing differentiation. These DLBCL subtypes also display different responses to therapy, with a significantly more favorable prognosis in the GCB patients.¹⁵ Based on these numerous evidences, GCB- and ABC-DLBCL are now identified as distinct molecular subtypes in the World Health Organization classification of lymphoid neoplasms. Nonetheless, more heterogeneity appears to be embedded under the DLBCL umbrella. A subset of DLBCL are characterized by a double-hit (DHIT) signature that is driven by *MYC* and *BCL2* (and/or *BCL6*) dysregulation. Although often classified as GCB, the DHIT DLBCL appear to have a DZ cell-of-origin and display poor outcome¹²⁸ (Figure 3). In addition, recent data dissecting DLBCL cell-of-origin based on single-cell transcriptomic analysis of GC B cells suggest that DLBCL normal counterparts can be found at every stage during GC development.⁸ A full integration of the recent efforts aiming to classify DLBCL based on their transcriptomic and genomic¹²⁵⁻¹²⁷ features will be instrumental to fully characterize this heterogeneous group of malignancies.

The malignant transformation of GC B cells

Several characteristics of GC B cells are easily found as features of tumor cells (Figure 4). GC B cells implement risky behaviors to acquire high affinity BCR, and the GC can become a supportive environment for malignant transformation.

DZ GC B cells display an extremely high rate of proliferation, a feature shared by aggressive lymphomas, in particular BL, which displays an extremely rapid duplication rate.

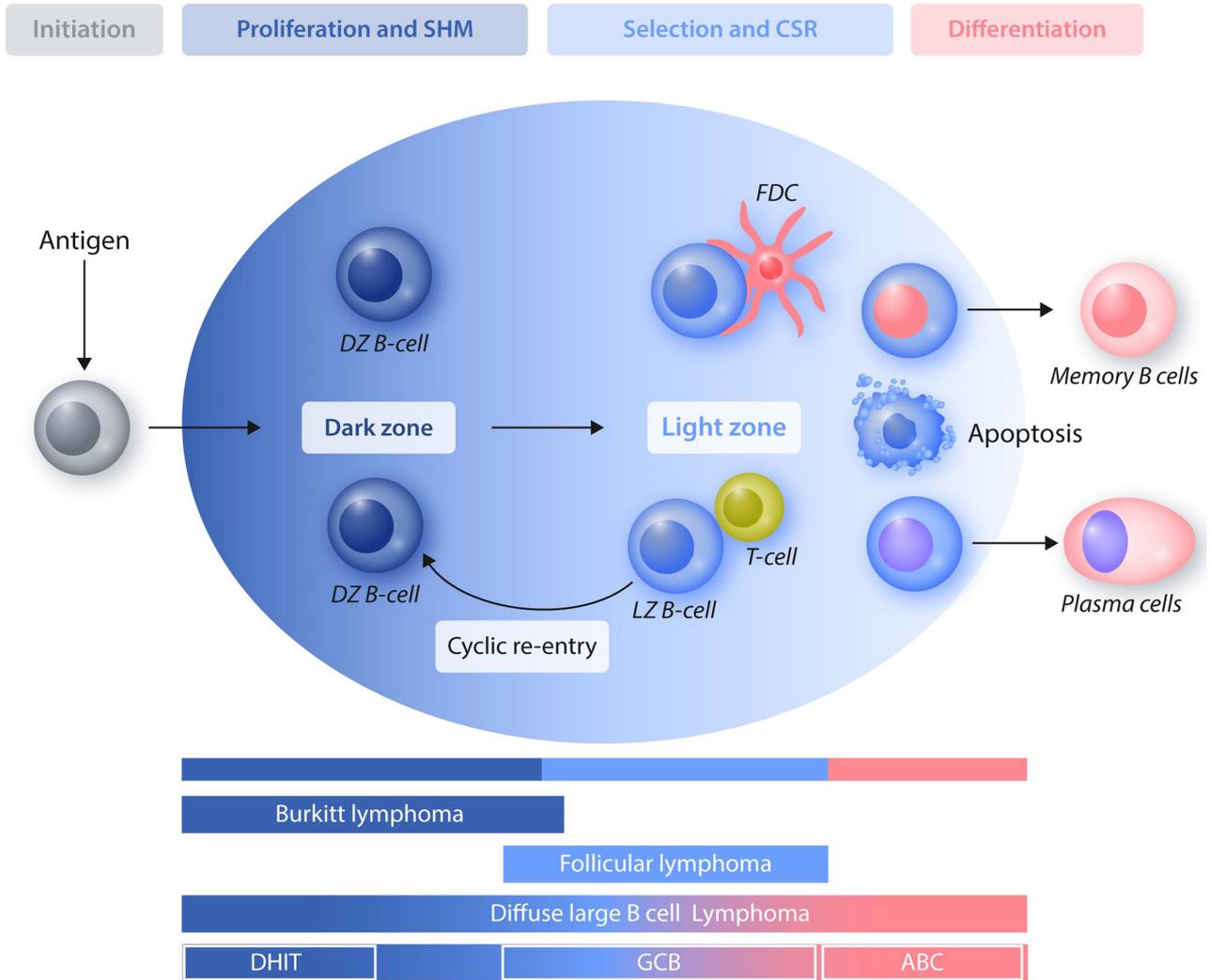


Figure 3. Cell-of-origin of GC-derived lymphomas. Distinct types of GC-derived lymphomas originate from cells that are blocked at different stages of maturation. Burkitt lymphomas resemble DZ B cells, follicular lymphomas and GCB-DLBCL originate from LZ B cells, and ABC-DLBCL display characteristics of late GC B cells that are committed to post-GC differentiation. A subset of DLBCL carrying a DHIT signature related to aberrant expression and/or activity of MYC proto-oncogene, bHLH transcription factor and BCL2 apoptosis regulator (and/or BCL6 transcription repressor) appear to originate from DZ GC B cells. ABC = activated B-cell like; CSR = class switch recombination; DHIT = double-hit; DLBCL = diffuse large B-cell lymphoma; DZ = dark zone; GC = germinal center; GCB = GC B-cell like; LZ = light zone; SHM = somatic hypermutation.

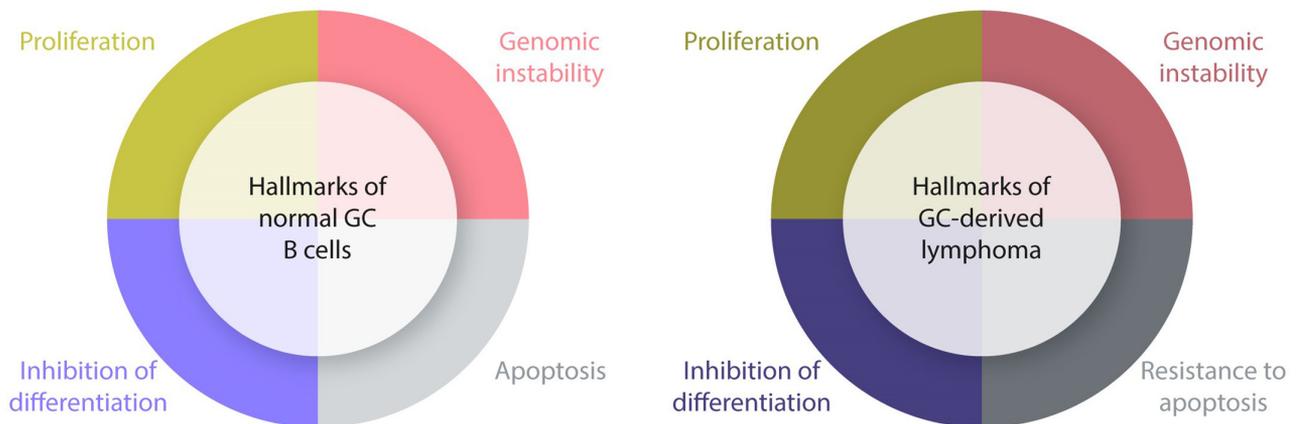


Figure 4. Hallmarks of normal and malignant GC B cells. This scheme summarizes the key features of normal (left) and malignant (right) GC B cells. The proliferative phenotype, the propensity to tolerate DNA breaks, and mechanisms aiming to inhibit terminal differentiation represent features that are often found in GC-derived lymphomas. The sensitivity of GC to apoptosis is overcome in lymphomas by diverse mechanisms including dysregulated expression of anti-apoptotic molecules (ie, BCL2 apoptosis regulator) and enforced activation of signaling pathways that promote survival. GC = germinal center.

GC B cells are targeted by SHM and CSR that are necessary to refine the affinity and functionality of the BCR but, at the same time, these physiologic DNA breaks provide a potentially deleterious environment for the generation of genetic lesions. In addition, for the immunoglobulin remodeling to occur, GC B cells implement a program aiming to impair the DNA damage response, providing the ideal setting for the accumulation of genetic lesions.

In the context of their risky behavior, GC B cells are efficiently programmed to die by apoptosis unless rescued by specific signals in the LZ. In order for lymphomagenesis to occur, cells must acquire mechanisms to overcome the pro-apoptotic GC program either by ectopic expression of anti-apoptotic molecules (ie, BCL2) or by enforcing the delivery of pro-survival signals that mimic those provided in the LZ.

Finally, GC B cells put on hold the process of terminal differentiation until the affinity-based selection is accomplished. Hacking the program that physiologically restrains GC B cells from differentiating is a dominant mechanism of malignant transformation.

Overall, GC B cells provide a suitable base for malignant transformation to occur, and lymphomagenesis has succeeded in figuring out ways to ensure that every detail is taken care of.

Disclosures

The author has no conflicts of interest to disclose.

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