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Pathogenesis of Human B Cell Lymphomas*

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Abstract

The mechanisms that drive normal B cell differentiation and activation are frequently subverted by B cell lymphomas for their unlimited growth and survival. B cells are particularly prone to malignant transformation because the machinery used for antibody diversification can cause chromosomal translocations and oncogenic mutations. The advent of functional and structural genomics has greatly accelerated our understanding of oncogenic mechanisms in lymphomagenesis. The signaling pathways that normal B cells utilize to sense antigens are frequently derailed in B cell malignancies, leading to constitutive activation of prosurvival pathways. These malignancies co-opt transcriptional regulatory systems that characterize their normal B cell counterparts and frequently alter epigenetic regulators of chromatin structure and gene expression. These mechanistic insights are ushering in an era of targeted therapies for these cancers based on the principles of pathogenesis.

Keywords

transcription factor; genomics; epigenetics; cancer; mutation; signaling

INTRODUCTION

The human mature B cell malignancies represent a medical challenge that is only partly met by current therapy, justifying concerted investigation into their molecular circuitry and pathogenesis. Reviews of individual lymphoma and leukemia subtypes have appeared recently (1–8). In the present review, our goal is to discuss general principles of molecular pathogenesis that are utilized recurrently by human mature B cell lymphomas and leukemias. Overarching themes include aberrant intracellular signaling, disordered transcriptional and epigenetic regulation, and immune evasion. The potent methods of functional and structural genomics have opened the genetic "playbook" of these malignancies, revealing genetic aberrations and oncogenic regulatory pathways that are responsible for these malignant phenotypes. Our ability to understand the pathogenesis of these malignancies has relied heavily on the rich literature regarding normal B cell biology.

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Increasingly, however, insights from B cell malignancies are revealing holes in our understanding of normal B cell function. Recent insights into lymphoma pathogenesis are leading to the rational development of targeted therapies that may achieve cures for more of these patients.

ORIGIN OF B CELL LYMPHOMAS

Some lymphoma subtypes can be distinguished using traditional clinical and pathological methods (9), but others are histologically indistinguishable and require gene expression profiling to be discerned (10) (Figure 1a). As is discussed in detail below, these lymphoma subtypes are characterized by recurrent genetic abnormalities and pathway dependencies that are present in a substantial fraction of cases (Figure 2). Each lymphoma subtype bears a phenotypic resemblance to B cells at a particular stage of differentiation, as judged by the presence or absence of immunoglobulin (Ig) variable (V) region mutations and by gene expression profiling (Figure 1b). This "normal B cell counterpart" has often been termed the "cell of origin" of a lymphoma, which we argue below is misleading terminology. The pre–germinal center mature B cell appears to be the normal B cell counterpart of most cases of mantle cell lymphoma, which have unmutated Ig V regions. One subtype of small lymphocytic lymphoma (SLL) and its leukemic relative, chronic lymphocytic leukemia (U-CLL), has unmutated V regions, and thus is pre–germinal center in derivation (11, 12), although gene expression profiling suggests an encounter with antigen (13, 14). The other CLL subtype has mutated Ig V regions (M-CLL) (11, 12) and thus may derive from a post–germinal center B cell (15). The germinal center B cell is the normal counterpart of follicular lymphoma, Burkitt lymphoma, and the germinal center B cell–like (GCB) subtype of diffuse large B cell lymphoma (DLBCL) (10, 16, 17). The activated B cell-like (ABC) subtype of DLBCL resembles post–germinal center plasmablasts (10, 16). Mucosa-associated lymphoid tissue (MALT) lymphomas are extranodal in origin and phenotypically related to post–germinal center marginal zone B cells (18). Hairy cell leukemia has mutated Ig genes and Ig heavy chain class switching, along with a gene expression profile resembling a post–germinal center memory B cell (19). Two lymphoma subtypes that arise in the thymus—primary mediastinal B cell lymphoma (PMBL) and nodular sclerosis classical Hodgkin lymphoma (HL)—may derive from a rare subset of B cells that resides in this organ (20).

The actual cell of origin for a lymphoma may be a B cell at an earlier stage of differentiation than its normal B cell counterpart. This can occur if the initiating oncogenic event happens early in hematopoietic development but allows further differentiation to take place before subsequent oncogenic hits are sustained. A fascinating case in point is CLL, a mature B cell neoplasm that may have the hematopoietic stem cell (HSC) as its cell of origin (21). All CLL samples share a common gene expression signature, despite the existence of the aforementioned CLL subtypes, suggesting a common pathogenesis for all cases (13, 14). Immunodeficient mice that receive HSCs from CLL patients develop mature B cells that are oligoclonal and have an lgV repertoire that is restricted to certain lgV subgroups, similar to human CLLs (see below) (21). Hence, CLL HSCs appear to harbor a genetic or epigenetic predisposition to develop a mature B cell lymphoproliferative disorder. In a similar vein, follicular lymphoma may originate from a bone marrow pre-B cell even though the

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malignant cells phenotypically resemble germinal center B cells. The t(14;18) translocation that defines follicular lymphoma is caused by the RAG recombinase, which is active in pre-B cells but not mature B cells. Most healthy adults have circulating B cells bearing the t(14;18) translocation, and surprisingly, these t(14;18)-bearing B cells reside within the memory B cell compartment, indicating that these B cells participated in a germinal center reaction (22). Clearly, the expression of BCL2 is not sufficient to transform B cells and apparently does not interfere with differentiation of premalignant B cells from the pre-B cell stage onward.

Many of the genetic lesions that initiate lymphomagenesis are the aberrant by-product of the enzymes that rearrange Ig segments to assemble the B cell receptor (BCR) in normal B cells. The mechanisms underlying translocations and somatic mutations caused by the RAG V(D)J recombinase and by activation-induced cytidine deaminase (AID) have been the subject of recent comprehensive reviews (23, 24). Here we instead focus on the signaling and regulatory pathways that lymphomas have borrowed from normal lymphocytes and misuse for their malignant purpose.

ONCOGENIC SIGNALING

Malignant B cells often commandeer normal B cell signaling pathways to sustain their growth and survival. This is accomplished through either gain-of-function mutations that activate signaling effectors, loss-of-function mutations that inactivate negative regulators of signaling, or autocrine receptor activation. Many oncogenic events in lymphomas converge on the antiapoptotic NF-κB pathway, but further survival signaling is provided by the phosphatidylinositol-3 kinase (PI3K) and JAK kinase pathways. Often, lymphomas simultaneously activate several interconnected signaling pathways, providing therapeutic opportunities and challenges (Figure 3).

Constitutive NF-κB Signaling

Perhaps the most common path taken by lymphoid malignancies to avoid cell death is constitutive activation of the NF-κB pathway (reviewed in Reference 25). NF-κB was first defined in B cells as a transcription factor regulating the expression of the Ig kappa light chain (26). It is now appreciated that this pathway is used by many normal cell types and cancers to promote cell survival. In B cells, NF-κB activation occurs transiently downstream of numerous receptors, including the BCR, CD40, the BAFF receptor, and various Toll-like receptors (TLRs). The lymphoid malignancies that have been associated with NF-κB most consistently are ABC DLBCL, HL, PMBL, gastric MALT lymphoma, and multiple myeloma. However, the potency of this pathway is such that sporadic cases belonging to other diagnostic categories can also activate NF-κB.

NF-κB refers to a family of transcription factors that are formed by homo- and heterodimerization of the subunits p65 (encoded by *RELA*), RelB, c-Rel, p50 (encoded by *NFKB1*), and p52 (encoded by *NFKB2*). These factors shuttle between the cytoplasm and the nucleus in a fashion that is regulated by diverse intracellular signaling events. The "classical" pathway of NF-κB activation utilizes an IκB kinase (IKK) complex consisting of α, β, and γ (NEMO) subunits. Activation of this IKK complex by upstream signals is

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complex, requiring the phosphorylation of IKKβ and the ubiquitination of IKKγ. Once active, IKKβ phosphorylates IκBα, an inhibitor that sequesters p50/p65 and p50/c-rel in the cytoplasm and also actively removes these transcriptional activators from chromatin. Phosphorylated IκBα is ubiquitinated by the βTrCP ubiquitin ligase and degraded by the proteasome, allowing NF-κB dimers to accumulate in the nucleus where they activate the transcription of target genes. The "alternative" pathway of NF-κB activation depends instead on an IKK complex consisting of two IKKα subunits. This form of IKK is typically activated by the kinase NIK, which is post-translationally controlled by a signaling pathway that regulates its stability (27, 28). This IKKα complex phosphorylates p100, the primary translation product of *NFKB2*, leading to its proteolytic processing into the NF-κB subunit p52, which pairs with RelB and travels to the nucleus. Both classical and alternative NF-κB pathways activate the transcription of an antiapoptotic transcriptional module, including the BCL2 family members BCL-XL and A1 as well as c-FLIP, c-IAP1, c-IAP2, and other proteins (29–32).

A recurring theme in lymphomas is the removal of negative regulators of NF-κB. Mutation and truncation of *IKBKA*, encoding IκBα, occurs in roughly one-quarter of HL cases, allowing constitutive migration of NF-κB dimers to the nucleus (reviewed in Reference 33). *TNFAIP3*, which encodes the NF-κB inhibitor A20, is inactivated by mutation, deletion, or epigenetic silencing in many lymphoma subtypes, including HL, PMBL, ABC DLBCL, and MALT lymphomas (34–37). A20, a deubiquitinating enzyme, inactivates IKKβ by removing regulatory K63-linked polyubiquitin chains from IKKγ. A20 is an NF-κB target gene and thus functions as a feedback inhibitor of NF-κB activation. Loss of A20 expression may not be sufficient to activate NF-κB, because the NF-κB-mediated inflammatory disease that develops in A20 knockout mice requires MyD88-mediated activation of NF-κB, presumably in response to commensal gut flora (38). Moreover, A20 inactivation often coexists with other genetic lesions that positively activate NF-κB, such as constitutive BCR and MYD88 signaling (39) (see below).

Oncogenic Engagement of NF-κB by the CARD11 Pathway

NF-κB activation through the classical pathway is a hallmark of the ABC DLBCL subtype (Figures 3, 4). Many NF-κB target genes are constitutively expressed in this DLBCL subtype compared with the GCB DLBCL subtype, and genetic inhibition of this pathway is lethal to ABC but not GCB DLBCL cell lines (40). Given the ability of NF-κB to antagonize the apoptotic action of chemotherapy (41), this observation may help to explain why ABC DLBCLs are more refractory to chemotherapy than are other DLBCL subtypes.

A functional genomics screen employing RNA interference revealed that the survival of most ABC DLBCL cell lines depends on the CBM complex, a signaling hub consisting of CARD11, BCL10, MALT1, and other proteins (42) (Figure 3). The CBM complex is required for the activation of IKKβ and the classical NF-κB pathway downstream of the antigen receptors in B and T lymphocytes (reviewed in Reference 43). CARD11 is a multidomain signaling adapter that contains an amino-terminal CARD and coiled-coil domains, an intervening linker domain, and a C-terminal MAGUK domain (Figure 4a). Prior to activation, CARD11 is located in the cytosol, where it is presumably kept in an inactive

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conformation through an intramolecular interaction between its coiled-coil and linker domains. PKCβ-dependent serine phosphorylation within the CARD11 linker domain is thought to relieve this intramolecular association (44). CARD11 is then able to translocate to the plasma membrane, where it binds BCL10 and MALT1. Subsequently, MALT1 recruits TRAF6, which ubiquitinates MALT1, TAB2, and IKKγ. Ubiquitinated TAB2 activates the kinase TAK1, which can then phosphorylate IKKβ in its activation loop. Full activation of IKK requires both phosphorylation and ubiquitination of IKKγ. RNA-interference screening identified casein kinase 1α (CK1α) as a new component of the CBM complex that is required for the survival of ABC DLBCL cells (45). CK1α has a dual function in this pathway. It is initially required for the formation of the CBM complex, a function that does not depend on its kinase activity. Thereafter, it attenuates NF-κB signaling by phosphorylating negative regulatory sites within the CARD11 linker domain.

Beyond its contribution to CBM-dependent IKK activation, MALT1 potentiates NF-κB signaling using its para-caspase domain (46), which is an unusual caspase-like protease that cleaves after arginine residues (47, 48). MALT1 proteolytically cleaves and inactivates A20 as well as CYLD, two negative regulators of NF-κB signaling (47, 49). Inhibition of MALT1 protease activity is lethal to ABC DLBCL cell lines and, therefore, represents an intriguing drug development target (50, 51).

Somatic mutations affecting the coiled-coil domain of CARD11 are present in ~10% of ABC DLBCL tumors, but they are less common in GCB DLBCLs and absent in other lymphoma subtypes (52) (Figure 4a). CARD11 coiled-coil domain mutations are also present in 16% of primary central nervous system lymphomas, a disease that shares gene expression features with ABC DLBCL (53). Inactivating mutations in the CARD11 coiled-coil domain had previously been identified in mouse mutagenesis studies, indicating that this domain is required for NF-κB signaling (54). By contrast, the lymphoma-derived coiled-coil mutants are gain of function, having the ability to stimulate NF-κB activity de novo and to render CARD11 hypersensitive to upstream signals (52).

Whereas wild-type CARD11 is diffusely distributed in the cytoplasm, these CARD11 coiled-coil domain mutants form discrete cytoplasmic clusters that colocalize with MALT1, CK1α, and the phosphorylated form of IKKβ, suggesting that these structures initiate NF-κB signaling (52). The degree to which each CARD11 mutant isoform aggregates correlates directly with its ability to activate the NF-κB pathway, supporting the view that these aggregates are coordinating centers for NF-κB signaling. It is worth noting that other MAGUK domain proteins also form microscopically visible proteinaceous structures, such as the postsynaptic density and the tight junction, presumably by forming a lattice-like structure. Hence, the lymphoma-derived CARD11 coiled-coil mutants may trigger the formation of a similar lattice-like structure, albeit in an unphysiological and deregulated fashion.

Mechanistically, mutations in the coiled-coil domain interfere with the association between the linker domain and the coiled-coil domain (55), potentially relieving the inhibitory influence of the linker domain and bypassing the need for PKCβ to phosphorylate and activate CARD11. Analysis of CARD11 mutations reported in multiple studies reveals hot

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spots that are more frequently mutated in lymphoma (Figure 4a). These hot spots may be points of contact with the linker domain, or they may regulate the three-dimensional structure of the coiled-coil domain so as to prevent linker domain interaction.

MALT1 is also central to the pathogenesis of gastric MALT lymphomas: Roughly one-quarter of cases harbor a t(11;18) translocation that fuses the N terminus of c-IAP2 to the C terminus of MALT1. This fusion protein activates NF-κB by recruiting TRAF6, leading to IKKβ phosphorylation and ubiquitination (56, 57). The association of c-IAP2-MALT1 with IKK is strengthened by a ubiquitin-binding domain in the c-IAP2 component, which latches on to the ubiquitinated IKKγ subunit (31). In addition, the cIAP2-MALT1 fusion protein oligomerizes, thereby stimulating the protease activity of the MALT1 component (58). A proteolytic target of cIAP2-MALT1 is NIK, a kinase that can activate the alternative NF-κB pathway (58). NIK is normally a highly unstable protein, but the cleavage product produced by cIAP2-MALT1 is stable, causing constitutive phosphorylation and activation of IKKα and engagement of the alternative NF-κB pathway. Hence, the development of inhibitors of MALT1 protease activity and/or NIK may open new treatment pathways for these patients.

B Cell Receptor Signaling

Despite accumulating Ig locus mutations and translocations, human malignant B cells typically maintain expression of the BCR on the cell surface, suggesting that they may utilize the ability of the BCR to engage downstream proliferation and survival pathways (reviewed in Reference 59). Arguably, the BCR is the organizing principle of B cell biology, playing an obligatory role in B cell development, antigen-driven clonal selection, and humoral immunity. The BCR consists of the antigen-binding IgH and IgL chains that are noncovalently coupled to the CD79A (Ig-α) and CD79B (Ig-β) subunits, which regulate BCR surface expression, internalization, and trafficking (reviewed in Reference 60). Upon antigen encounter and BCR clustering, CD79A and CD79B transmit signals to a variety of downstream signaling pathways. Each of these subunits has an immunoreceptor tyrosine-based activation motif (ITAM) consisting of two tyrosines in a conserved amino acid setting (61). BCR aggregation promotes tyrosine phosphorylation of these ITAMs by Src-family kinases, principally LYN, FYN, and BLK. Dually phosphorylated ITAMs recruit SYK through interactions with its tandem SH2 domains (62). SYK activation initiates a signaling cascade that engages the NF-κB, PI3K, NF-AT, MAP kinase, and RAS signaling pathways, leading to cell survival and proliferation (reviewed in Reference 63).

Conditional ablation of either IgM (64) or CD79A (65) in mature B cells in the mouse results in the demise of all mature B cells in the spleen and lymph nodes over the course of several weeks. Moreover, substitution of CD79A with a truncated version lacking the ITAM reconstitutes surface BCR expression but does not support the survival of mature B cells, directly implicating BCR signaling in the maintenance of mature B cells (65). These observations led to the notion of "tonic" BCR signaling, which is a BCR-dependent process that does not require antigen engagement (64). In mice with conditional deletion of IgM, concomitant expression of a constitutively active p110α subunit of PI3K rescues mature B cell survival (66). Tonic BCR signaling thus appears to depend on PI3K-mediated activation of the kinase AKT, which coordinates several downstream survival pathways. By contrast,

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constitutive NF-κB signaling, mediated by an activated form of IKKβ, is unable to rescue B cell survival following BCR ablation (66). However, strong NF-κB pathway activation via the CBM complex is apparently required for the differentiation and/or survival of B-1 and marginal zone B cells, because mice lacking CBM components also lack these subpopulations but have relatively normal numbers of follicular B cells (reviewed in Reference 67).

Mouse lymphoma models support the notion that BCR-derived survival signaling can drive malignant transformation. Eμ-MYC mice, which possess a MYC transgene under the control of the IgH enhancer, develop tumors that are most often derived from pre-B cells (68). When these mice are crossed with transgenic mice expressing a BCR specific for hen egg lysozyme (HEL), more aggressive lymphomas of mature B cell origin are produced, and lymphoma development is further hastened by transgenic expression of HEL, the cognate antigen for this BCR (69). Ex vivo ablation of CD79A (69) or SYK (70) kills lymphoma cells derived from these mice, in the presence or absence of cognate antigen. These results implicate both tonic and antigen-dependent BCR signals in lymphomagenesis.

Indirect evidence of a role for the BCR in human lymphomas comes from analysis of Ig V regions. IgH and IgL V regions are highly mutated in Burkitt lymphoma (71, 72), MALT lymphoma (73, 74), and DLBCL (75). In some instances, the mutational profile is uniform within the malignant clone, whereas, in others, somatic hypermutation (SHM) of the V region is ongoing. For instance, the GCB DLBCL subtype has ongoing Ig V region mutation, whereas the ABC DLBCL subtype has a fixed load of Ig V region mutations (76). An excess of replacement mutations in the complementarity-determining regions compared with the framework regions of the BCRs supports the notion that malignant B cells are selected to preserve the integrity of the BCR (77, 78).

IgH V region repertoire analysis in CLL cells revealed that these malignant B cells utilize V segments different from those used by nonmalignant B cells (79). Almost one-third of CLL samples possess “stereotyped” BCRs, which are nearly identical receptors that arise independently in different patients (80, 81). Stereotyped BCRs were also recently described in 10% of mantle cell lymphomas (82). These stereotyped receptors often exactly preserve the junctions between V, D, and J segments in both the Ig heavy and light chain, suggesting selection by an antigen. Indeed, many CLL BCRs, particularly U-CLL BCRs, recognize autoantigens present on dead or dying cells (83, 84). CLL cells may encounter such antigens in the lymph node microenvironment because CLL cells isolated from this compartment show evidence of BCR engagement by both gene expression profiling and phosphoprotein analysis (85). In theory, CLL cells may receive transient BCR signals in the lymph node that promote their proliferation as well as their survival while they migrate through the bloodstream.

Viral and bacterial agents in the tumor microenvironment contribute to the pathogenesis of several types of lymphoma, especially those derived from marginal zone B cells (reviewed in Reference 86). Gastric MALT lymphoma is frequently associated with *Helicobacter pylori* infection and spontaneously resolves in ~75% of patients following antibiotic treatment to eradicate the bacteria (87). MALT lymphomas with the t(11;18) translocation are resistant to

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antibiotic treatment, presumably because the cIAP2-MALT1 fusion protein activates NF-κB signaling constitutively (18). MALT lymphoma B cells do not seem to recognize bacterial antigens with high affinity. Instead, the BCRs of some MALT lymphomas have rheumatoid factor activity and may, therefore, be activated by antigen-antibody complexes generated by a polyclonal B cell response to the bacteria (88). Another analysis of both human and mouse MALT lymphomas suggests that the BCRs are polyreactive to diverse antigens (89). The malignant B cells may also receive signals from T cells responding to the bacteria within the tumor microenvironment (90).

Likewise, viral antigens may also promote BCR signaling. Hepatitis C virus (HCV) infection has been causally linked to the development of splenic marginal zone lymphoma (SMZL) (reviewed in Reference 91). Treatment with type I IFN to resolve HCV infection results in tumor remission in more than 75% of patients with HCV-related SMZL lymphoma (92, 93). Moreover, the BCR of an HCV-related lymphoma directly binds the HCV-E2 envelope glycoprotein, indicating that HCV proteins may directly act as an antigen (94).

Many germinal center and post-germinal center B cell lymphomas selectively retain expression of an IgM BCR. Naive, mature B cells express an IgM BCR and/or an IgD BCR and typically switch to an IgG BCR within the germinal center. In follicular lymphoma, the canonical t(14;18) translocation places BCL2 under control of the heavy-chain locus, destroying the ability of the affected IgH allele to transcribe an IgH chain. In most cases, the IgH allele with a productive V(D)J rearrangement remains IgM, even though the BCL2-translocated IgH allele has undergone class switch recombination (CSR) (95). Although it is possible that follicular lymphomas use the surface IgM to bind to a self or foreign antigen, analysis of Ig V region sequences revealed the frequent acquisition by SHM of a site for N-linked glycosylation (96). The BCRs in such cases have sugars attached via these sites, which may bind to lectins on the surface of immune cells in the tumor microenvironment, potentially causing BCR clustering and signaling (97).

In DLBCL, the ABC subtype often expresses an IgM BCR, whereas the GCB subtype preferentially expresses an IgG BCR (98). ABC DLBCLs maintain IgM BCR expression apparently by blocking CSR as a result of a variety of internal deletions of the Sμ and Sγ switch regions (98). These Sμ deletions predominantly occur on the productive IgH allele, whereas the nonproductive allele is usually Ig class switched (99). These results are consistent with positive selection for an IgM BCR, potentially because IgM BCRs and IgG BCRs deliver qualitatively different signals (100–103). Membrane IgM possesses only two amino acids on its cytoplasmic end; thus, most signals from IgM BCRs emanate from CD79A and CD79B. Membrane IgG has a relatively long C-terminal tail jutting into the cytosol, containing both a PDZ domain binding motif and a tyrosine phosphorylation site, allowing it to interact differentially with signaling effectors (100, 104) and to avoid negative regulation by CD22 (105). Signals downstream of the IgG BCR are qualitatively different from those coming from the IgM BCR. IgG BCRs produce strong ERK, MAPK, and calcium responses that promote differentiation of B cells into marginal zone B cells and plasma cells (101–103). By contrast, IgM BCRs deliver mitogenic signals and have a lower propensity to induce differentiation. Hence, follicular lymphomas and ABC DLBCLs may

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retain IgM BCR signaling because it promotes proliferation and survival of the malignant clone rather than terminal plasmacytic differentiation.

The molecular and genetic foundation of pathogenic BCR signaling in lymphoma is perhaps best understood in the case of ABC DLBCL, which utilizes a "chronic active" form of BCR signaling (106) (Figure 3). The aberrant activation of NF-κB in this subtype can be explained in ~ 10% of cases by activating mutations in CARD11 (52). However, ABC DLBCLs with wild-type CARD11 nonetheless bear a gene expression signature of NF-κB activation (40, 52), and ABC DLBCL cell lines with wild-type CARD11 still depend on CARD11 for survival (42). An RNA interference screen revealed that ABC DLBCL lines with wild-type CARD11 require Bruton's tyrosine kinase (BTK) for survival, but this is not the case for an ABC DLBCL line with mutant CARD11 or for GCB DLBCL lines (106). BTK is a tyrosine kinase that functions downstream of the BCR to activate IKK and the classical NF-κB pathway (107). BTK is recruited to the plasma membrane via its PH domain, which recognizes phosphatidylinositol (3,4,5)-trisphosphate (PIP3) that is generated as a consequence of BCR-dependent PI3K activation (108). Once recruited to the BCR signaling complex, LYN or SYK can phosphorylate and activate BTK (108). BTK forms a complex with the adapter BLNK and phosphorylates phospholipase C γ2 (PLCγ2), activating this enzyme to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) into diacyl glycerol (DAG) and inositol trisphosphate (IP3). IP3 induces release of calcium stores via the IP3 receptor on the endoplasmic reticulum. Together, calcium and DAG activate PKCβ, leading to phosphorylation of CARD11 and activation of NF-κB. Consistent with this model, a selective BTK kinase inhibitor is toxic to ABC DLBCL cell lines with wild-type CARD11 (106).

The importance of BTK in normal BCR signaling led to the discovery that the BCR is required for the survival of ABC DLBCL lines with wild-type CARD11 (106) (Figure 3). Knockdown of any BCR component (IgH, IgL, CD79A, or CD79B) kills these ABC DLBCL lines, as does knockdown of effectors acting downstream of the BCR (SYK, BLNK, PLCγ2, PI3Kδ, and PKCβ). Abrogation of BCR signaling in ABC DLBCL diminishes NF-κB, AKT, ERK, MAPK, and NF-AT signaling, in keeping with the known activities of the BCR in normal B cells (106). These results suggest that ABC DLBCLs rely on multiple pathways to survive, which could be exploited therapeutically using rational combinations of signaling inhibitors (see below).

Examination of ABC DLBCL lines and patient samples using total internal reflection fluorescence microscopy revealed that their surface BCRs form prominent clusters that are immobile, a phenotype that sets them apart from cell-line models of other lymphoma subtypes (106). These BCR clusters are reminiscent of those on normal B cells shortly following antigen stimulation (109). By contrast, the BCRs in GCB DLBCL cell lines are diffusely distributed in the plasma membrane (106), akin to a resting normal B cell (109). BCR clusters on ABC DLBCL lines coincide with phosphotyrosine staining, indicating that they are sites of active signaling (106). It is not clear at present what causes the BCRs to cluster in ABC DLBCL. One possibility is the presence of an antigen. Because BCR clusters are observed in cell lines and patient samples removed from the tumor microenvironment, a self-antigen expressed within the tumor cell is a possibility. Alternatively, the BCR

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clustering in ABC DLBCLs could be antigen independent, perhaps reflecting changes in the structure of the BCR or in other coreceptors that can influence clustering (110, 111).

Chronic active BCR signaling in ABC DLBCL is distinct from tonic BCR survival signaling in normal mature B cells by several criteria. First, chronic active BCR signaling engages NF-κB through the CBM complex (106), which is also a hallmark of antigen-stimulated BCR signaling in normal mature B cells (63). Conversely, tonic BCR signaling does not rely on NF-κB signaling, but rather on PI3K-dependent pathways (66). Second, given the dependence of ABC DLBCLs on the CBM complex, it is notable that CBM components are essential only in marginal zone and B-1 B cells in the mouse (67), whereas tonic BCR signaling is required for the survival of all mature B cells (64). Third, resting B cells that rely on tonic BCR survival signaling have diffusely distributed BCRs, in contrast to the clustered BCRs in ABC DLBCLs. Thus, BCR signaling in ABC DLBCL is more akin to antigen-stimulated BCR signaling than to tonic BCR signaling.

It is certainly conceivable that some human lymphomas depend on tonic BCR signaling rather than on the more active form of BCR signaling that characterizes ABC DLBCL. Some GCB DLBCL cell lines are sensitive to a pharmacologic inhibitor of SYK, leading to the proposal that they depend on tonic BCR signaling (112). However, several cell-line models of GCB DLBCL are not affected by knockdown of the BCR or downstream signaling components (106). This apparent discrepancy may relate to the fact that the SYK inhibitor used also targets other kinases (113, 114), or it may reflect differences between lymphoma cell lines. The search for lymphoma cell lines that depend on tonic BCR signaling should rely on BCR knockdown experiments as well as biochemical studies, such as analysis of the AKT phosphorylation that characterizes tonic BCR signaling in mouse B cells (66).

More than 20% of ABC DLBCL tumors have mutations affecting the ITAMs of CD79B and CD79A, providing the genetic “smoking gun” that chronic active BCR signaling is of pathogenetic importance in this lymphoma subtype (106). A majority of these mutations alter the first ITAM tyrosine residue in CD79B by substitutions of various other amino acids or by deletion of this residue altogether (Figure 4b). Less frequently, other conserved residues in the CD79B ITAM are mutated in ABC DLBCL, and occasionally, the ITAM of CD79B or CD79A can be partially or completed deleted. Mutations in CD79A/B are rarely, if ever, observed in GCB DLBCL, Burkitt lymphoma, or mantle cell lymphoma. It is important to note that any given ABC DLBCL tumor retains at least one complete ITAM on either CD79A or CD79B, presumably to serve as a signaling platform for SYK and other effectors to bind. Unlike the CARD11 coiled-coil domain mutations in ABC DLBCL, the CD79B mutations do not produce a pronounced phenotype when overexpressed in heterologous cell types and, in particular, do not drive BCR clustering (106). Nevertheless, it is evident that the CD79 mutations are gain-of-function “driver” mutations by several criteria: (*a*) They recurrently affect one amino acid required for signaling, (*b*) they occur preferentially in one cancer subtype, and (*c*) the survival of ABC DLBCLs can be sustained by the mutant form of CD79B (106).

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Insight into the function of the CD79B mutants is provided by studies of mice in which the signaling tails of CD79B or CD79A have been deleted or mutated (115–117). Somewhat contrary to expectation, these mice develop mature B cells and appear to have enhanced BCR signaling. In the case of knockin mice in which both ITAM tyrosine residues of CD79B are mutated to alanine, mature B cells produce an exaggerated response to T-independent antigens, and the mice have elevated levels of circulating IgM (115). B cells from these mice also have increased surface IgM levels relative to wild-type littermates owing to decreased BCR internalization (115). Anti-IgM stimulation of the mutant B cells evokes prolonged phosphotyrosine and phospho-AKT responses compared to wild-type B cells, even though the levels of phospho-Src (i.e., phospho-Lyn) and phospho-Syk are decreased in these mutant B cells (115). In mice with comparable substitutions (118) or mutations (117) in CD79A, B cells also have higher levels of surface IgM, potentially implicating similar mechanisms.

Several parallels exist between the phenotype of these mutant mice and the effect of CD79B mutations in ABC DLBCL. First, the mutant CD79A and B isoforms increase surface BCR levels in ABC DLBCL (106). Interestingly, a CD79B isoform in which the second ITAM tyrosine is mutated, which has never been observed in a patient sample, does not increase surface BCR expression, pointing to a selective role of the first ITAM tyrosine of CD79B (106). Second, pharmacologic inhibition of Src kinase activity increases BCR surface expression in ABC DLBCL cells expressing wild-type CD79B to levels observed with mutant CD79B, presumably by altering the internalization of the BCR (106).

As with most receptors, BCR signaling evokes several feedback mechanisms to limit the extent and duration of signaling. Among the Src-family kinases, LYN acts as both a positive and negative regulator of BCR activity. LYN initiates BCR signaling by phosphorylating ITAM tyrosines following BCR aggregation. However, LYN is unique among Src-family kinases in its ability to limit BCR activation by phosphorylating immunoreceptor tyrosine-based inhibitory motifs (ITIMs) present on FcγRIIb or CD22 (reviewed in Reference 119). Phosphorylated ITIMs recruit SHP-1, which can dephosphorylate CD79A/B ITAMs and quench BCR signaling. As evidence of LYN’s potent negative role in BCR signaling, LYN knockout mice develop a B cell-driven autoimmune disease as a result of uncontrolled BCR activity (120). In ABC DLBCL cell lines expressing mutant CD79B, LYN kinase activity is lower than in cells expressing wild-type CD79B (106, 121). LYN promotes BCR internalization in normal B cells (122), suggesting that the reduced activity of LYN in ABC DLBCLs with mutant CD79B may contribute to their increased surface BCR expression and constitutive BCR signaling.

An attractive model to explain the selection of CD79A/B mutations in ABC DLBCLs postulates that these mutations may allow a premalignant B cell to escape anergy. B cell anergy is a state of cellular quiescence defined by attenuated BCR signaling and low levels of surface BCR (123, 124). Self-reactive B cells are silenced by clonal deletion, receptor editing, or anergy (125, 126). Autoreactive B cells, particularly those with a low affinity toward their cognate antigen, can evade receptor editing and clonal deletion in the bone marrow and migrate to the periphery. Chronic contact with either foreign or self-antigen can push these cells into anergy. It has been estimated that 20% of naive mature human B cells

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express autoreactive BCRs (127) and 30% have an anergic phenotype (128). LYN kinase activity in mature follicular B cells promotes peripheral self-tolerance through anergy (129). Hence, by suppressing LYN kinase activity, CD79B mutants may allow a self-reactive B cell to avoid anergy and clonally expand in the germinal center microenvironment. There, the premalignant cell may mutate its BCR, potentially improving its affinity for a self-antigen, allowing further clonal expansion. Full-blown ABC DLBCL tumors necessarily acquire a variety of further oncogenic hits to promote their malignant growth and survival (Figure 2).

Recurrent Oncogenic Mutations in MYD88

The involvement of innate immune signaling pathways in the pathogenesis of lymphomas was uncovered by an RNA interference screen, in which knockdown of the signaling adapter MYD88 proved toxic to ABC DLBCL lines, but not to cell-line models of other lymphoma subtypes (39) (Figure 3). MYD88 is an adapter protein that couples TIR-containing receptors, such as the TLRs, to a variety of downstream signaling circuits, including the NF-κB, p38 MAP kinase, and type I interferon pathways (130). MYD88 accomplishes this by coordinating the IRAK-family kinases into a helical signaling complex via interaction between death domains found in MYD88 and the IRAK kinases (131). IRAK4 phosphorylates IRAK1, allowing recruitment of the ubiquitin ligase TRAF6 and the engagement of downstream pathways. In keeping with this model, ABC DLBCL lines also depend on IRAK1 and IRAK4 for survival (39). IRAK4 kinase activity is essential in ABC DLBCL cell lines, but IRAK1 kinase activity is not required. This parallels other settings in which IRAK1 can sustain NF-κB signaling independent of its kinase activity, suggesting that it serves as a signaling adapter (132, 133). Small-molecule inhibitors of IRAK4 kinase are selectively toxic to ABC DLBCL lines, opening up therapeutic opportunities (see below).

High-throughput RNA sequencing of lymphomas revealed recurrent oncogenic mutations in MYD88 (39). Nearly 30% of ABC DLBCL tumors possess a single nucleotide substitution in the TIR domain of MYD88 that replaces leucine at position 265 with proline (L265P) (39) (Figure 4c). This mutation is rare or absent among GCB DLBCL, PMBL, and Burkitt lymphoma tumors, but it is present in 9% of gastric MALT lymphoma biopsies (39). MYD88 L265P is also present in 3% of CLL cases, occurring preferentially in younger patients with aggressive disease (134). Other recurrent mutations affecting the MYD88 TIR domain are present in an additional 10% of ABC DLBCL tumors and are present at a similar frequency in GCB DLBCL tumors. The L265P mutation occurs at a highly conserved residue in a β-sheet at the hydrophobic core of the TIR domain, and another mutation occurs at a structurally adjacent methionine residue (M232T) (Figure 4c). These mutations would be predicted to distort the tertiary structure of the TIR domain. Many of the other MYD88 mutations cluster in the “B-B loop” of the TIR domain, a region that participates in interactions with TLR TIR domains (135), suggesting that TLRs may play a role in pathogenic MYD88 signaling in lymphomas. Indirect evidence in favor of this view comes from experiments with CLL cells exposed to a variety of TLR agonists. CLL cells with the MYD88 L265P mutation secrete greater amounts of cytokines and chemokines than do CLL cells with wild-type MYD88 (134). However, the biochemical and functional relationship between the MYD88 mutants and specific TLRs has not yet been established.

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MYD88 mutations in lymphomas are oncogenic and gain of function. All extant ABC DLBCL cell lines harbor a MYD88 mutation, generally L265P. These lines die upon MYD88 knockdown and can be rescued by the MYD88 L265P mutant but not by wild-type MYD88, demonstrating the oncogenic potential of this mutant (39). Ectopic expression of mutant isoforms of MYD88 in GCB DLBCL lines induces NF-κB activity to varying degrees, whereas wild-type MYD88 has little effect (39). Some other MYD88 mutations are capable of activating NF-κB as well as MYD88 L265P, suggesting that other features of MYD88 L265P beyond NF-κB activation account for its more frequent selection in lymphoid malignancies. Biochemically, the MYD88 L265P isoform nucleates the formation of a tight complex involving IRAK4 and phosphorylated IRAK1, which is not observed with wild-type MYD88 (39). The existence of a stable, phosphorylated form of IRAK1 in this setting is remarkable because phosphorylated IRAK1 is normally rapidly ubiquitinated and targeted for proteasomal degradation following association with MYD88 in normal cells (136). Of note, the other MYD88 mutant isoforms do not form this stable complex detectably, perhaps explaining why the L265P mutant plays the most prominent role in lymphoma pathogenesis.

In ABC DLBCL, MYD88 L265P upregulates gene expression signatures of NF-κB, JAK-STAT3, and type I interferon signaling (39). MYD88 L265P signaling induces secretion of IL-6 and IL-10, two cytokines that are produced in roughly half of ABC DLBCL cases (137, 138). Binding of these cytokines to their respective receptors activates a JAK-family kinase to phosphorylate STAT3, causing STAT3 to enter the nucleus and activate a large set of genes (137). STAT3 potentiates NF-κB signaling in ABC DLBCLs (137), presumably due to a physical interaction between STAT3 and NF-κB dimers (139). Accordingly, a JAK kinase inhibitor synergizes with an IKKβ inhibitor in killing ABC DLBCL cell lines (137). The presence of a MYD88 mutation was significantly associated with the subgroup of ABC DLBCL tumors with STAT3 activation (39). However, some ABC DLBCL tumors display the gene expression signature of STAT3 activity but yet have wild-type MYD88, raising the interesting possibility that, in the absence of a MYD88 mutation, some ABC DLBCL tumors may use TLRs to engage wild-type MYD88.

Oncogenic MYD88 signaling in ABC DLBCL also causes IFNβ secretion and subsequent activation of type I interferon target genes (106). It is conceivable that ABC DLBCL cells benefit from the production of IFNβ, perhaps by inhibiting and/or altering immune cells in the tumor microenvironment. Type I interferon enhances expression of TLR7 and TLR9 (140, 141), which could potentiate MYD88 signaling in a feed-forward fashion if oncogenic MYD88 isoforms rely on TLRs. Alternatively, the production of type I interferon may be the "cost of doing business" with MYD88 mutant isoforms, originating from the normal function of MYD88 to induce type I interferon production in response to TLR ligands.

Genetic and functional data suggest that chronic active BCR signaling and MYD88-dependent signaling cooperate to sustain the survival of ABC DLBCL cells. Among ABC DLBCL tumors, those bearing a CD79A/B ITAM mutation overlap significantly with those bearing a MYD88 L265P mutation (39). Simultaneous knockdown of MYD88 and CD79A synergizes in killing ABC DLBCL cell lines, indicating that these two pathways are not redundant (39). Many lines of evidence suggest that the BCR and TLR-MYD88 pathways

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can collaborate during certain normal immune responses and in autoimmunity. In the immune response to retroviruses, MYD88-dependent signals in B cells are necessary to mount a germinal center response (142). This is presumably due to the need for TLRs to recognize viral RNA complexes and thereby accentuate antiviral BCR signaling. In autoimmunity, a model has developed in which DNA- or RNA-containing autoantigens are recognized by the BCR in LAMP-1+late endosomes/autophagosomes, where they are delivered to either TLR7 or TLR9 (143–146). Late endosomes are acidic compartments where antigens are processed and where TLR3, TLR7, and TLR9 are modified by proteases to enable ligand binding (147–149). In normal B cells, BCR signaling is required for TLR9 to move from early endosomes to late endosomes (144). In anergic B cells, by contrast, responses to TLR9 ligands are blunted (150), and BCR stimulation does not cause TLR9 to enter the late endosomal compartment (145). As discussed above, the CD79A/B ITAM mutations in ABC DLBCLs may help lymphoma cells avoid anergy. In so doing, these mutations may also permit access of TLRs to activating ligands, facilitating signaling that may be enhanced by oncogenic MYD88 mutants.

TRANSCRIPTIONAL REGULATORS AND LYMPHOMAGENESIS

B cells integrate inputs from receptors for antigen, cytokines, chemokines, adhesion molecules, and death inducers to initiate a transcriptional response in the nucleus, thereby reprogramming the cell to proliferate, migrate, differentiate, or die, as appropriate. Transcription factors play a key role in these responses in normal B cells (151–153); consequently, they are exploited by B cell malignancies (Figure 1b). In some instances, these factors are modified or overexpressed by genetic alterations in these cancers. In other cases, master regulatory transcription factors control a vast network of downstream targets in both normal and malignant B cells, and these networks can be required to sustain the phenotype and survival of the malignant cell (Figure 2).

BCL6

The transcriptional repressor BCL6 is a master regulator of germinal center B differentiation (154–156) and, accordingly, plays an important role in the pathogenesis of germinal center–derived lymphomas (Figure 1b) (157, 158). BCL6 activity is dysregulated by translocation or mutation in a remarkably high proportion of DLBCL cases (50%) and in a subset of follicular lymphoma cases (10%) (reviewed in Reference 158). B cell lymphomas alter the expression of BCL6 by replacing or removing its normal regulatory sequences, sparing the BCL6 coding region. The most common way in which BCL6 expression is altered is by translocation, which replaces its normal 5′ regulatory regions with promoters from more than 20 Ig and non-Ig genes that are active in post–germinal center B cells (159, 160). Germinal center B cells in normal individuals harbor an Ig-BCL6 translocation at a low frequency, suggesting that deregulated BCL6 is insufficient to cause malignancy by itself (161). AID mutates upstream regulatory regions of *BCL6* in normal germinal center B cells and in lymphomas (162–164). In lymphomas, these mutations can destroy regulatory motifs in the *BCL6* 5′ end, in some cases preventing BCL6 from repressing its own transcription, whereas in other cases blocking the repressive effects of IRF4 or STAT5 (164–166).

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A mouse knockin model in which BCL6 expression is driven by the Iμ promoter in the IgH locus develops a B cell malignancy akin to human DLBCL (167). These mice develop a monoclonal lymphoma, again indicating that BCL6 deregulation alone is insufficient for transformation and that other genetic hits are required. These mice develop lymphomas only if AID is functional in B cells (168), implying that BCL6 dysregulation provides a permissive setting where AID mutagenic activity can produce other oncogenic "hits" leading to malignancy.

BCL6 is a BTB/POZ domain–containing protein that represses transcription (169, 170). BCL6 represses genes by directly binding a DNA motif that loosely resembles STAT-family binding sites (154) and can be indirectly recruited to DNA by other factors (171–173). Once bound, BCL6 recruits several corepressors with unique, nonoverlapping functions. BCL6 can bind to the corepressors CTBP1 and CTBP2, and this interaction is uniquely necessary for BCL6 to regulate its own expression negatively via BCL6 binding sites in its first exon (164, 171, 174). The corepressors NCOR and SMRT can bind the BCL6 BTB domain and recruit histone deacetylase (HDAC) activity to silence target genes involved in the DNA damage response. When the corepressor BCOR is recruited to the BCL6 BTB domain, it forms a distinct repression complex that mediates histone ubiquitination and demethylation at DNA damage response genes (175–177). BCL6 can also associate with the germinal center B cell-restricted protein MTA3, a subunit of the Mi-2/NuRD corepressor complex that recruits HDAC activity to BCL6 target genes that encode regulators of plasmacytic differentiation (178).

Given the crucial role of BCL6 in normal germinal center B cell differentiation and lymphomagenesis, it is important to understand the genetic network controlled by this transcriptional repressor. Genetic manipulation of BCL6 and subsequent gene expression profiling revealed three important biological processes controlled by BCL6 (179). First, BCL6 blocks terminal differentiation by repressing *PRDM1*, which encodes a master regulator of plasmacytic differentiation, Blimp-1 (179, 180). In this regard, another important target of BCL6 is IRF4 (181), which induces Blimp-1 during plasmacytic differentiation (182). Second, BCL6 enables cell-cycle progression by repressing key inhibitors of cyclin-dependent kinases, *CDKN1A* (p21) and *CDKN1B* (p27kip1) (179, 183). In this way, BCL6 action may make the cell cycle in germinal center B cells a "free-running clock," unchecked by normal cell-cycle regulatory mechanisms. Third, BCL6 blocks the expression of many genes associated with inflammation, including chemokines (IL-10, CCL3) and interferon regulators (STAT1). Further work in macrophages has confirmed and extended this regulatory circuit, demonstrating a profound effect of BCL6 on the response to LPS, which may help to explain the fatal inflammatory disease that occurs in BCL6 knockout mice (184, 185).

Additional biological insights into BCL6 have been derived from analysis of BCL6 binding sites in the genome. Several thousand genes are bound by BCL6 in normal germinal center B cells, a subset of which is repressed as a consequence. To allow germinal center B cells to cope with persistent DNA damage inflicted by SHM and CSR, BCL6 represses genes encoding important regulators of the DNA damage response (e.g., TP53, ATR, CHEK1, p21, p27kip1, GADD45A, SUB1) (183, 186–189). Another crucial germinal center B cell

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phenotype is apoptosis, which is a default fate that enables the positive selection of B cells that have acquired high-affinity antigen receptors as a result of SHM. BCL6 aids and abets this process by repressing the genes encoding antiapoptotic proteins, notably BCL2 (171, 181). Germinal center B cell centroblasts also have very low activity of the NF-κB pathway (190, 191). BCL6 represses *NFKB1*, encoding the NF-κB p50 subunit (192), and it also appears to colocalize with the NF-κB p65 subunit on many NF-κB target genes, thereby blocking their expression (185).

Given the central role of BCL6 in germinal center biology, it is not surprising that inhibition of BCL6 function is lethal to germinal center–derived lymphomas (177, 189). As discussed below, peptide or small-molecule inhibitors of BCL6 repression are toxic to many germinal center–derived lymphoma cell lines, regardless of whether they have a BCL6 translocation. This suggests that the regulatory network controlled by BCL6 in normal germinal center B cells is “inherited” by these malignancies and must be retained for their survival. That said, comparative analysis of genomic binding sites of BCL6 in germinal center–derived lymphomas and normal germinal center B cells revealed substantial differences, suggesting that BCL6 may adopt a somewhat modified function in these cancers (181).

It is important to emphasize that some of the normal functions of BCL6 in germinal center B cells must be circumvented to enable malignant transformation. BCL6 directly represses *MYC* (181), possibly to bias centroblasts toward cell division rather than cell growth. In all Burkitt lymphomas and in a subset of DLBCLs, *MYC* is translocated, thereby relieving BCL6 repression. Similarly, the t(14;18) translocation in follicular lymphoma relieves repression of *BCL2* by BCL6. The BCL6-mediated repression of the NF-κB pathway is likely overcome in ABC DLBCL by the variety of genetic aberrations that constitutively activate this pathway (see above). Moreover, ABC DLBCLs downmodulate BCL6 expression (10, 16), likely as a result of their partial plasmacytic differentiation, thereby limiting the action of BCL6.

Blimp-1

ABC DLBCL is a post-germinal center malignancy because it has mutated Ig V genes (76) and bears a gene expression signature that includes many plasma cell-associated genes, such as *XBP1* and *IRF4* (16) (Figure 1b.) Unlike multiple myeloma, however, ABC DLBCL maintains expression of many mature B cell genes, suggesting that it is blocked in plasmacytic differentiation at the plasmablast stage. This blockade is achieved by inactivating Blimp-1, a key transcription factor that is necessary and sufficient to drive mature B cells into terminal differentiation and cell-cycle arrest (193–195). Blimp-1 extinguishes the expression of most genes that are characteristically expressed in mature B cells by directly repressing genes encoding key B cell transcription factors, such as PAX5 and SPIB (196, 197).

Blimp-1 acts as a tumor suppressor in ABC DLBCL (198–200). In a substantial fraction of ABC DLBCL tumors, the *PRDM1* gene is disrupted by truncation, deletion, and mutation, or it is epigenetically silenced. *PRDM1* is directly repressed by BCL6, and fittingly, translocations that cause constitutive expression of BCL6 occur in ~30% of ABC DLBCLs but in only ~10% of GCB DLBCLs (198, 201). *PRDM1* is also repressed by the ETS-family

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transcription factor SPIB (202), which is also a target of Blimp-1 repression (196), constituting a negative regulatory loop. Compared with other DLBCL subtypes, ABC DLBCLs express SPIB highly (see below). In addition to BCL6 and SPIB, other mechanisms may likely block Blimp-1 expression in ABC DLBCL because many of these tumors fail to express Blimp-1 protein (198).

Deletion of *PRDM1* in mouse germinal center B cells results in a lymphoproliferative disease with marginal zone hyperplasia that gives rise to DLBCLs with a long latency, confirming the tumor-suppressor function of Blimp-1 (198, 203). When such mice are crossed to transgenic mice expressing a constitutively active form of IκB kinase to engage NF-κB, lymphomas with an ABC DLBCL phenotype develop (203). These findings support the simplifying hypothesis that ABC DLBCL oncogenesis requires, at a minimum, NF-κB activity and a block in terminal plasmacytic differentiation (Figure 1b.).

IRF4

The transcription factor IRF4 is critical for plasmacytic differentiation and is required for the survival of several post-germinal center malignancies, as we discuss below. However, IRF4 is not genetically aberrant in most of these cancers, suggesting that IRF4 dependence is not a classical case of "oncogene addiction," which typically stems from genomic abnormalities that activate the expression or alter the function of an oncogene (204). A recent body of research in cancer biology has revealed that cancer cells can develop a novel or exaggerated dependence on genes whose regulatory regions and coding sequences are not affected by structural alterations. In these cases, the dependence has been termed nononcogene addiction (205, 206). Generally speaking, nononcogene addiction comes in three varieties. First, cancer cells may depend on transcription factors that are characteristic of the lineage from which they originate, and such regulators have been termed lineage-restricted survival factors (207). Second, cancer cells can depend on signaling or regulatory factors that are "downstream" of oncogenic alterations in the cancer cell. Third, cancer cells can develop an exaggerated dependence on cellular stress pathways, which are activated by the deregulated proliferation and metabolism of cancer cells (208).

IRF4 plays critical roles in the development of the lymphoid, myeloid, and dendritic cell lineages, together with its closely related IRF-family member IRF8 (reviewed in Reference 209). IRF4 is infrequently modified by structural genomic alterations in lymphoid malignancies. In rare cases of multiple myeloma, IRF4 is deregulated by translocations involving the IgH locus (210). In ~2% of multiple myeloma cases, a recurrent amino acid substitution is introduced into the DNA-binding domain of IRF4 (lysine 123 to arginine) (211). The functional significance of this mutation has yet to be determined, but its recurrence suggests a selective advantage for multiple myeloma cells bearing this change. IRF4 translocation is a relatively uncommon finding in GCB DLBCL, occurring in ~5% of cases, which are mostly pediatric (212). IRF4-positive GCB DLBCLs have a distinct gene expression signature that distinguishes them from both GCB and ABC DLBCL (212). Thus, IRF4 may function as a classical oncogene in rare cases of multiple myeloma and DLBCL, but in most cases of these malignancies, the *IRF4* gene is not affected by structural aberrations.

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Genomic-scale loss-of-function screening using RNA interference libraries is an effective means to identify both oncogene and nononcogene addiction in cancer (42, 213, 214). An RNA interference screen of multiple myeloma cell lines revealed that all cell lines, regardless of genetic etiology, require the transcription factor IRF4 for survival (215). Likewise, IRF4 is required for the survival of ABC DLBCL cells (A. Shaffer & L. Staudt, unpublished observations). Because *IRF4* is wild type in structure in most cases of multiple myeloma and ABC DLBCL, their IRF4 dependence is a case of nononcogene addiction.

IRF4 regulates largely distinct molecular networks in myeloma and ABC DLBCL (A. Shaffer & L. Staudt, unpublished observations). ABC DLBCLs share a gene expression program with mitogenically activated mature B cells, with high expression of NF-κB and its targets (10, 40). IRF4 is highly expressed in ABC DLBCL, owing to constitutive NF-κB activity (217). Although IRF4 acts mainly as a transcriptional activator in myeloma cells, it can both activate and repress transcription in ABC DLBCL cells. In ABC DLBCL, IRF4 directly transactivates many genes that are characteristically expressed in this lymphoma subtype while directly repressing genes that are more highly expressed in the GCB DLBCL subtype (A. Shaffer & L. Staudt, unpublished observations). IRF4 positively regulates a number of genes that are induced by NF-κB pathway activation, yet it represses many genes that are induced by IL-6, IL-10, and type I interferon.

These differences in IRF4 function in ABC DLBCL and myeloma can be traced to the interaction of IRF4 with SPIB in ABC DLBCLs (A. Shaffer & L. Staudt, unpublished observations). IRF4 binds to an ETS-IRF composite DNA motif along with the ETS family members PU.1 or SPIB (218–222). In mature B cells, SPIB expression is critical for BCR signaling, and in its absence, BCR-cross-linked B cells proliferate poorly, die more readily by apoptosis, and are impaired in their ability to form germinal centers (223, 224). SPIB is highly expressed in ABC DLBCL, and its expression is further increased by recurrent gains and amplifications of the *SPIB* locus in this malignancy (225). In one ABC DLBCL cell line, *SPIB* is the direct target of a chromosomal translocation in which Ig enhancers drive high expression of the gene (98), nominating *SPIB* as an oncogene in ABC DLBCL. Indeed, knockdown of *SP1B* is lethal to ABC DLBCL cells (225). Notably, the ETS-IRF motif to which IRF4 and SPIB bind is the single most overrepresented motif underlying IRF4 binding sites in ABC DLBCL (A. Shaffer & L. Staudt, unpublished observations). One key target gene of IRF4 and SPIB in ABC DLBCL is *CARD11*, which contains an ETS-IRF motif to which these factors bind (A. Shaffer & L. Staudt, unpublished observations). Because NF-κB upregulates IRF4 expression, the survival of ABC DLBCLs relies on a positive-feedback loop involving CARD11, NF-κB, and IRF4/SPIB (Figure 1b).

An unexpected role for IRF4 in mantle cell lymphoma has emerged from studies of the proteasome inhibitor bortezomib, which can induce clinical responses in ~50% of patients (14). Mantle cell lymphoma cell lines selected to be bortezomib resistant have elevated expression of transcription factors (IRF4, Blimp-1) and cell-surface markers (CD38, CD138) characteristic of plasma cells (14). Biopsy samples from patients who are resistant to bortezomib also have a plasmacytic profile, characterized by high IRF4 and CD38 expression (14). Short-term knockdown of IRF4 sensitizes mantle cell lymphoma lines to bortezomib. Over time, IRF4 knockdown kills mantle cell lymphoma lines, thus extending

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the scope of IRF4 addiction to mantle cell lymphoma. Further work is needed to determine whether IRF4 requires other transcription factors to promote mantle cell lymphoma survival and to understand which IRF4 target genes mediate IRF4 function in this setting.

microRNAs AS ONCOGENES AND TUMOR SUPPRESSORS IN LYMPHOID MALIGNANCIES

The first example of a microRNA functioning as a tumor suppressor was provided by analysis of mono- and bi-allelic deletions of chromosome region 13p14 in CLL (226). This region harbors the noncoding RNA DLEU2, which encodes microRNA-15a (miR-15a) and miR-16-1 in its fourth intron. 13q deletions occur preferentially in the M-CLL subtype (227), which has an indolent clinical course and is marked by low ZAP70 expression (13). This genomic region is occasionally affected by translocations in CLL, one of which was reported to have a breakpoint within 3 kb of miR-15a (226). The targets of these microRNAs have not been fully delineated but have been reported to include cell-cycle regulators (cyclin D3, CDK6, cyclin E1) (228, 229), and BCL2 (230). Interestingly, miR-15a/miR-16-1 also represses p53, a positive regulator of the miR-34b/miR-34c cluster, which represses ZAP70 (231). Thus, deletion of miR-15a/miR-16-1 may indirectly lower ZAP70 levels, consistent with the enrichment of 13q deletions in the ZAP70-negative M-CLL subtype.

Two mouse models emphasize the biological importance of these microRNAs in preventing B cell malignancy. One is a well-studied natural experiment, the autoimmune NZB mouse, which harbors a point mutation flanking the 3′ end of miR-16-1, lowering its expression (232). This miR-16-1 allele is one of three genetic loci that tracks with the characteristic lymphoproliferative disease in this mouse strain. In mice engineered to delete miR-16-1 and miR-15a, a relatively rapid and aggressive CLL-like disease develops (228). The predominant phenotype caused by deletion of these microRNAs is enhanced proliferation, with little effect on cell survival. This observation is consistent with recent studies that have emphasized the role of ongoing proliferation in human CLL, despite the fact that most CLL cells in the blood are in the G0/G1 phase of the cell cycle (233). CD5+ lymphomas arising in miR-15a/miR-16-1-deleted mice are characterized by stereotyped BCRs, derived from a restricted repertoire of Ig Vgenes (228), a phenotype shared by human CLLs (79).

The miR-17-92 cluster has oncogenic activity and has, therefore, been dubbed "oncomir-1" (234, 235). The locus that encodes the miR-17-92 cluster is recurrently amplified in human B cell malignancies and other cancers, causing the overexpression of these microRNAs in cases of mantle cell lymphoma, follicular lymphoma, Burkitt lymphoma, GCB DLBCL, and multiple myeloma (225, 234, 236–238). Within DLBCL, amplification and overexpression of miR-17-92 occurs in 12.5% of GCB DLBCL tumors but never occurs in ABC DLBCL tumors (225). Those GCB DLBCL tumors with miR-17-92 amplification have higher expression of MYC and its target genes, suggesting a collaboration between MYC and miR-17-92 in GCB DLBCL pathogenesis (225).

Mice with transgenic overexpression of the miR-17-92 cluster develop a lymphoproliferative disease in which the B cells proliferate more readily in response to various activation stimuli and resist Fas-induced cell death (239). These phenotypes were ascribed to downmodulation

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of PTEN and BIM by the miR-17-92 cluster (239). Introduction of the miR-17-92 cluster or miR-19 alone together with a MYC transgene accelerates the development of B cell lymphomas in mice, apparently by reducing the degree of apoptosis that is induced by MYC overexpression (234, 235, 240). The reduction in PTEN caused by miR-19 is associated with enhanced activity of the Akt/mTOR pathway, which both blocks cell death and promotes cell growth (235). The oncogenic action of miR-17-92 may also be promoted by its repression of *CDKN1A*, encoding p21, which could allow tumor cells to escape senescence and cell-cycle checkpoints (241, 242).

miR-17-92 expression is controlled by oncogenes and tumor suppressors. An elaborate regulatory cascade is initiated by the oncogene MYC, which binds to the *MIR17HG* promoter and strongly induces the expression of the miR-17-92 cluster (243). In turn, miR-17-92 represses E2F1, an important positive regulator of gene expression during S phase of the cell cycle. MYC transactivates several members of the E2F family of transcription factors, including E2F1, E2F2, and E2F3, which positively regulate MYC in a feed-forward loop. Although each of these factors promotes proliferation, E2F1 is unique in promoting apoptosis under certain conditions (244). Hence, miR-17-92-mediated repression of E2F1 may allow MYC-expressing tumor cells to evade apoptosis. Moreover, E2F3 binds to the *MIR17HG* promoter and induces miR-17-92 expression (245). Thus, a working model is that MYC and E2F3 collaborate to produce high expression of miR-17-92, which serves to promote proliferation and prevent apoptosis (245). In addition to this MYC network, miR-17-92 is a component of the p53 tumor-suppressor pathway. p53 represses miR-17-92 expression under hypoxic conditions, and accordingly, ectopic expression of miR-17-92 can block hypoxia-induced apoptosis (234, 245, 246).

Knockdown of miR-34a causes an accumulation of mature B cells, whereas transgenic overexpression of this microRNA blocks early B cell differentiation (247). These phenotypes have been traced to miR-34a-mediated repression of the transcriptional factor FOXP1 (247), an oncogene that is translocated and amplified in lymphoma and is characteristically expressed at high levels in ABC DLBCL (16, 225, 248, 249). miR-34a is repressed by MYC and induced by p53, which ties this microRNA to the pathogenesis of several lymphoma subtypes (247, 250).

Cancer cells can evade microRNA regulation of oncogenes by deleting the microRNA recognition sequences encoded in their untranslated regions (UTRs). A prime example of this mechanism relates to cyclin D1, the prototypic oncogene of mantle cell lymphoma. The 3′ UTR of *CCND1*, encoding cyclin D1, contains several RNA regulatory motifs that control its expression. This region includes an ARE element that controls RNA stability as well as binding sites for miR-15, miR-16, miR-17-5, and miR-20. Some mantle cell lymphoma cases have elevated cyclin D1 mRNA levels, and these are associated with increased proliferation and inferior overall survival (251). These cases express short cyclin D1 mRNA isoforms, lacking the regulatory elements in the 3′ UTR (252). In some cases, mantle cell lymphoma tumors delete all or part of the 3′ UTR, whereas other tumors introduce mutations in the 3′ UTR that create alteRNAtive polyadenylation and cleavage sites, again producing shorter mRNAs lacking the regulatory motifs. These structural abnormalities may stabilize cyclin D1 mRNA by removing the ARE element and by

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removing the binding site for miR-16-1 (252, 253). The removal of 3′ UTRs containing microRNA binding sites may be a general mechanism by which cancer cells elude homeostatic regulation by microRNAs (254). The mechanism underlying 3′ UTR shortening in cancer may be related to the shortening of 3′ UTRs in proliferating cells (255) and presumably involves the regulation of alteRNAtive polyadenylation and cleavage during mRNA synthesis (254).

EPIGENETIC DYSREGULATION IN LYMPHOID MALIGNANCIES

Recent cancer genomics studies have uncovered recurrent structural alterations affecting enzymes that alter the transcriptional output of the genome by modifying chromatin. Many epigenetic regulators alter histones by adding or removing methyl and acetyl groups, with certain modifications associated with active chromatin [e.g., histone H3, lysine 4 (H3K4), methylation and histone H3/H4 acetylation] and others associated with repressive chromatin (e.g., H3K27 and H3K9 methylation) (256–258). Histone-modifying enzymes can function either as oncogenes or tumor suppressors, but the precise downstream benefit for the cancer cell in altering their activity is generally unknown. One relatively clear example is BMI1, which is a component of the polycomb repression complex-1 (PRC1) that introduces repressive H3K27 methylation marks. Early mouse-model studies revealed BMI1 to be an oncogene that cooperates with MYC in lymphomagenesis, in part by repressing transcription from the INK4a/Arf locus, that encodes p16 and $INK4^{ARF}$, which block the cell cycle and activate p53, respectively (259, 260). The INK4A/Arf locus is frequently deleted in mantle cell lymphoma (251), but in some cases, it is epigenetically silenced by BMI1 amplification and overexpression (261).

Mutation of EZH2, a component of the polycomb repression complex-2 (PRC2), is a frequent event in GCB DLBCL and occurs less commonly in follicular lymphoma and PMBL (262–264). The PRC2 complex methylates H3K27, thereby promoting transcriptional repression. In embryonic stem cells, PRC2 activity represses gene expression and maintains their self-renewing phenotype (265). In normal germinal center B cells, EZH2 associates with ~1800 promoter regions and is generally associated with H3K27 methylation of these loci (266). Heterozygous mutations in EZH2 are present in approximately ~21% of GCB DLBCL tumors but never in ABC DLBCL cases (262, 263). Most often, these mutations affect an evolutionarily conserved tyrosine (Tyr641) in the catalytic SET domain, substituting serine, histidine, phenylalanine, or asparagine residues (262). Less often, other residues in the SET domain may be mutated in GCB DLBCL (263). Initial functional investigation of the EZH2 Tyr641 mutant isoform showed that it is less able to catalyze the addition of the first methyl group onto H3K27 than the wild-type EZH2, implying a loss of function (262). However, this observation is not easily reconciled with the fact that EZH2 mutations are invariably heterozygous in lymphomas. More recent studies have revealed that Tyr641 mutations are gain of function, allowing the enzyme to more readily add the second and third methyl groups onto monomethylated H3K27 (267, 268). The retention of the wild-type EZH2 allele in lymphomas is presumably necessary to put the first methyl group on H3K27, enabling the Tyr641 mutant enzyme to methylate this residue fully. The critical downstream targets of mutant EZH2 in DLBCL have not yet been elucidated.

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Lymphomas also sustain frequent inactivating mutations and deletions in *CREBBP* and *EP300*, which encode the histone acetyltransferases (HATs) CBP and p300, respectively (269, 270). HATs facilitate transcription by acetylating histone tails but also acetylate other proteins and modify their function. For some proteins, HAT-mediated acetylation enhances function (e.g., p53, NF-κB), but for others, acetylation is inhibitory (e.g., BCL6) (270–275). In DLBCL, 41% of GCB DLBCL tumors have *CREBBP* aberrations compared with only 17% of ABC DLBCL tumors. *CREBBP* aberrations are frequent in follicular lymphoma (31%) and in relapsed B cell acute lymphoblastic leukemia (18%), but they are rare or absent in CLL, Burkitt lymphoma, and MALT lymphomas (269, 276). *EP300* mutations or deletions are present in 10% and 9% of DLBCL and follicular lymphoma cases, respectively, and are largely nonoverlapping with *CREBBP* mutations, suggesting that these two HATs perform similar tumor-suppressor functions in lymphoma. The missense mutations that occur in CBP impair enzymatic activity by decreasing affinity for the cofactor acetyl-CoA, resulting in reduced acetylation of BCL6 and p53 (269). Given that CBP/p300 acetylates and inactivates BCL6, which is a transcriptional repressor of p53, and also acetylates and activates p53, it is possible that loss of CBP/p300 may attenuate the p53-mediated response to DNA damage or other stresses in DLBCL (269). However, the precise tumor-suppressor mechanism of CBP and p300 is not clear at present and is complicated by the fact that a large number of cellular transcription factors recruit these enzymes to their target genes, thereby modifying chromatin structure and expression (277).

Recent sequencing studies have uncovered frequent genetic events that inactivate *MLL2*, encoding a histone H3K4 methyl transferase that is associated with transcriptional activity (263, 278). *MLL2* mutations and deletions are present in 24-32% of DLBCL cases and in 89% of follicular lymphoma cases, a frequency on par with the frequency of BCL2 translocations in follicular lymphoma. Different inactivating mutations are often present on both *MLL2* alleles, suggesting that MLL2 is a classical tumor suppressor. Indeed, inactivating mutations in *MLL2* and the related gene *MLL3* occur in 16% of childhood medulloblastomas (279). DLBCLs sustain deletions in *MLL, MLL3*, and *MLL5* in addition to *MLL2*, suggesting that each of these genes may have tumor-suppressive function (263, 278). Given these compelling genetic data, the task is now to understand the nature of the regulatory disturbance that ensues following loss of MLL proteins in lymphoma pathogenesis.

Epigenetic dysregulation plays a key role in the pathogenesis of HL and PMBL through the coordinated action of the histone H3K9 demethylase JMJD2C (also known as GASC1 or KDM4C) and the kinase JAK2 (280). These two malignancies presumably arise from the same normal precursor cell, the thymic B cell, share a common gene expression profile, and frequently depend on the NF-κB pathway for survival (217, 281–283). Their molecular pathogenesis is also similar, with both malignancies harboring DNA copy-number gain or amplification affecting chromosome band 9p24 in 30-40% of cases (225, 281, 284–287). Originally, attention focused on JAK2 as the pathogenetic culprit in this amplicon because it is a key kinase that delivers signals emanating from anumber of cytokine receptors (281, 284, 286–288). The suspicion that JAK2 signaling plays a role in these cancers was heIgHtened with the discovery of recurrent inactivating mutations in SOCS1, a negative regulator of cytokine receptor signaling (289–292).

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However, the 9p24 amplicon encompasses at least 3 megabases and includes many other genes that may contribute to lymphoma pathogenesis (280). An RNA interference-based screen in PMBL and HL identified three essential genes in this amplicon: *JAK2, JMJD2C,* and *RANBP6.* Whereas RANBP6 is a gene of unknown function, both JMJD2C and JAK2 can modify histones. JMJD2C is a H3K9 demethylase that is amplified, translocated, and overexpressed in several cancer types (293–301). H3K9 trimethylation is a hallmark of heterochromatin, a state typically associated with gene silencing. JAK2 not only functions in the cytoplasm but also transits to the nucleus to phosphorylate histone H3 on tyrosine 41 (H3Y41) (302). In so doing, JAK2 decreases heterochromatin, similar to its *Drosophila* counterpart *Hopscotch* (303). In PMBL and HL cell lines, JAK2 and JMJD2C cooperate to decrease heterochromatin throughout the genome (280). The presumed mechanism is by preventing the recruitment of the heterochromatin-associated protein HP-1a. HP-1α uses its chromodomain to interact with methylated H3K9 and uses its chromoshadow domain to interact with the unphosphorylated histone H3 tail near tyrosine 41. Hence, by demethylating H3K9 and phosphorylating H3Y41, JMJD2C and JAK2 conspire to prevent HP-1α from binding to chromatin through either of its interaction domains.

Hundreds of genes are induced in PMBL and HL cells by JAK2-mediated chromatin remodeling (280). This gene expression signature of JAK2 action in the nucleus is a pervasive feature of PMBL tumors, evident in most cases irrespective of whether they harbor a 9p24 amplification. Activation of JAK2 may be a universal feature of PMBL and HL, stemming from the autocrine IL-13 signaling that typifies these lymphomas (280, 304, 305). The *MYC* locus is a critical target of epigenetic activation by both JAK2 and JMJD2C. Knockdown of either JAK2 or JMJD2C stimulates the formation of heterochromatin at the *MYC* locus, lowering expression of MYC and its downstream target genes (280). Another interesting target of JAK2-mediated histone modification is *PDCD1LG2*, which encodes PD-L2, a ligand for the inhibitory PD-1 receptor on T cells (280, 306). PD-L2 is characteristically expressed on the surface of PMBL and HL cell lines at levels that are capable of blocking T cell activation (307). Because these lymphomas both arise in the thymus, the upregulation of PD-L2 may be selected to elude recognition by T cells.

IMMUNE EVASION

Tumor cells must avoid immune surveillance; consequently, lymphomas accumulate genetic lesions in genes necessary for immune recognition. Translocations involving *CIITA* encoding a transactivator of MHC class II genes (308) occur in the malignant cells of a significant proportion of HL (15%) and PMBL (38%) tumors, but they are rare in other DLBCL subtypes (307). These translocations invariably fuse the N terminus of CIITA in frame with a variety of other genes. As a result, one copy of *CIITA* is inactivated, and the fusion protein can also act in a dominant-negative manner to extinguish MHC class II expression, thereby limiting the ability of the tumor cells to interact with T cells (307). Loss of MHC class II is associated with inferior survival in PMBL and HL (309, 310), as is the presence of CIITA translocation in PMBL (307). Similarly, loss of MHC class II expression on the malignant cells of DLBCL tumors is associated with inferior survival and fewer tumor-infiltrating $CD8^+$ cells, suggesting that loss of immune surveillance is an important mechanism in some DLBCLs (311).

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In some cases of PMBL and HL, *CIITA* is fused to the genes encoding PD-L1 or PD-L2, two ligands for PD-1, a receptor on T cells that inhibits activation through the antigen receptor (307). The resultant fusion protein is displayed on the cell surface and functionally impairs T cell activation. As mentioned above, the PD-L1 and PD-L2 genomic loci are epigenetically modified by JAK2 and JMJD2C in these lymphomas, perhaps facilitating their recurrent translocation to *CIITA*. Together, these data suggest that, under the selective pressure of thymic T cells, malignant thymic B cells acquire genetic lesions that prevent T cell recognition by impairing MHC class II expression and/or by blocking T cell activation.

In 12% of DLBCL tumors, inactivating mutations and deletions occur in *B2M*, which encode β2-microglobulin, a protein necessary for the cell-surface expression of MHC class I dimers (263, 278). These lesions would presumably impair recognition of the malignant cells by cytotoxic T cells. It is also notable that DLBCL tumors sustain inactivating genetic events in *CD58,* which encodes a cell-surface receptor for CD2 on T and NK cells (278). CD58 (LFA-3) cooperates with ICAM-1 to enhance adhesion of target cells to T and NK cells (312–314), so its loss in DLBCL may allow the malignant cells to avoid immune surveillance.

Finally, the secretion of immunomodulatory cytokines and chemokines by malignant lymphoma cells is likely to alter the tumor microenvironment substantially. In HL, the majority of cells in the tumor are nonmalignant immune cells that are presumably present because of the cytokines and chemokines secreted by the malignant Hodgkin Reed-Sternberg cell (reviewed in Reference 4). IL-10 and IL-6 are secreted by a subset of ABC DLBCLs, often in association with oncogenic MYD88 L265P mutations (39, 137, 138). The MYD88 L265P mutation also causes the secretion of type I interferon, which together with IL-10 could be potently immunosuppressive.

THERAPIES TARGETING LYMPHOMA PATHOPHYSIOLOGY

Currently, chemotherapy, radiation, and the anti-CD20 antibody Rituximab are the mainstays of lymphoma treatment. However, optimal cancer therapies target the abnormal state of the cancer cell while sparing normal cells. Ideally, one hopes to identify and exploit "synthetic lethality" with therapies that target the oncogenic rewiring of the malignant cell (205, 315). The constitutive activation of signaling pathways in lymphoma offers opportunities to intervene with tolerable effects on normal immune function. The B cell-restricted transcription factors that are essential for lymphoma survival may also be amenable to attack because a short-term loss of normal B cells can be managed without risk to the patient.

Signaling Inhibitors

Given the pervasive role of NF-κB pathway activation in human lymphomas, NF-κB inhibitors should be developed and tested in these cancers. Such inhibitors may invoke apoptosis in the malignant lymphoma cell on their own, but they may also synergize with cytotoxic chemotherapy because NF-κB blocks the ability of these agents to induce apoptosis (41). A relatively blunt instrument to block NF-κB is the proteasome inhibitor bortezomib, which blocks the degradation of EBa. Phase 2 clinical trials in DLBCL suggest

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that the combination of bortezomib plus chemotherapy is preferentially active in the ABC DLBCL subtype relative to GCB DLBCL (316, 317). This finding is currently being validated in several randomized phase 3 trials. A natural target for therapeutic development would be IKKβ, given that the classical pathway is activated in many lymphoma types. Drugs targeting IKKβ have been developed with autoimmune and inflammatory diseases as the target, but they have not entered multicenter trials, possibly because of concerns that long-term inhibition of NF-κB may be associated with unacceptable toxicities, including inhibition of innate immune function (reviewed in Reference 318). However, short-term treatment of susceptible lymphomas with an IKKβ inhibitor may be achievable with manageable side effects and should still be considered. A mechanism of resistance to inhibition of IKKβ is the compensatory activation of IKKα (319). Both enzymes are in the same molecular complex, but IKKβ is a superior kinase for EBa. However, under conditions of IKKβ inhibition, IKKα can take over as an effective EBa kinase, suggesting that targeting both enzymes may be synergistic.

An orthogonal strategy to inhibit the NF-κB pathway arose from the observation that the ubiquitin ligase for EBa, βTrCP, requires modification by a neddyl peptide for activity (320). MLN4924, a small molecule targeting the neddylation-activating enzyme NAE, blocks NF-κB activity by inhibiting βTrCP and stabilizing EBa, causing apoptosis of ABC DLBCL cell lines (320). However, this agent may also have activity against GCB DLBCL and other cancers because it also targets an ubiquitin ligase required for DNA synthesis during S phase of the cell cycle (321).

A parallel approach to targeting NF-κB in lymphomas is to block upstream pathways that feed into IKK, such as the BCR pathway. Given the direct and indirect evidence for BCR signaling in lymphomas, it is encouraging that a host of new agents targeting this pathway are coming on line, including inhibitors of SYK, BTK, PI3Kδ, and PKCβ. The SYK inhibitor R406 has shown promise in phase II clinical trials against a variety of B cell lymphomas, including DLBCL, follicular lymphoma, CLL, and mantle cell lymphoma (322). This drug can kill some ABC DLBCL cell lines that genetically depend on SYK, but it also has activity against other cell lines that do not die upon knockdown of the BCR or SYK (70, 106, 112). Because R406 targets other kinases besides SYK (113,114), it is possible that some toxicity of this drug may be off-target. Nonetheless, the high response rate in CLL/SLL (55%) is encouraging, supporting continued trials of this agent and the development of more specific SYK inhibitors.

A highly selective small-molecule inhibitor of BTK, PCI-32765 (323), is showing activity in early-phase clinical trials in B cell malignancies, including CLL and ABC DLBCL. A unique feature of this drug is its covalent attachment to the active site of BTK through a cysteine that is present in only 10 kinases in the human genome, thereby endowing the drug with excellent pharmacodynamic characteristics and specificity. PCI-32765 can extinguish chronic active BCR signaling and kill ABC DLBCL cell lines in vitro at nanomolar concentrations, but it does not kill other lymphoma cell lines that lack BCR signaling (106).

PI3Kδ, the lymphoid-restricted catalytic subunit of PI3K, is the target of the drug CAL-101, which has shown activity in early-phase clinical trials in CLL (324). Interestingly, CAL-101

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decreases chemokine secretion following BCR stimulation, and plasma levels of CCL3, CCL4, and CXCL13 decline in patients with CLL who are treated with this drug. Concomitantly, CLL cells are released from the lymph node microenvironment into the circulation (324), potentially depriving the cells of antigenic stimulation (85). PI3Kδ is also required for the survival of ABC DLBCL lines with chronic active BCR signaling (106), suggesting that CAL-101 should be developed in this setting. In addition to engaging the AKT pathway, which blocks apoptosis by phosphorylating and inactivating the BH3-only protein BAD, PI3K signaling augments NF-κB signaling in ABC DLBCL, adding to its prosurvival function (325).

Oncogenic MYD88 mutations, which occur in ABC DLBCL, MALT lymphoma, and CLL, offer several entry points for targeted therapy. The MYD88 L265P mutation activates IRAK4 kinase; accordingly, a small-molecule inhibitor of IRAK4 kinase is selectively toxic for ABC DLBCL lines (39). Inherited IRAK4 deficiency in humans is associated with recurrent bacterial infections early in life, but this susceptibility wanes by adolescence (326, 327), suggesting that IRAK4 inhibitors could be delivered without severe immune compromise. Inhibitors of IRAK4 kinase are in development because of the presumed role of this kinase in chronic autoimmune and inflammatory diseases (reviewed in Reference 328) and should be cross-purposed for treatment of lymphomas with MYD88 mutations. Because coinhibition of the BCR and MYD88 pathways is more toxic than inhibition of either pathway alone (39), it will be interesting to conduct clinical trials combining drugs that inhibit these two pathways.

Signaling downstream of oncogenic MYD88 mutations induces IL-6 and IL-10 secretion in ABC DLBCL (39) and IL-6 secretion in response to TLR agonists in CLL (134). IL-6 and IL-10 signaling through their respective receptors activates JAK-family kinases to phosphorylate STAT3. An inhibitor of JAK kinase signaling synergizes with an IKKβ inhibitor in killing ABC DLBCL lines (137). Several selective JAK kinase inhibitors are in clinical development for the treatment of myeloproliferative disorders with JAK2 mutations as well as autoimmune and inflammatory disorders (reviewed in Reference 329). ABC DLBCLs are not killed by a selective JAK2 inhibitor (280), suggesting that inhibitors targeting other JAK family members will be needed for successful JAK/STAT3 pathway inhibition in this lymphoma subtype.

JAK2 is an outstanding target in PMBL and HL given the role of autocrine IL-13 signaling and JAK2 gain/amplification in these lymphomas (280, 305). Drugs that block IL-13 association with its receptor are in development for allergic conditions (330) and could conceivably be harnessed for treatment of these lymphomas. The cooperation between JAK2 and JMJD2C in modifying the epigenome in these lymphomas is a rationale for the development of drugs targeting this histone demethylase. Of note, knockdown of JMJD2C is lethal to many cell lines (280), suggesting that PMBL and HL have developed an addiction to this epigenetic regulator that could be exploited therapeutically.

Recently described signaling abnormalities in lymphoid malignancies offer additional new therapeutic possibilities. NIK kinase is a rational target in MALT lymphomas because the c-IAP2-MALT1 fusion oncoprotein proteolytically clips off the NIKN terminus, thereby

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stabilizing the kinase (58). NIK inhibitors should also be evaluated in multiple myeloma because NIK is highly expressed as a result of various genetic aberrations, including NIK amplification and translocation as well as deletion of its negative regulators, TRAF3 and c-IAP1/c-IAP2 (331, 332). Cancer gene resequencing revealed that 100% of hairy cell leukemia cases harbor the oncogenic B-RAF V600E mutation, which was originally described in malignant melanoma (333). This mutant B-RAF isoform activates the RAS-MEK pathway and can be inhibited by PLX-4720, a drug that preferentially inhibits this mutant and that has shown clinical activity in melanoma (334).

Drugging Transcription Factors

As one of the most commonly dysregulated proteins in lymphoma pathogenesis, BCL6 has garnered a great deal of attention as a therapeutic target (335, 336). One way to attack BCL6 is to decrease its stability using an inhibitor of the chaperone HSP90 (337), which kills DLBCL lines both in vitro and in vivo. Several HSP90 inhibitors are in clinical development (338), but their rational development is complicated by the multitude of HSP90 client proteins. A more targeted approach to BCL6 inhibition is to disrupt the interaction between BCL6 and its corepressors (SMRT, NCOR, BCOR) by targeting the BCL6 BTB domain (177). Cell-permeable BCL6 peptide inhibitors (BPIs) bind BCL6 and block its interaction with SMRT, resulting in chromatin reactivation at BCL6-repressed loci and reexpression of BCL6 target genes (177, 339). Using the crystal structure of the BCL6 BTB domain complexed with a corepressor, investigators designed a small-molecule inhibitor to disrupt this interaction (189). This agent induces apoptosis of many but not all DLBCL cell lines, suggesting that a subset of DLBCLs do not rely on BCL6. Although BCL6 knockout mice suffer from an intense inflammatory disease, administration of BCL6 inhibitors to mice over a short time frame does not appear to evoke such pathology, favoring the further clinical development of BCL6-targeted therapies.

The downstream targets of BCL6 repression offer opportunities for synergistic drug combinations. BCL6 represses p53 expression; hence, BPI, an inhibitor of BCL6, derepresses p53, induces p53 target genes, and promotes cell death (340). A small-molecule inducer of p53 activity, PRIMA-1, cooperates with BPI in killing DLBCL cells, presumably by eliciting a more potent transactivation of p53 target genes. Another intriguing target of BCL6 repression is EP300, an enzyme that acetylates and inactivates BCL6 (270). Hence, BCL6 and EP300 form a negative regulatory loop. As mentioned above, DLBCL tumors accumulate EP300 mutations (269, 270), potentially to negate this regulatory relationship. BPI inhibition of BCL6 induces EP300 acetyltransferase activity, and inhibition of EP300 reverses BPI toxicity, suggesting that aberrant protein acetylation contributes to the lethal effect of BPI (270). SAHA, a histone deacetylase inhibitor, can also induce aberrant protein acetylation and synergizes with BPI in killing lymphoma cells (270).

MYC is a prototypic human oncogene that plays an especially important role in lymphoid malignancies, owing to its recurrent translocation and amplification in DLBCL, Burkitt lymphoma, and multiple myeloma. Although small-molecule inhibitors of the interaction between the MYC and its bHLH dimerization partner MAX have been reported (341), they have not been developed as drug candidates. An unexpected avenue to block the

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transcription of *MYC* has emerged recently from investigations of epigenetic regulators using chemical biology and functional genomics methods. BRD4 is a chromatin protein that has been implicated in the recruitment of the transcriptional elongation complex pTEFb (342). BRD4 is also recruited to active genes via its bromodomain, a domain that binds lysine-acetylated histone tails (343). As such, BRD4 can be classified as an epigenetic "reader" of the state of chromatin (344). A recent RNA interference screen targeting epigenetic regulators revealed that BRD4 is essential for the development of acute myeloid leukemia (AML) in a mouse model (345). Small-molecule inhibitors of the interaction between BRD4 and acetylated lysine have been developed (346, 347), one of which (JQ-1) suppresses AML proliferation (345). Molecular profiling revealed that JQ-1 inhibits MYC mRNA and protein expression and silences the MYC transcriptional network in AML (345). JQ-1 has potent effects on the viability of Burkitt lymphoma cell lines in vitro and blocks MYC expression in this setting (348), which is remarkable given that these lines express *MYC* under the control of the IgH enhancer. JQ-1 is not overtly toxic to mice, suggesting that BRD4 has a relatively restricted repertoire of genes that it regulates.

Promoting Apoptosis

The antiapoptotic BCL2 family members have attracted considerable attention as therapeutic targets since their role in lymphomagenesis was discovered (349–352). BCL2 is ectopically expressed as a consequence of the t(14;18) translocation, which characterizes >90% of follicular lymphomas and ~25% of DLBCLs, virtually all of which belong to the GCB DLBCL subtype (353). In addition, the BCL2 genomic locus can be amplified, which is a recurrent lesion in ABC DLBCL (225). BCL2 is transcriptionally upregulated by an unknown mechanism to very high levels in most cases of CLL/SLL and mantle cell lymphoma (13, 354). Structure-based design led to the discovery of small-molecule BH3 mimetics that bind to the proapoptotic BCL2 family members and promote apoptosis (355). The first-in-class version, ABT-263, has shown activity in early-phase clinical trials in relapsed/refractory lymphoma, especially CLL/SLL (356). ABT-263 reduces platelet numbers owing to on-target inhibition of BCL-XL, which platelets require for survival (357), prompting the development of new BH3-mimetics that spare BCL-XL platelets by ABT-263. ABT-263 does not bind to MCL1 or A1, which limits its activity in tumors that highly express these antiapoptotic BCL2 family members (358–360). The clinical activity of these various inhibitors is likely to be governed in part by the quantitative relationship between proapoptotic and antiapoptotic BCL2 family members (361).

PERSPECTIVE

The malignant biology of lymphomas has evolved into a rich field of inquiry, powered by advances in genomics. Our deep knowledge of normal B cell differentiation has accelerated our understanding of the molecular circuitry of lymphomas. The diversity of lymphomas is initially daunting but becomes tractable as we discover their shared genetic aberrations and identify common mechanisms of signaling, transcriptional regulation, and survival (Figures 2–4). Although we currently appreciate many basic pathogenetic mechanisms in lymphoma, recent high-throughput gene-resequencing data from lymphomas show that we have only glimpsed the complexity of lymphoma pathogenesis. Soon, the era of mutation discovery

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will wane and online sites will serve up thousands of genetic lesions that recur in lymphomas. Functional investigations of these mutant alleles will then take center stage, revealing unanticipated structure-function relationships in key immune regulatory molecules as well as uncovering new basic principles in lymphomagenesis. Functionalgenomics methods will continue to make an important contribution, helping us to decipher which somatic mutations are critical drivers and to define regulatory pathways to which lymphomas are addicted. Conceptual distillation of this complexity will be the key to new therapies for our patients suffering with lymphoma.

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Glossary

Ig	immunoglobulin
V	variable
CLL	chronic lymphocytic leukemia
DLBCL	diffuse large B cell lymphoma
GCB DLBCL	germinal center B cell-like subtype of DLBCL
ABC DLBCL	activated B cell-like subtype of DLBCL
MALT	mucosa-associated lymphoid tissue
HL	nodular sclerosis classical Hodgkin lymphoma
BCR	B cell receptor
AID	activation-induced cytidine deaminase, also known as AICDA
SHM	somatic hypermutation
SMZL	splenic marginal zone lymphoma
CSR	class switch recombination
HDAC	histone deacetylase
miR	microRNA
HAT	histone acetyl transferase

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	Lymphoma subtype	Immuno-phenotype	Diagnostic molecular features	Etiology	Frequency	Clinical features
MCL	Mantle cell lymphoma (MCL)	CD20, CD5, Cyclin D1, CD10⁻, CD23⁻	Cyclin D1 t(11;14) (>95%)	Unknown	7%	Median age 55 Nodal, intestinal, blood, bone marrow Survival 50% at ~3–5 years Curable <10%
CLL SLL	Chronic lymphocytic leukemia/ small lymphocytic lymphoma (CLL/SLL)	CD79a, CD23, CD5, CD20 (weak), CD22 (weak)	ZAP70 (50%)	Unknown; rare familial cases	8%	Median age 65 Blood, bone marrow, spleen, nodal (SLL) Survival 50% at 7 years Curable <10%
FL	Follicular lymphoma (FL)	CD20, CD10, BCL2, BCL6	BCL2 t(14;18) (>80%)	Unknown	25%	Median age 57 Nodal and bone marrow Survival 73% at 10 years Curable <20%
BL	Burkitt lymphoma (BL)	CD20, CD10, BCL6	MYC t(8;14), CD10, Ki67; gene expression profiling	EBV (Endemic: 100%, sporadic: 30%); immunodeficiency	<1%	Median age 11 (children), 24 (adults) Extranodal > nodal Survival >80% at 5 years Curable >80%
GCB	Germinal center B cell–like diffuse large B cell lymphoma (GCB DLBCL)	CD20, CD10, BCL6, GCET, LMO2, IRF4⁻, FOXP1⁻	Gene expression profiling	Unknown	17%	Median age 61 Nodal and extranodal Survival ~60% at 5 years Curable >50%
ABC	Activated B cell–like diffuse large B cell lymphoma (ABC DLBCL)	CD20, IRF4, FOXP1, CD10⁻, BCL6⁻ GCET1⁻, LMO2⁻	Gene expression profiling	Unknown	15%	Median age 66 Nodal and extranodal Survival ~40% at 5 years Curable >30%
MALT	Mucosa-associated lymphoid tissue lymphoma (MALT)	CD20, CD79a, CD5⁻, CD10⁻, CD23⁻	AP12-MALT1 t(11;18) (60%)	*H. pylori*; autoimmune disease	9%	Median age 60 Extranodal and bone marrow (20%) Survival >80% at 5 years Curable >50%
HCL	Hairy cell leukemia (HCL)	CD20, CD22, CD79a	BRAF V600E	Unknown	<1%	Median age 55 Spleen, liver, blood Survival >75% at 5 years Curable <30%
PMBL	Primary mediastinal B cell lymphoma (PMBL)	CD30, CD20, CD10⁻	Gene expression profiling	Unknown	6%	Median age 33 Mediastinal, thoracic > nodal Survival >60% at 5 years Curable >60%
HL	Hodgkin lymphoma (HL)	CD30, CD15, CD20⁻	None	Mononucleosis (EBV); rare familial cases	11%	Bimodal median age: 25 and 60 years Mediastinal, nodal Survival 85% at 15 years Curable >80%

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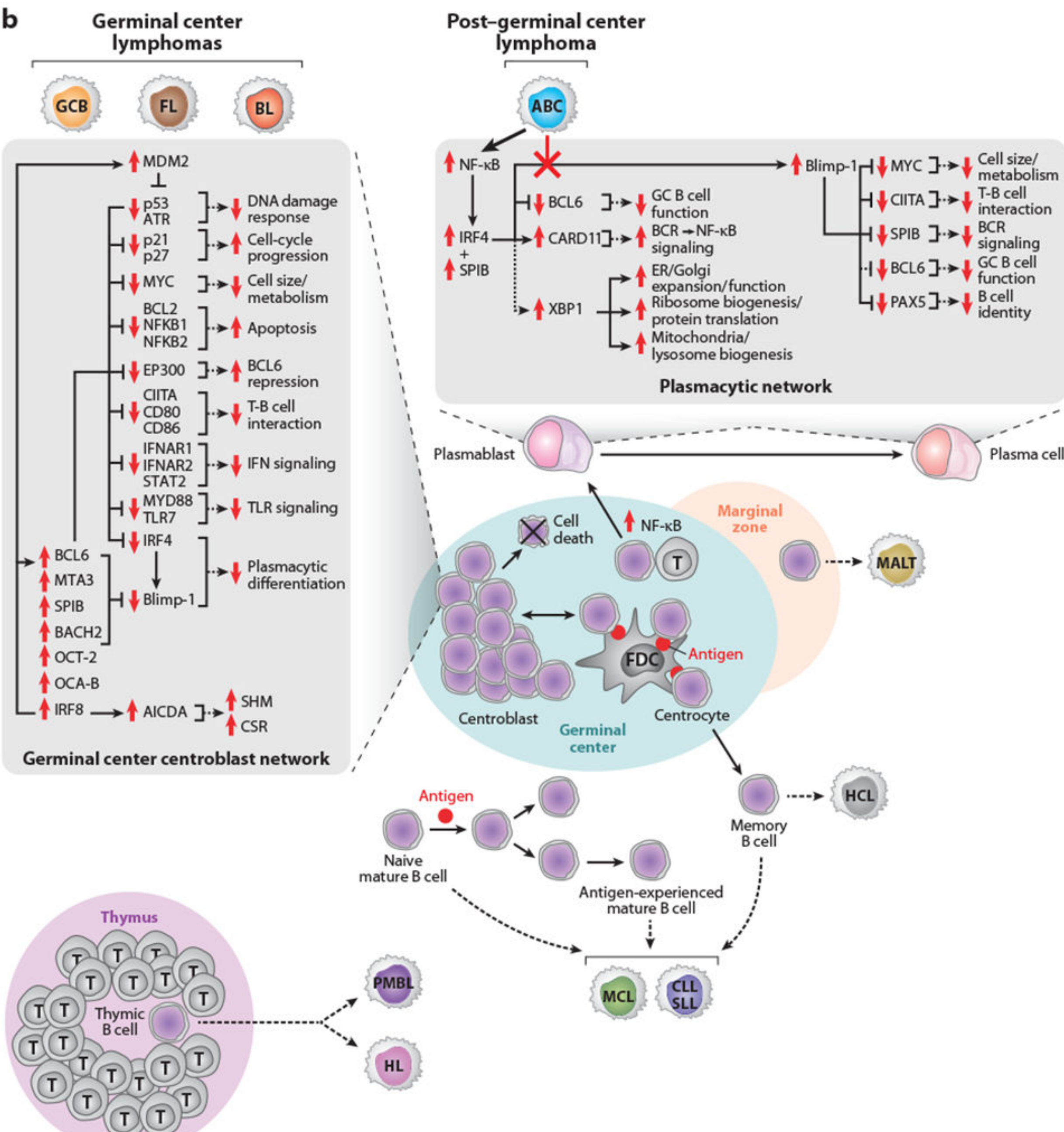

Figure 1.

Summary of clinical, pathological, and molecular characteristics of mature B cell malignancies. (*a*) The major lymphoma subtypes discussed in this review are listed with their approximate frequency among B cell neoplasms indicated. See text for details. (*b*) Transcription factor networks in normal and malignant B cells. The most similar normal B cell counterpart for each malignancy is shown. Follicular lymphoma, Burkitt lymphoma, and germinal center B cell–like (GCB) diffuse large B cell lymphoma (DLBCL) have gene expression profiles that resemble those of normal germinal center B cells. A suite of

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transcription factors are required to generate and/or maintain normal germinal center B cells. These factors control the indicated cellular processes, which favor the proliferation and selection of germinal center B cells bearing B cell receptors (BCRs) with high affinity for antigen while promoting the apoptosis of B cells with low-affinity BCRs. Activated B cell–like (ABC) DLBCL resembles the post-germinal center plasmablast, a normally transient state en route to terminal plasmacytic differentiation. Constitutive NF-κB activity in ABC DLBCL upregulates expression of the transcription factor IRF4, which drives plasmacytic differentiation. Terminal differentiation is blocked at the plasmablast stage in ABC DLBCL by inactivation of the transcription factor Blimp-1 (see text for details).

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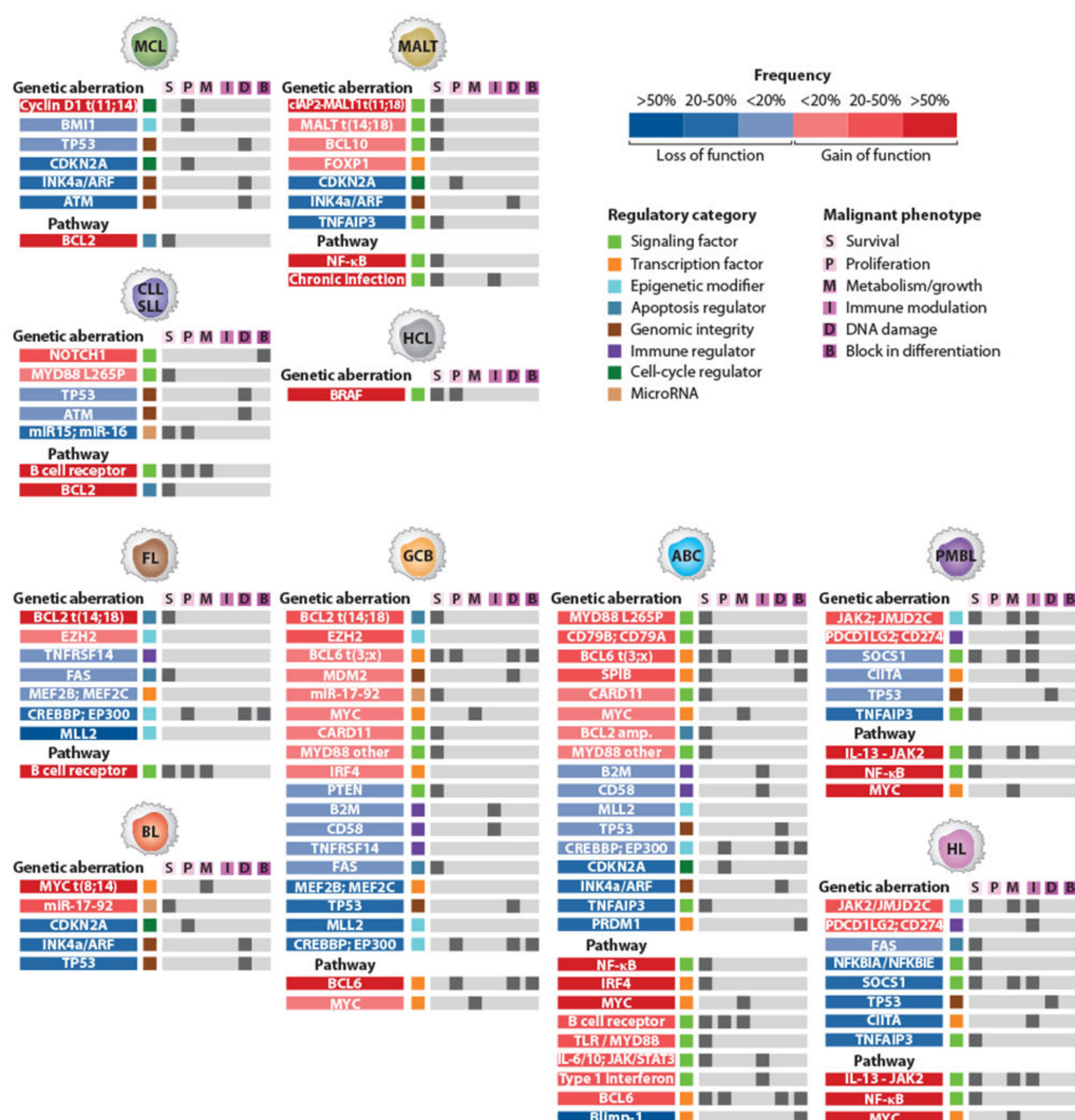

Figure 2.

Summary of the characteristic molecular features of mature B cell malignancies. For each disease, the top panels represent recurrent genetic aberrations (copy number gain/loss, translocation, mutation, DNA methylation) that produce gain-of-function *(red)* or loss-of-function *(blue)* phenotypes. The bottom panels illustrate pathways or genes that are critical for the survival, proliferation, or other malignant phenotypes of each cancer subtype. Genes and pathways are classified according to regulatory category and malignant phenotypes as indicated. For abbreviations of lymphoma subtypes, see Figure 1a. Information for this

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figure was gleaned from the following references: MCL (3, 251, 354), MALT (1, 18), CLL/SLL (5, 134), HCL (333), FL (263, 268, 269, 362), BL (6, 236, 363), GCB (39, 157, 201, 225, 234, 263, 268, 269, 278, 353, 364, 365),ABC (35, 36, 39, 52, 53, 98, 106, 137, 199, 201, 225, 263, 278, 366, 367; A. Shaffer & L. Staudt, unpublished observations), PMBL (2), HL (4, 280, 306).

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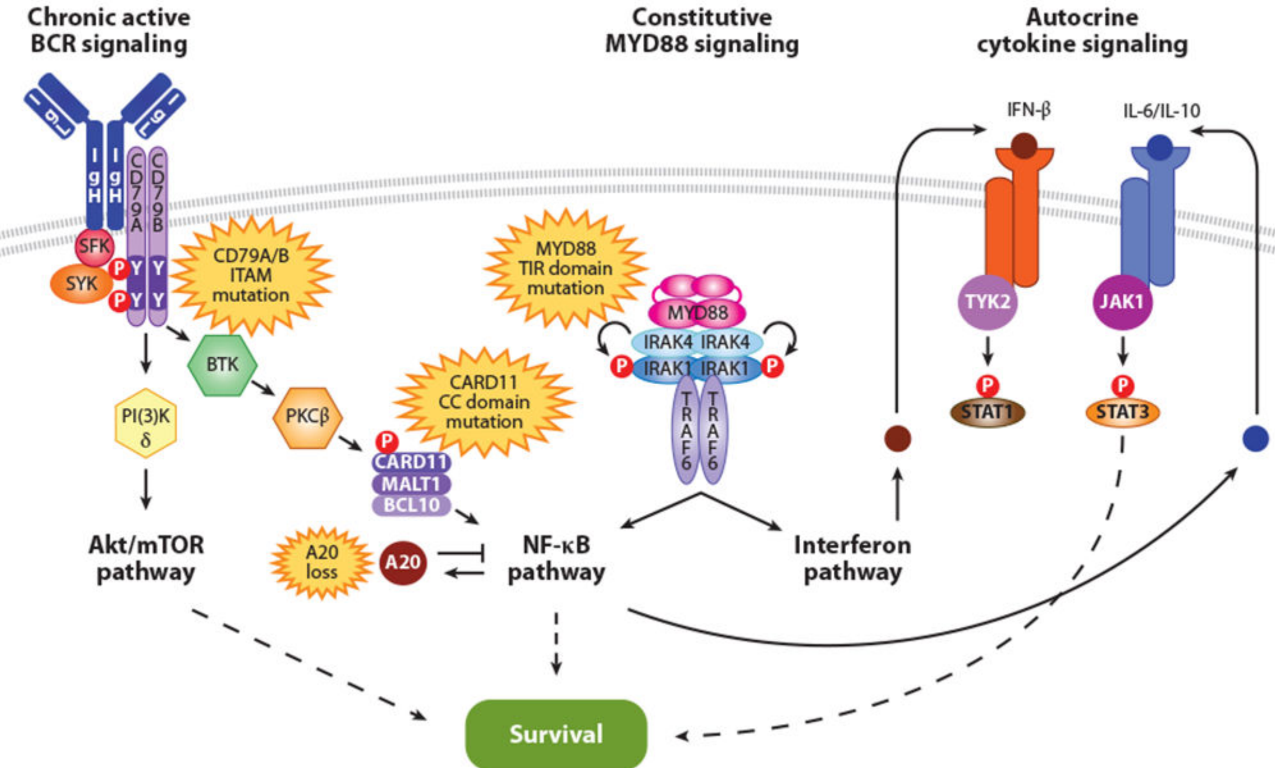

Figure 3.

Deregulated intracellular signaling in activated B cell–like (ABC) diffuse large B cell lymphoma (DLBCL). Constitutive activation of the NF-κB pathway promotes survival in ABC DLBCL and can be achieved by several oncogenic mechanisms, as indicated. Survival of ABC DLBCL cells is also sustained by PI(3)K signaling and autocrine IL-6/IL-10 signaling to the JAK/STAT3 pathways. Mutant MYD88 can also stimulate IFN-β production, which may have both autocrine and immunomodulatory function. See text for details.

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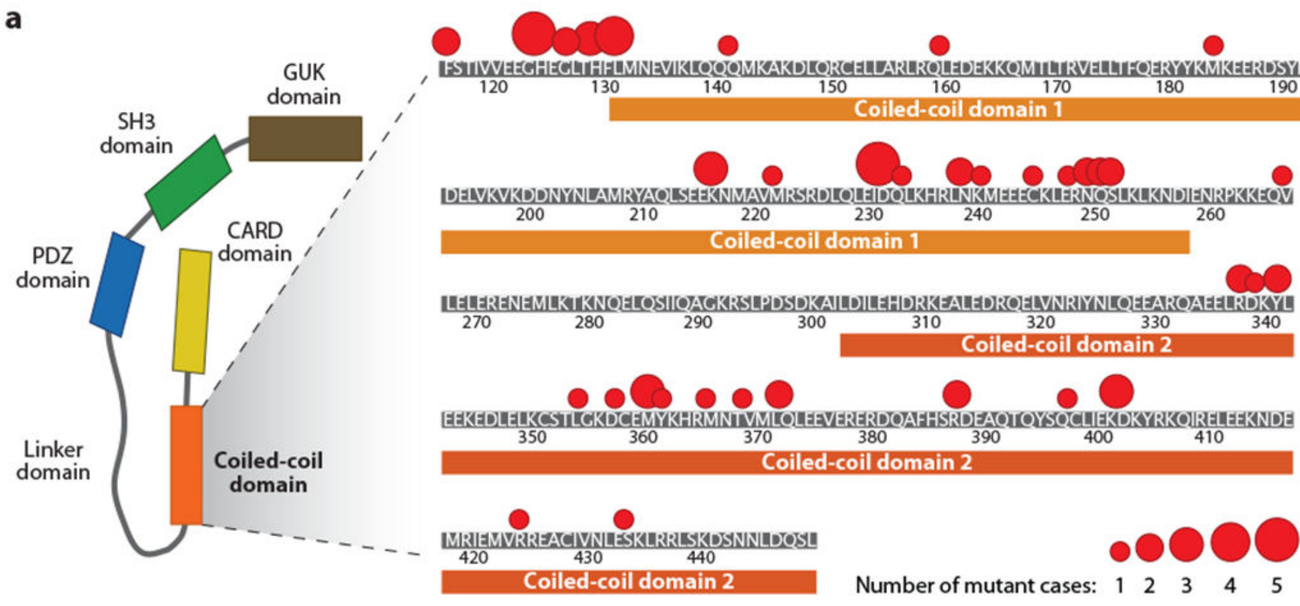

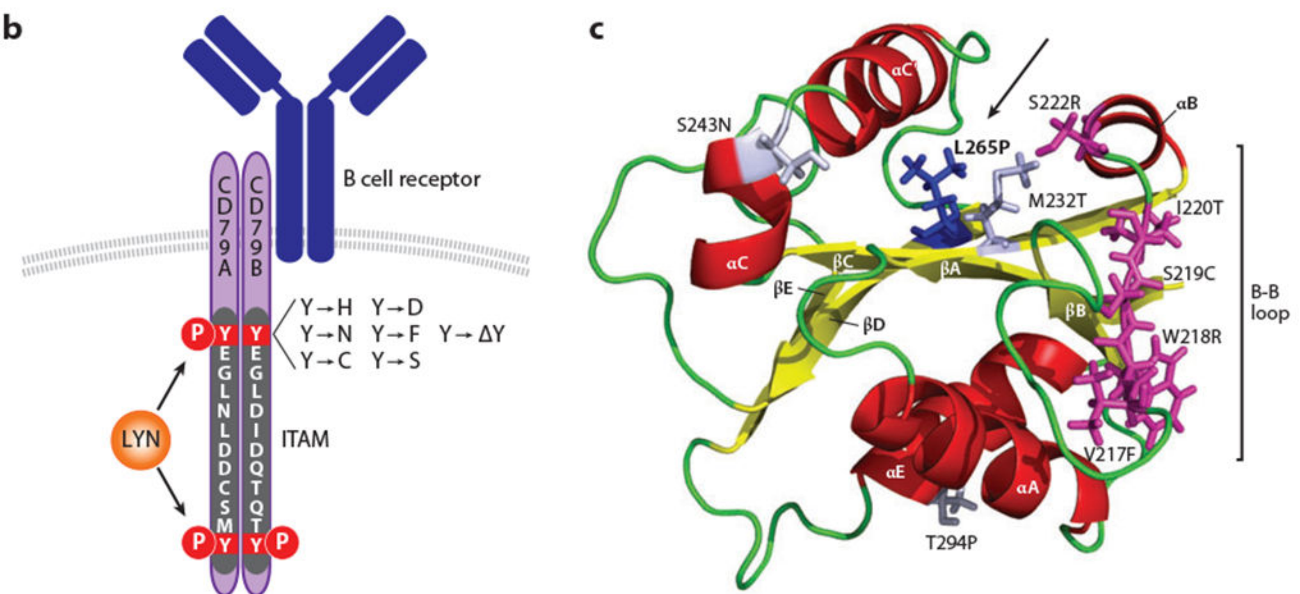

Figure 4.

Recurrent gain-of-function mutations affecting signaling pathways in lymphoma. (a) Activating mutations in the CARD11 coiled-coil (CC) domain. Shown are frequencies of mutations affecting amino acids within the CC domain in DLBCL, gastric lymphoma, and primary central nervous system lymphoma, summarized from References 52, 53, 263, 278, 368. (*b*) Mutations in the B cell receptor (BCR) subunit CD79B in ABC DLBCL. Shown are mutations affecting the first tyrosine of the CD79B immunoreceptor tyrosine-based activation motif (ITAM) signaling module. These mutations are present in 18% of ABC DLBCL tumors, but other mutations or deletions in the CD79B or CD79A ITAM region

occur in an additional 3% of cases (not shown) (106). (*c*) Location of MYD88 mutations acquired by human lymphomas. Shown is the NMR structure of the MYD88 Toll/interleukin-1 receptor (TIR) domain, with the amino acids that are mutated in lymphoma indicated by their side chains (39). The most prevalent mutation, L265P (*arrow*), is located in the hydrophobic core of this domain. Several other recurrent mutants reside in the B-B loop that is involved in interactions with other TIR domains present in Toll-like receptors (TLRs). See text for details.

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