Genomewide molecular profiling has revealed new subtypes of lymphoma that originate from lymphocytes that differ in developmental stage and that use distinct oncogenic programs, yet are indistinguishable under the microscope. In this review, we discuss recent progress in the molecular genetics of aggressive lymphomas and focus on the most common form of this disease, diffuse large-B-cell lymphoma, which accounts for 30 to 40% of newly diagnosed lymphomas.

B-CELL DEVELOPMENT AND LYMPHOMAGENESIS

Non-Hodgkin’s B-cell lymphomas co-opt the regulatory biologic features of normal B cells for their own malignant purpose. This means that the function of such neoplasms depends considerably on the differentiation state of the B cells from which they originate (Fig. 1). During B-cell development in the bone marrow, recombination of V, D, and J gene segments assembles immunoglobulin heavy-chain (IgH) and light-chain (IgL) genes. In this process, two enzymes encoded by recombinase-activating genes (RAG1 and RAG2) cause breaks in double-stranded DNA, but DNA repair processes (nonhomologous end-joining) resolve them. However, such breaks can contribute to chromosomal translocations in lymphoma.\(^1\)

The germinal center is the probable source of many types of lymphoma.\(^2\) The germinal-center reaction begins when antigen, in conjunction with signals from T cells, activates mature B cells. Antigen-specific T cells and follicular dendritic cells bearing antigen surround germinal-center B cells. Centroblasts (rapidly dividing B cells with a noncleaved nucleus) in the dark zone of the germinal center expand at a remarkable rate. These cells periodically enter the light zone of the germinal center, where they morph into centrocytes (nondividing B cells with a cleaved nucleus). These centrocytes strip antigen from follicular dendritic cells and process it for presentation to nearby T cells.\(^3\) Centrocytes can revert to centroblasts and reinitiate proliferation, or they can differentiate into memory B cells or plasma cells.

During the germinal-center reaction, two distinct modifications of B-cell DNA alter the B-cell receptor: somatic hypermutation and class-switch recombination, both of which require activation-induced cytidine deaminase (AID).\(^4\) Class-switch recombination changes the immunoglobulin heavy-chain class from IgM to IgG, IgA, or IgE, whereas somatic hypermutation entails immunoglobulin-variable-region mutations, which create a population of B cells with increased (or decreased) affinity for a particular antigen. These genetic modifications are essential for a normal immune response, but they are also a source of DNA damage that can become pathologic in lymphomas.

A suite of transcription factors shapes the phenotype of the germinal-center B cell (Fig. 1). These cells selectively express B-cell lymphoma 6 (BCL6), a repressor
of transcription. Genetic translocations in diffuse large-B-cell lymphoma and other lymphomas\(^5\) deregulate BCL6, and disruption of the gene abolishes the germinal-center response in mice.\(^6\)\(^7\)\(^8\) BCL6 protein represses many genes involved in the differentiation of plasma cells, cell-cycle progression, responses to DNA damage, and cell death. Notably, one of these genes is Blimp-1,\(^9\)\(^10\) a master regulator of plasma-cell differentiation that extinguishes the gene-expression program of mature B cells.\(^11\)\(^12\) As germinal-center B cells begin to differentiate into plasma cells, they upregulate interferon regulatory factor 4 (IRF4), a transcription factor required for the conversion to plasma cells. IRF4 in turn increases the expression of Blimp-1,\(^13\)\(^14\)\(^15\) and this increase represses BCL6, thus tipping differentiation in favor of plasma cells.\(^11\)

Germinal-center B cells give rise to many types of lymphoma, including diffuse large-B-cell
Malignant lymphomas can arise at multiple stages of normal B-cell development. After the stimulation of a mature naive B cell with a T-cell–dependent antigen, the germinal-center reaction is initiated. The germinal-center B cell represents a discrete, quasi-stable differentiation stage that is characterized by a unique regulatory network and the activation of cytokine deaminase (AID), which induces both immunoglobulin (Ig) somatic hypermutation and heavy-chain class switching. Several transcription factors are required to establish and maintain the identity and function of the germinal-center B cell, including BCL6, MTA3, SPIB, BACH2, OCT2, OCAB, and IRF8. Red lines indicate that a regulatory factor inhibits the indicated gene or cellular function, and blue lines indicate positive regulation. In concert, these factors block plasmacytic differentiation by repressing Blimp-1. They also promote cell-cycle progression without cell growth while blocking the DNA damage response evoked by AID-dependent mutations and DNA breaks. Within the germinal center, the rapidly proliferating centroblasts are prone to cell death. Periodically, centroblasts travel to a subcompartment of the germinal center that is rich in follicular dendritic cells and follicular helper T cells, where they become centrocytes. Centrocytes may be rescued from cell death as a result of stimulation by antigen on follicular dendritic cells and CD40 ligand on T cells and may then revert to the centroblast state and resume proliferation. IRF4 initiates plasmacytic differentiation by establishing a characteristic regulatory network, which extinguishes the mature B-cell program while promoting terminal differentiation and immunoglobulin secretion. The putative origins of various non-Hodgkin’s lymphomas — including the germinal-center B-cell–like (GCB) and activated B-cell–like (ABC) subtypes of diffuse large-B-cell lymphoma (DLBCL) — are indicated. Lymphomas that are derived from germinal-center B cells have recurrent genetic abnormalities that circumvent the normal genetic program in order to block plasmacytic differentiation, promote cell growth, and evade apoptosis. NF-κB denotes nuclear factor-κB.

lymphoma, follicular lymphoma, and Burkitt’s lymphoma. These types of lymphoma carry the differentiation program of the normal B cell from which they arise,16-18 but oncogenic abnormalities in the lymphoma often subvert the normal program. For example, normal centroblasts lack the antiapoptotic activities of B-cell lymphoma 2 (BCL2) and the nuclear factor-κB (NF-κB) pathway and hence are poised to die.17,19 Malignant centroblasts, however, avoid cell death by acquiring activating translocations of BCL2 or by constitutively activating NF-κB. Likewise, BCL6 suppresses the MYC oncogene in most normal centroblasts,17,18,20 but malignant centroblasts evade this control by translocating or amplifying MYC, thereby allowing expression of a potent regulator of cell metabolism and growth.

ALTERATIONS OF B-CELL DNA IN LYMPHOMAS

RAG RECOMBINASE

The normal mechanisms of V(D)J recombination, somatic hypermutation, and class-switch recombination can alter the genome of lymphomas. RAG-mediated chromosomal breaks in pro-B cells can persist over many cell divisions, thereby creating the potential for a translocation in which immunoglobulin loci fuse to DNA breaks in other genes.21 The activation of RAG recombinase in mature B cells during immunoglobulin-receptor editing can also promote translocations.22 The t(14;18) chromosome translocation, which occurs in most follicular lymphomas and some diffuse large-B-cell lymphomas, places BCL2 under the control of IgH locus enhancers. This translocation requires RAG recombinase, which cleaves DNA in the IgH locus and in an unusual DNA structure in BCL2.23 In mantle-cell lymphoma, RAG recombinase allows a t(11;14) translocation that joins the CCND1 gene to the IgH locus, causing overexpression of cyclin D1 and hence deregulation of the cell cycle. The translocation breakpoints in t(14;18) and t(11;14) suggest that AID conspires with RAG recombinase to create double-stranded DNA breaks in BCL2 and CCND1.24

AID

The mutator enzyme AID plays several roles in lymphomagenesis. In mouse models, the development of diffuse large-B-cell lymphoma requires AID,25 and transgenic overexpression of AID causes B-cell lymphomas.26 AID can introduce mutations into nonimmunoglobulin genes of germinal-center B cells in mice at frequencies that are many orders of magnitude above the background rate of mutation.27-29 Diffuse large-B-cell lymphomas accumulate AID-dependent mutations in many genes, including MYC and PIM1 oncogenes.30 These mutations may accrue because of a deficiency in DNA mismatch repair or selection for cells with advantageous mutations.29 Regions that incur AID-mediated mutations coincide with chromosomal translocation breakpoints, suggesting that AID introduces double-stranded breaks that promote translocations.30
Pathogenesis of Diffuse Large-B-Cell Lymphoma

Diffuse large-B-cell lymphoma, the most common form of lymphoma, accounts for 30 to 40% of newly diagnosed lymphomas. Combination chemotherapy plus rituximab can cure approximately 50% of cases. Gene-expression profiling can divide this type of lymphoma into three histologically indistinguishable molecular subtypes: the ABC subtype, the germinal-center B-cell–like (GCB) subtype, and primary mediastinal B-cell lymphoma (PMBL).16,37-40 These subtypes differ in the expression of thousands of genes and apparently arise from B cells that are at separate stages of differentiation. In addition, the process of malignant transformation differs for each subtype, as evidenced by distinctive genetic abnormalities, and the three subtypes differ in clinical presentation, in cure rates after chemotherapy, and in responsiveness to targeted therapies. For these reasons, we view each of the subtypes as a distinct neoplasm.

GCB lymphomas express hundreds of genes that define germinal-center B cells.16,37,41 The malignant clone continues to undergo somatic hypermutation,42 and the cells have often switched IgH classes.32 By contrast, ABC lymphomas have the plasma-cell expression program, including the transcription factor XBP1, the master regulator of immunoglobulin secretion.38,43 Constitutive activation of the NF-κB pathway causes ABC lymphomas to express the transcription factor IRF4, and this may push them toward differentiation into plasma cells.44,45 However, ABC lymphomas acquire genetic lesions that interfere with Blimp-1, thereby blocking full differentiation into plasma cells.9,46-50

A block in differentiation seems to be an important early step in the pathogenesis of the ABC subtype, but the nature of the precursor cell is unclear. These lymphomas contain high amounts of AID, and their IgH genes have been heavily mutated.32,38,42,51 Nevertheless, most ABC lymphomas have not undergone class-switch recombination, and they express IgM, unlike most normal germinal-center B cells and other GCB lymphomas.32,38 They could originate from IgM-positive post–germinal-center memory cells52 or from a pre–germinal-center B cell that expresses AID, a characteristic of certain extrafollicular B cells.53

PMBL, the third subtype, typically presents as a mediastinal mass in a young woman (median age, 30 to 35 years). The tumor mass often contains a thymic remnant, suggesting that it originates from a rare thymic B cell. Although the ABC and GCB subtypes can seed the gastrointestinal tract and bone marrow, PMBL spreads by direct extension to adjacent thoracic structures.39 Clinical features alone cannot reliably distinguish PMBL from other subtypes, but gene-expression profiling can readily distinguish them.39,40 The gene-expression signature of PMBL contains a molecular link with Hodgkin’s lymphoma, which may also arise from a thymic B cell.39,40 However, these two lymphomas differ because PMBL typically expresses genes of mature B cells, whereas Hodgkin’s lymphoma does not.39

Genetic Aberrations

Oncogenic Pathways in Lymphoma Subtypes

Some oncogenic abnormalities occur in more than one subtype of diffuse large-B-cell lymphoma,50,54 but many oncogenic pathways are predominantly or exclusively used by only one subtype (Fig. 2). Genetic lesions that are specific to GCB lymphoma are the t(14;18) translocation,37,50 deletion of the tumor suppressor PTEN, amplification of the microRNA cluster miR-17-92 (which down-regulates PTEN55), and p53 mutations.56

Numerous genetic abnormalities in the ABC subtype are rare or absent in the other subtypes. Most ABC lymphomas overexpress BCL2 and many amplify the BCL2 locus.50 Many delete the INK4a–ARF locus, which encodes p16, an inhibitor of senescence, and p14Arf57, an inhibitor of p53 activation.50,57 Loss of these tumor suppressors blocks the action of chemotherapy, and this
could contribute to the poor prognosis that is associated with the ABC subtype.\(^{50}\)

A genetic hallmark of PMBL is amplification of a region on chromosome 9p24, which occurs in almost half the patients with this subtype and also in those with Hodgkin’s lymphoma.\(^{39,50,58}\)

This region encodes JAK2, a tyrosine kinase that phosphorylates and activates the transcription factor STAT6.\(^{59}\) Moreover, SOCS1, a suppressor of JAK signaling, is regularly deleted in PMBL and Hodgkin’s lymphoma.\(^{60-62}\) The amplified region in 9p24 encompasses many megabases of DNA,
suggesting involvement of other genes. For example, the amplification of PDL1 (also called CD274) and PDL2 (also called CD273), which encode inhibitors of T-cell responses, could allow the malignant clone to survive in the thymus.39

**CONSTITUTIVE NF-κB SIGNALING**

The ABC subtype has gene-expression characteristics of normal B cells that were activated by cross-linking the B-cell receptor.16 Subsequent studies directly implicated B-cell-receptor signaling in the pathogenesis of this lymphoma. In resting normal B cells, the inhibitory protein IκBα sequesters NF-κB transcription factors in the cytoplasm.65 The stimulation of the cells through certain surface receptors leads to phosphorylation of IκBα, causing its degradation in proteasomes. Cytoplasmic NF-κB factors can then move to the nucleus and activate their target genes. In normal B cells, cross-linking the B-cell receptor transiently engages the NF-κB pathway, but in the ABC subtype, the NF-κB pathway is constitutively active (Fig. 2 and 3A).44 Interference with NF-κB signaling kills ABC but not GCB cells, which shows that the ABC subtype is dependent on the constitutive activity of this pathway.44,45

The many downstream targets of NF-κB collectively prevent apoptosis and thereby block the action of many forms of chemotherapy.64 The constitutive activation of NF-κB pathways may thus contribute to the poor response of the ABC subtype to chemotherapy.16,37,65 NF-κB signaling in the ABC subtype also induces the cytokines interleukin-6 and interleukin-10, which act through JAK kinases and the STAT3 transcription factor as autocrine signals to the cell.56,57 Blockade of JAK signaling acts synergistically with the inhibition of NF-κB in killing ABC cells.56

An “Achilles heel” screen has been developed in the hope of finding new targets for therapeutic development by identifying genes that are required for the proliferation or survival of cancer cells.66 This screen revealed that the ABC subtype relies on three molecules to activate NF-κB: CARD11, BCL10, and MALT1 (CBM complex). When normal lymphocytes encounter antigen, B-cell–receptor signaling causes phosphorylation of CARD11, which allows it to assemble with BCL10 and MALT1 into a complex that activates IκB kinase (IKK), the enzyme that phos-
Various genetic abnormalities activate CARD11 in the ABC subtype. In approximately 10% of patients, CARD11 is a bona fide oncogene that acquires mutations that activate NF-κB and prolong cell survival. Mutant CARD11 isoforms create large protein aggregates in the cytoplasm, the probable sites of constitutive NF-κB activation (Fig. 3B).

**CHRONIC ACTIVE B-CELL–RECEPTOR SIGNALING**

Many ABC lymphomas have wild-type CARD11 yet rely on CARD11 to activate NF-κB. In these lymphomas, there is a chronic active form of B-cell–receptor signaling that engages the CBM pathway. The B-cell receptor consists of the antigen-binding IgH and IgL chains and two signaling subunits, CD79A and CD79B (Fig. 3A). Engagement of the receptor by antigen triggers a cascade of kinases that activate multiple downstream pathways. The survival of the ABC subtype with wild-type CARD11 depends on the B-cell receptor and downstream kinases. Like antigen-stimulated normal B cells, these ABC cells display prominent clusters of B-cell receptors on their surface. About 20% of ABC lymphomas have mutations in CD79A or CD79B, which are rare or absent in GCB and other lymphoma subtypes. The mutant CD79 proteins increase expression of the B-cell receptor and reduce activation of LYN, a negative regulator of B-cell–receptor signaling.

It appears from this evidence that chronic active B-cell–receptor signaling is a critical step in the pathogenesis of the ABC subtype. Conceivably, ABC lymphomas could originate from a B cell that acquires a mutation in CD79A or CD79B, thereby fostering clonal expansion and increasing the probability of additional oncogenic events. Inactivation of A20, a negative regulator of NF-κB signaling, increases the activity of this pathway in the ABC subtype. A20 aberrations do not occur commonly in the GCB subtype, but they are present in other lymphomas with NF-κB activity. The expression of A20 requires NF-κB signaling, suggesting that tumors with A20 inactivation rely on additional mechanisms to activate NF-κB signaling, such as chronic active B-cell–receptor signaling or a CARD11 mutation.

**MOLECULAR SIGNATURES OF THERAPEUTIC RESPONSE**

The subtypes of diffuse large-B-cell lymphoma are associated with distinctly different overall rates of survival after anthracycline-based chemotherapy: overall survival is favorable in patients with the GCB subtype and PMBL and inferior in those with the ABC subtype. The addition of rituximab to standard chemotherapy combining cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) has improved survival in patients with the ABC subtype, but this subtype remains less curable than the GCB subtype (Fig. 4B).

Biologic attributes that influence overall survival have been identified by gene-expression signatures. In one such approach, the signatures are integrated into a statistical model in which the risk for each patient is estimated by a survival predictor score (Fig. 4A and 4C). The expression of a signature for a germinal-center B cell in the GCB subtype is associated with favorable survival and mirrors the distinction between the GCB and ABC subtypes. Two other signatures reveal differences in nonmalignant cells in the microenvironment of tumors. A favorable stromal-1 signature reflects extracellular matrix deposition and infiltration of the tumors with macrophages. The fibrosis may be caused by connective-tissue growth factor, a cytokine that stimulates fibrotic responses of mesenchymal cells. The infiltrating macrophages may transmit trophic signals to the tumor, suppress antitumor immune responses, or both. Conversely, a stromal-2 signature is associated with inferior survival and identifies tumors with a high density of blood vessels. The angiogenesis of these tumors may be due to low expression of the angiogenesis inhibitor thrombospondin 2 and high expression of the chemokine stromal-cell–derived factor 1 (SDF-1), which can recruit endothelial precursors to the tumor.

**THERAPEUTIC OPPORTUNITIES**

**NF-ΚΒ PATHWAY**

Therapies targeting the antiapoptotic NF-κB pathway seem especially attractive, given the evidence implicating this pathway in the ABC subtype, PMBL, marginal-zone lymphoma, and Hodgkin’s lymphoma. These tumors engage the classic NF-κB pathway, in which the IKK β sub-
The IKKβ phosphorylates IκBα, triggering its ubiquitination by ubiquitin ligase SCFβ-TrCP (Skp1–Cul1–F-box ubiquitin ligase with a beta-transducin repeat-containing protein subunit) and then degradation in the proteasome. Selective IKKβ inhibitors hold promise in the treatment of lymphoma but have yet to enter clinical trials. Inhibitors of heat shock protein 90 (HSP90) can

**Figure 4.** Prediction of Survival According to Gene Expression in Diffuse Large-B-Cell Lymphoma.

Panel A shows three gene-expression signatures that are associated with overall survival in patients with diffuse large-B-cell lymphoma (DLBCL) who have been treated with chemotherapy combining cyclophosphamide, doxorubicin, vincristine, and prednisone with rituximab (R-CHOP). Shown are the average expression levels of the genes in each signature in 233 biopsy specimens; each vertical line represents a single specimen. The germinal-center B-cell signature is prognostically favorable and mirrors the distinction between the two most prevalent subtypes, activated B-cell–like (ABC) and germinal-center B-cell–like (GCB) subtypes, as indicated. The stromal-1 and stromal-2 signatures reflect the character of the nonmalignant cells infiltrating the biopsy specimens. The stromal-1 signature is associated with favorable survival, and the stromal-2 signature is associated with inferior survival. These three signatures are combined in a mathematical model that provides a survival predictor score (bottom bar), which can be used to assess risk for each patient treated with R-CHOP. Gene-expression values and the survival predictor scores are shown over a range that varies by a factor of 16, according to a red–green color scale. For gene expression, red indicates high expression, and green indicates low expression. For the survival predictor score, red indicates unfavorable survival, and green indicates favorable survival. Shown are representative signature genes that are upregulated in expression in association with the indicated signature. Panel B shows survival rates for patients with a molecular diagnosis of the GCB or ABC subtype after R-CHOP therapy. Panel C shows a model for prediction of survival according to gene expression. Patients were ranked according to their survival predictor scores (as shown in Panel A) and divided into quartiles. In this Kaplan–Meier analysis, the gene-expression–based model defines differences in 3-year progression-free survival among patients treated with R-CHOP.
also block IKK, since HSP90 is a component of the IKK macromolecular complex. Another strategy to inhibit NF-κB is to prevent the degradation of IκBα by the blockade of ubiquitin ligase SCFβ-TRCP by means of a small-molecule antagonist of the NEDD8 activating enzyme. Recently, MALT1 has emerged as a therapeutic target in this pathway since it has protease activity that is required for NF-κB signaling and the survival of ABC lymphoma cells.

An indirect way to inhibit NF-κB is to block the degradation of IκBα in proteasomes with the use of bortezomib. In a phase 2 trial of bortezomib plus chemotherapy in patients with relapsed or refractory diffuse large-B-cell lymphoma, the response rate was higher in patients with the ABC subtype than in those with the GCB subtype (85% vs. 13%), and patients with the ABC subtype had superior overall survival. These results are notable, given the consistently poor response of ABC lymphoma to chemotherapy alone. The inhibition of NF-κB may have sensitized the tumor to the cytotoxic action of chemotherapy, although bortezomib affects many additional pathways that may be used differently by ABC and GCB subtypes.

**B-CELL–RECEPTOR SIGNALING**

Chronic active B-cell–receptor signaling in patients with the ABC subtype can be interrupted by inhibiting the SRC-family kinases BTK, SYK, and PKCβ, or the phosphatidylinositol 3-kinase (PI3K)–mTOR pathway (Fig. 3A and 3B). A selective BTK inhibitor kills ABC cells with chronic active B-cell–receptor signaling, as does the multi-kinase inhibitor dasatinib, which inhibits BTK and SRC-family kinases. A SYK inhibitor, R406, can produce remissions in patients with diffuse large-B-cell lymphoma that last for several months. However, R406 has activity in vitro against diffuse large-B-cell lymphomas that are not SYK-dependent, suggesting that the drug’s in vivo activity may in some cases stem from the inhibition of other kinases. A small-molecule inhibitor of PKCβ produced complete and partial remissions in a small fraction of patients, but whether these tumors were ABC lymphomas was not determined.

Since both PI3K and NF-κB signaling supply survival signals to ABC cells, combination therapies that block both pathways may prove synergistic in patients with chronic active B-cell–receptor signaling.

**BCL2**

Preclinical data from lymphoma cell lines and primary tumor samples indicate high efficacy of BCL2 inhibitor ABT-737 against lymphoma. The sensitivity to ABT-737 treatment in vitro can be predicted by the nature and relative abundance of the antiapoptotic BCL2 family members and the proapoptotic BH3-only proteins.

**BCL6**

Many cells lines for diffuse large-B-cell lymphoma and Burkitt’s lymphoma undergo growth arrest and apoptosis after inhibition of BCL6. BCL6 dependency occurs in ABC and GCB cell lines, with or without a BCL6 translocation, and thus probably reflects the dependency of normal germinal-center B cells on BCL6. The inhibition of BCL6 by a cell-permeable peptide that disrupts its interaction with corepressor proteins kills xenografts but is not overtly toxic to mice, suggesting that therapeutic targeting of BCL6 holds promise.

**TUMOR MICROENVIRONMENT**

An unexplored opportunity in lymphoma therapy is modulation of the tumor microenvironment. A subgroup of patients with high expression of the stromal-2 signature and increased blood-vessel density in tumors might respond to therapy against vascular endothelial growth factor. A component of the stromal-2 signature, SDF-1, is angiogenic, suggesting that agents that block its receptor (CXCR4) should be evaluated in patients with diffuse large-B-cell lymphoma. The myeloid-lineage cells that infiltrate some of these lymphomas may provide trophic stimuli to the malignant cells, raising the possibility that monoclonal antibodies against these cells might be efficacious. The fibrosis in some tumors could promote cytokine or chemokine signaling, and a monoclonal antibody against the profibrotic cytokine CTGF might block this effect.

**SUMMARY**

Given the emergence of many new therapeutic agents that affect essential regulatory pathways in lymphomas, the challenge is to identify rational combinations that kill lymphoma cells synergistically. This effort requires both preclinical evaluation and the development of clinical-trial strategies that include molecular profiling. Gene-
expression profiling could be used to determine the lymphoma subtype and to estimate the risk associated with standard therapy. Cancer-gene resequencing could be used to identify tumors that are likely to be dependent on a particular signaling pathway and to determine which step in the pathway to inhibit. Finally, the activity of the targeted pathways before and during therapy could be used to assess the efficacy of the treatment. We expect that this “divide-and-conquer” approach will provide increasingly effective and nontoxic therapies for patients with lymphoma.

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