Focus on lymphomas

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Epidemiology

Lymphomas comprise the fifth most common cancer type in the United States, with approximately 55,000 cases of non-Hodgkin's lymphoma (NHL) and 7,400 cases of Hodgkin's lymphoma (HL) each year. An unexplained finding is the 80% rise in NHL between 1973 and 1997 (http://www.seer.cancer.gov). The strongest known risk factors are genetic and acquired immunodeficiencies (Knowles, 1999). Epstein-Barr virus (EBV) is an important cofactor in the development of B cell lymphoma among immunosuppressed patients, but also plays a role in immunocompetent patients with Burkitt's lymphoma and HL. Human herpes virus 8 and human T-lymphotropic virus-1 are associated with primary effusion lymphoma and peripheral T cell lymphoma, respectively. Chronic immune stimulation by *H. pylori* is an etiologic factor in gastric MALT lymphoma (Wotherspoon et al., 1993). Autoimmune conditions such as Sjogren's syndrome are associated with an excess risk of marginal zone lymphomas (Mariette, 1999).

Molecular pathogenesis of lymphomas

Diverse molecular abnormalities in lymphomas converge on common pathways that promote proliferation, block differentiation, inhibit cell death, or permit genomic instability (Figure 1). Chromosomal translocations are frequent oncogenic events in lymphomas and can be aberrant byproducts of the molecular machinery that remodels the immunoglobulin (Ig) loci during normal lymphocyte differentiation (Kuppers and Dalla-Favera, 2001). Typically, these translocations do not alter the coding region of the oncogene at the translocation breakpoint, but rather deregulate its expression.

Molecular cloning of the recurrent t(14;18) translocation in follicular lymphoma (FL) led to the discovery of BCL-2 and to an appreciation that cancer cells frequently evade apoptosis (Chao and Korsmeyer, 1998). Antiapoptotic members of the BCL-2 family are overexpressed in many lymphomas due to chromosomal translocation or amplification, or by transcriptional activation (Figure 1). Exceedingly high BCL-2 expression is characteristic of small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and mantle cell lymphoma (MCL), but the mechanisms underlying this transcriptional overexpression are unknown. In other lymphomas, apoptosis can be blocked by the NF-κB signaling pathway (Baldwin, 2001). Normally, NF-κB transcription factors are sequestered in the cytoplasm by IκBα. Signaling through various receptors activates IκB kinase (IKK) to phosphorylate IκBα, leading to its degradation in the proteosome and the release of NF-κB to the nucleus. In lymphomas, the NF-κB pathway can be abnormally activated by translocations involving the *NFkB2*, *BCL10*, and *MALT1* genes, by IκBα mutations or deletions, and by activation of IKK by viruses or intracellular signaling pathways that have not been fully characterized (Figure 1).

Roughly one-sixth of all NHLs have translocations of *BCL-6*, which encodes a transcriptional repressor required for the development and function of normal germinal center (GC) B cells (Dalla-Favera et al., 1999; Staudt et al., 1999). BCL-6 blocks terminal differentiation of GC B cells to plasma cells by repressing Blimp-1 (Shaffer et al., 2000). BCL-6 translocations may therefore cause lymphomas by trapping cells at the GC stage of differentiation, exposing them to the potentially mutagenic effects of the Ig somatic hypermutation apparatus (Pasqualucci et al., 2001). BCL-6 can also block cellular senescence (Shvarts et al., 2002) and repress transcription of the cyclin-dependent kinase inhibitor p27kip1 (Shaffer et al., 2000), which may extend the replicative capacity of cells with BCL-6 translocations.

Cell growth and proliferation is deregulated in various lymphomas by translocation, mutation, and overexpression of *c-myc* (Figure 1). Likewise, G1-S phase progression can be promoted by translocation or overexpression of the genes encoding D-type cyclins. The senescence checkpoint that limits proliferation may be abrogated in mantle cell lymphoma (MCL) by genomic deletion of $p16^{INK4A}$ or by genomic amplification of *bmi-1*, which encodes a repressor of the INK4A locus (Bea et al., 2001).

An important acquired trait of lymphomas is functional inactivation of genes that monitor genome integrity. ATM, which senses DNA damage and telomere integrity, is frequently inactivated by genomic deletions and mutations in MCL (Schaffner et al., 2000). Inactivation of p53 occurs in many lymphomas and is associated with adverse outcome in MCL (Louie et al., 1995) and with transformation of FL to diffuse large B cell lymphoma (DLBCL) (Lo Coco et al., 1993; Sander et al., 1993).

Molecular diagnosis of lymphomas

The standard for the pathological identification of lymphomas is the World Heath Organization (WHO) classification, which integrates tumor morphology, immunophenotype, recurrent genetic abnormalities, and clinical features (Supplemental Table S1 at http://www.cancercell.org/cgi/content/full/2/5/363/DC1) (Jaffe et al., 2001). This classification recognizes over 30 different lymphoma types derived from virtually every stage of normal lymphocyte differentiation and has proven useful in patient management and treatment. Nonetheless, clinical outcomes of patients assigned to the same diagnostic category can be quite heterogeneous. For instance, the survival of patients with follicular lymphoma can range from 20 years to less than 1 year. In DLBCL, roughly 40% of patients are cured by multiagent chemotherapy, whereas the others succumb to this disease.

Such clinical heterogeneity often stems from molecular differences between tumors. For example, gene expression profiling has revealed that DLBCL is comprised of molecularly distinct subgroups that are derived from different stages of normal B cell differentiation and have different cure rates (Figure 2A)

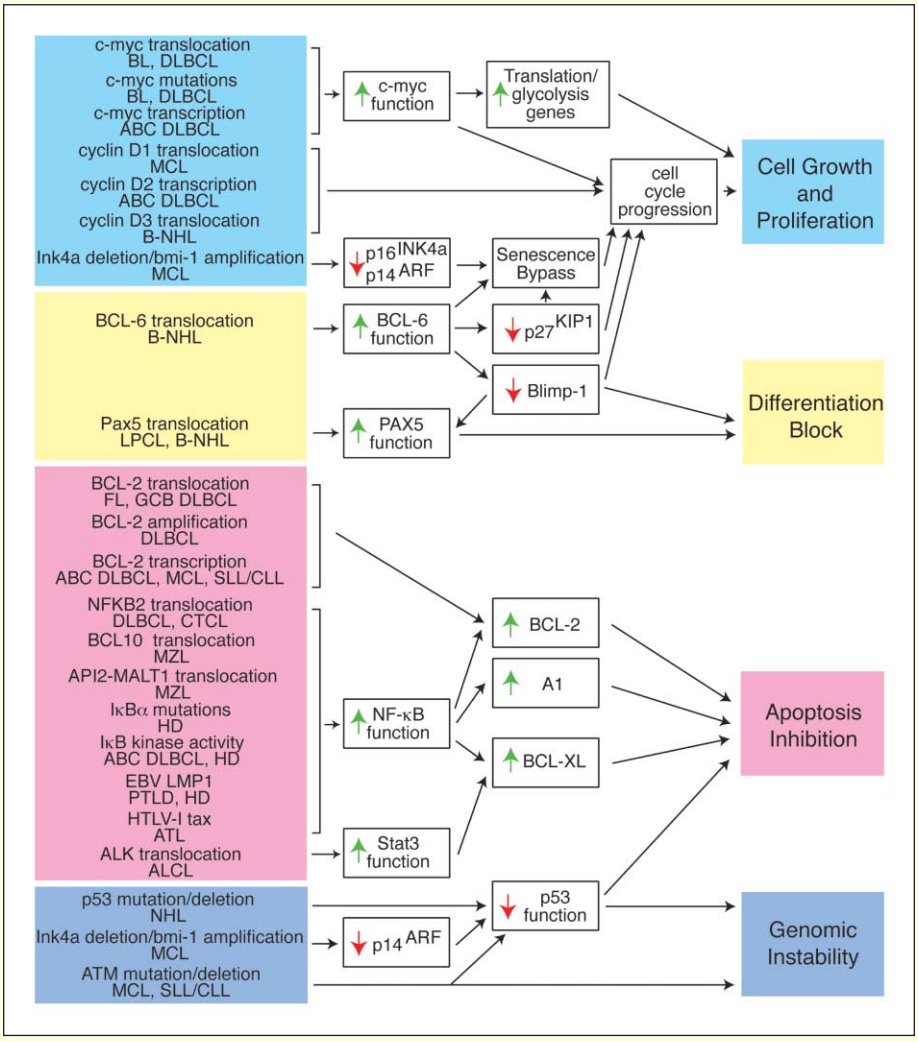

Figure 1. Pathogenetic mechanisms in lymphomas

Arrows indicate presumptive target genes and pathways affected by oncogenic events in various lymphoma types. An enhanced version of this figure with references is available as Supplemental Figure S1 at http://www.cancercell.org/cgi/content/full/2/5/363/DC1. Abbreviations: BL, Burkitt's lymphoma; DLBCL, diffuse large B cell lymphoma; ABC, activated B cell; MCL, mantle cell lymphoma; B-NHL, B cell non-Hodgkin's lymphoma; LPCL, lymphoplasmacytoid lymphoma; FL, follicular lymphoma, GCB, germinal center B cell; SLL, small lymphocytic lymphoma; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T cell lymphoma; MZL, marginal zone lymphoma; HD, Hodgkin's disease; EBV, Epstein-Barr virus; PTLD, posttransplant lymphoproliferative disorder; ATL, adult T cell lymphoma; ALCL, anaplastic large cell lymphoma.

(Alizadeh et al., 2000; Rosenwald et al., 2002). Roughly half of the DLBCL tumors express genes that are characteristic of normal GC B cells, and this subgroup has been termed germinal center B cell-like (GCB) DLBCL. Another ~30% of DLBCL tumors lack expression of these genes, but instead express genes characteristic of mitogenically activated peripheral blood B cells, and this subgroup has been termed activated B cell-like (ABC) DLBCL. Finally, ~20% of DLBCL tumors do not resemble either GCB or ABC DLBCL in gene expression, and this heterogeneous subgroup has been termed Type 3 DLBCL. Clinically, patients with GCB DLBCL have the best prognosis (Figure 2A).

Analysis of recurrent chromosomal aberrations demonstrated that the DLBCL gene expression subgroups can be considered pathogenetically distinct diseases. *BCL-2* translocations and *c-rel* amplifications occur only in GCB DLBCLs (Rosenwald et al., 2002). Conversely, ABC DLBCLs utilize the antiapoptotic NF-κB pathway by constitutive activation of the IKK (Davis et al., 2001).

The DLBCL gene expression subgroup distinction explains some, but not all, of the variable survival of these patients. Clinical outcome data were used to discover genes whose expression patterns correlate with survival following chemotherapy for DLBCL, and these genes were combined to form multivariate predictors of survival (Rosenwald et al., 2002; Shipp et al., 2002). Clinical parameters also predict survival (INHLPFP, 1993), but the gene expression-based predictors were independent of the clinical predictor. Thus, the effectiveness of chemotherapy in DLBCL is dictated both by intrinsic molecular properties of DLBCL tumors and by clinical features of these patients, such as the extent of tumor bulk at diagnosis.

Four variable biological features of DLBCL tumors have the most influence on clinical outcome, and these biological features are reflected in characteristic gene expression signatures (Figure 2B) (Rosenwald et al., 2002). The GC B cell gene expression signature predicts favorable survival, in keeping with the relatively favorable course of GCB DLBCL patients. The proliferation gene expression signature, which reflects the rate of tumor cell division, predicts poor outcome. The expression of major histocompatibility complex class II genes predicts favorable survival, as do genes in the lymph node signature, which reflects a reactive stromal and innate immune response to the DLBCL tumor cells. The predictive power of these two gene expression signatures suggests that the immune response to the DLBCL cells may be an important determinant of overall survival following chemotherapy.

Recent treatment advances

Lymphomas, being malignancies of immune cells, are usually systemic and require chemotherapy. Some lymphoma types are potentially curable, whereas others may respond to therapy but are incurable (Supplemental Table S1). In DLBCL, the most common curable lymphoma, the addition of rituximab, a monoclonal antibody against CD20, to standard CHOP chemotherapy has significantly increased the survival and probable cure of older patients (Coiffier et al., 2002). Dose-adjusted EPOCH, a rationally designed chemotherapy based on principals of pharmacodynamics and drug resistance, may be more effective in highly proliferative DLBCLs (Wilson et al., 2002).

Monoclonal antibodies are also effective in other lymphoma types. Rituximab alone or in combination with chemotherapy has improved the treatment outcome of low-grade B cell lymphomas

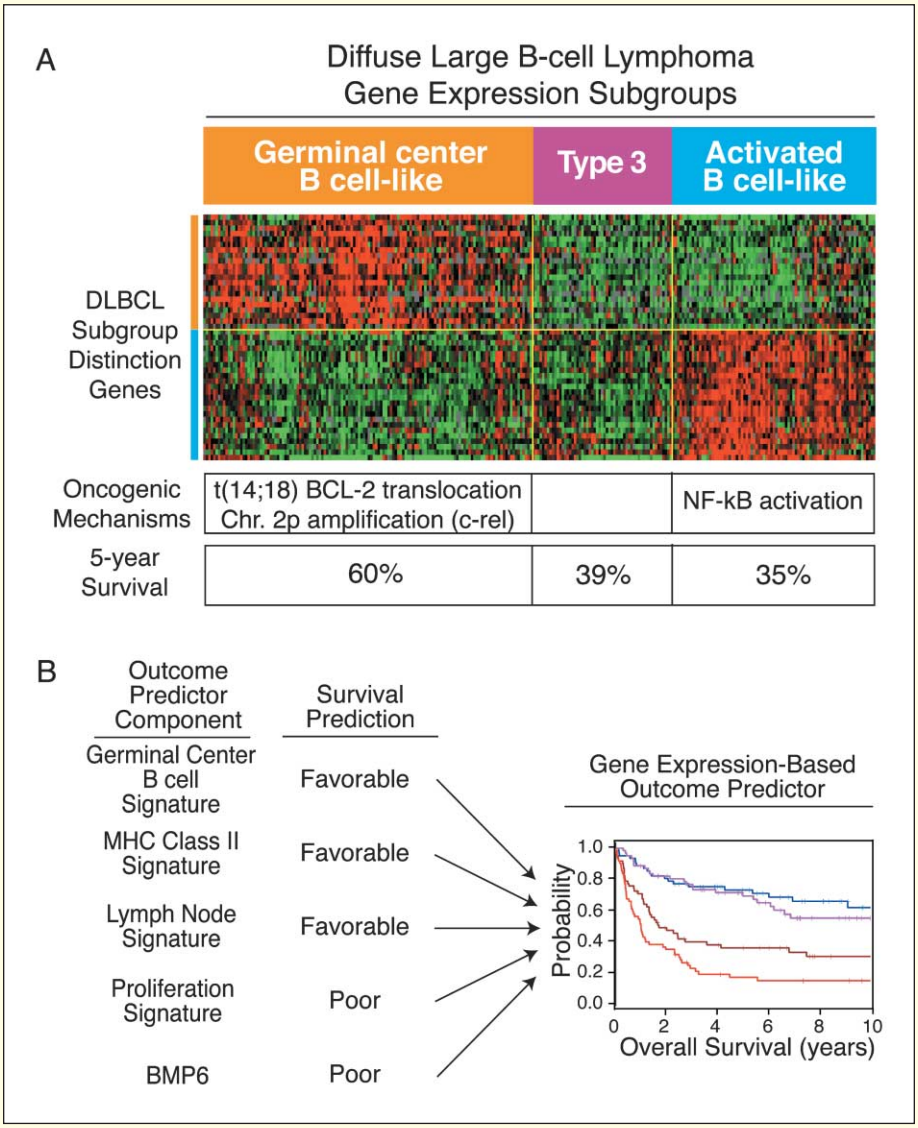

Figure 2. Molecular diagnosis of diffuse large B cell lymphoma (DLBCL)

A: Subgroups of DLBCL defined by hierarchical clustering of gene expression data have distinct clinical and pathogenetic features (Davis et al., 2001; Rosenwald et al., 2002). Relative gene expression is depicted on a color scale in which red represents higher expression, green represents lower expression, and black represents median expression. Each column represents a different lymphoma biopsy sample, and each row a different gene.

B: A gene expression-based outcome predictor of survival following chemotherapy for DLBCL based on four gene expression signatures (see text for details) and the expression of a single gene, *BMP6* (Rosenwald et al., 2002). High expression of a predictor component in a DLBCL tumor correlated with favorable or poor survival as indicated. Patients with DLBCL were assigned an outcome predictor score based on these 5 gene expression variables. The patients were ranked according to their outcome predictor scores and divided into 4 quartiles that are represented by different colors in the Kaplan-Meier plot of overall survival.

(Czuczman et al., 1999; Maloney et al., 1997). Alemtuzumab, a monoclonal antibody against the CD52 antigen, achieved a 33% response rate in refractory CLL and a 60% complete response rate in T cell prolymphocytic leukemia (Keating et al., 2002). Immunotoxin fusion proteins are also promising. A fusion of diphtheria toxin and interleukin-2 is effective in 30% of cutaneous T cell lymphomas (Olsen et al., 2001), and a fusion of an anti-CD22 variable domain with pseudomonas exotoxin induces complete remissions in patients with resistant hairy-cell leukemia (Kreitman et al., 2001).

Augmentation of immunity against lymphomas is another promising approach. Vaccines using Ig idiotype-keyhole limpet hemocyanin conjugates or Ig idiotype-pulsed dendritic cells produce clinical responses and could be potentially curative in FL (Bendandi et al., 1999; Timmerman et al., 2002).

Eradication of an infectious agent can be curative in some lymphomas. More than half of patients with gastric MALT lymphoma can be cured by antibiotic treatment of *H. pylori* (Wotherspoon et al., 1993). Treatment of hepatitis C with interferon α can cure some patients with splenic lymphoma with villous lymphocytes (Hermine et al., 2002).

Future challenges in the lymphomas

An intriguing new therapeutic target in several lymphoma types is the NF-κB pathway (Figure 2). Inhibition of this pathway is lethal to cell line models of ABC DLBCL (Davis et al., 2001), HL (Bargou et al., 1997), and EBV-associated lymphoproliferative disorders (Cahir-McFarland et al., 2000). An inhibitor of the proteosome, velcade/PS-341, blocks the NF-κB pathway and has been used successfully in Phase II clinical trials of multiple myeloma (Adams, 2002). This agent could have activity in certain lymphomas as a single agent, and could synergize with conventional chemotherapy, given that the NF-κB pathway blocks the induction of apoptosis by DNA damaging agents (Baldwin, 2001).

Therapeutic strategies targeting BCL-2 are promising, given its frequent overexpression in lymphomas. One approach involves antisense inhibition of BCL-2 (Waters et al., 2000). Future strategies may exploit the fact that BCL-2 uses its BH3 domain to bind proapoptotic BH3-only proteins, preventing them from interacting with BAX/BAK and initiating apoptosis (Letai et al., 2002). BH3 mimetics could conceivably fill the BCL-2 BH3 pocket, thus sensitizing tumor cells to the apoptotic action of BH3-only proteins.

We must remember that the lymphomas constitute a diverse collection of diseases, and that successful therapy will depend on detailed knowledge of the pathogenetic pathways involved in each lymphoma. Given the clinical and molecular heterogeneity within the current diagnostic categories of lymphomas, it will be essential to refine lymphoma diagnosis by including quantitative molecular assays based on the results of gene expression profiling. Such efforts will certainly add to the large number of potential molecular targets in the lymphomas that have already been defined.

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