Molecular features of B-cell lymphoma

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Malignant transformation of B cells can occur at various steps of lymphocyte development, starting from early B-cell progenitors up to mature B cells, which reflects the heterogeneity of B-cell malignancies with regard to their biologic and clinical behavior. The genetic characterization of B-cell neoplasms during the past two decades has elucidated the mechanisms underlying B-cell lymphomagenesis and led to a more precise definition of lymphoma subgroups. This progress is reflected in the upcoming World Health Organization classification for hematologic neoplasms, which stresses the diagnostic importance of recurrent genetic alterations in leukemias and lymphomas. In the recent past, several genes deregulated by such recurrent chromosomal aberrations have been identified. In addition, the recent introduction of microarray technology has now allowed a more global assessment of gene dysregulation in B-cell oncogenesis and provided a new means for more exactly defining the molecular hallmarks of distinct lymphoma subtypes. This review will focus on recently described molecular features of B-cell lymphomas discovered by the application of new molecular cytogenetic techniques, advanced breakpoint cloning strategies, and microarray approaches. Curr Opin Oncol 2001, 13:316–324 © 2001 Lippincott Williams & Wilkins, Inc.

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Abbreviations

CLL	chronic lymphocytic leukemia
DLBCL	diffuse large B-cell lymphoma
FISH	fluorescence *in situ* hybridization
FL	follicular lymphoma
MALT	mucosa-associated lymphoid tissue
NHL	non-Hodgkin lymphoma

Chromosomal aberrations in B-cell malignancies

Since the first description of a characteristic chromosomal aberration in B-cell lymphomas, the t(8;14) in Burkitt lymphomas by *Zech et al.* in 1976 [1], significant progress has been achieved in the molecular characterization of B-cell neoplasms. These advances have been spurred by the realization that characteristic chromosomal translocations, or rearrangements, tend to occur specifically in a given type of non-Hodgkin lymphoma (NHL), consistently altering the regulation of a particular gene [2••,3••]. For example, the description of the t(8;14) led not only to identification of the dysregulation of one of the key players in cell homeostasis, *c-MYC* but also to the genetic definition of a tumor subtype. The upcoming World Health Organization classification for hematologic neoplasms regards detection of t(8;14) or one of its variants, or *c-MYC* rearrangement, to be the "gold standard" for the diagnosis of Burkitt lymphoma [2••].

Although characteristic genetic changes have been detected by chromosome analyses in many subtypes of NHL (Table 1) [3••], several of which are discussed subsequently in detail, the application of conventional cytogenetics is hampered by the low mitotic index characteristic of mature B-cell neoplasms like chronic lymphocytic leukemia (CLL) or multiple myeloma, or by the low number of tumor cells found in Hodgkin disease. Nevertheless, new insights into the genetic subgrouping of these neoplasms have been gathered using molecular cytogenetic techniques like fluorescence *in situ* hybridization (FISH) and comparative genomic hybridization. Owing to space limitations, this review is restricted to recently identified genetic abnormalities in Hodgkin disease and B-cell NHL, although Table 1 also includes several genes shown to be important in the genesis of other B-cell neoplasms, such as CLL and multiple myeloma, for the readers' interest.

Genetic alterations in Hodgkin disease

Based on *Ig* hypermutation [4] and gene expression studies [5], it is now widely accepted that with very few exceptions [6,7], Hodgkin disease is a B-cell lymphoma [8••]. Unfortunately, as compared to B-cell NHL, hardly any data exist concerning the genetic changes underlying the pathogenesis of this neoplasm, as the scarcity of tumor cells in Hodgkin disease tissues renders chromosomal analyses of these cells largely unsuccessful. Exciting new data on the chromosomal changes in Hodgkin dis-

Table 1. Genes involved in B-cell oncogenesis via translocations with *Ig* loci

Cytogenetic abnormality*	Disease (predominant subtypes)	Involved/presumed target gene(s) (aliases)	Presumed function of target gene(s)†
t(1;14)(p22;q32)	MALT lymphoma	*BCL10* (*mE10, CIPER, CARMEN, CLAP*)	Antigen receptor-induced NFκB-activation
t(1;14)(q21;q32)	BCP-ALL, B-NHL	*BCL9*	Unknown
t(1;14)(q21;q32)	Myeloma, also BL with dup(1q)	*IRTA1/2*	Immunoglobulin receptor superfamily
t(1;14)(q21;q32)	DLBCL	*MUC1* (*EMA, CD227, PEM, PEMT, H23AG, PUM*)	Mucin (episialin); cell surface transmembrane glycoprotein
		MDC15 (*ADAM15*)	Metalloproteinase-like disintegrin-like and cysteine-rich protein
t(1;22)(q21;q11)	Transformed follicular B-NHL	*FCGR2B* (*CD32B*)	Low affinity Fc gamma receptor IIB; ITIM-containing receptor for the Fc domain of IgG; binds IgG immune complexes; member of the immunoglobulin superfamily
t(2;14)(p13;q32)	B-CLL, IC, DLBCL	*BCL11A* (*EVI9*)	Zinc finger transcriptional repressor
t(3;14)(q27;q32)	DLBCL and others	*BCL6* (*BCL5, LAZ3*)	Zinc finger transcriptional repressor
t(4;14)(p16;q32)	Myeloma	*FGFR3* (*CEK2, ACH, JTK4*)	Fibroblast growth factor receptor 3; receptor tyrosine kinase that binds acidic and basic FGF
		MMSET (*WHSC1*)	Wolf-Hirschhorn syndrome candidate 1; contains a SET domain, an HMG box, and PHD fingers
t(5;14)(q31;q32)	BCP-ALL	*IL3* (*MCGF*)	Interleukin-3 (colony-stimulating factor); hematopoietic growth factor
t(6;14)(p25;q32)	Myeloma	*IRF4* (*NF-EM5, MUM1, LSIRF*)	Interferon regulatory factor 4; transcription factor that stimulates B-cell proliferation
t(6;14)(p21;q32)	DLBCL, myeloma, SLVL, MZL	*CCND3*	Cyclin D3, essential for control of the cell cycle at the G1/S (start) transition; interacts with the CDC2 protein kinase
t(2;7)(p12;q21)	SLVL	*CDK6* (*PLSTIRE*)	Cyclin-dependent protein kinase 6; interacts with D-type cyclins and phosphorylates RB in G1-phase
t(8;14)(q24;q32)	BL, DLBCL, B-PLL, myeloma	*c-MYC*	Transcription factor; activates/represses expression of multiple target genes
t(9;14)(p13;q32)	Lymphoplasmacytoid lymphoma, myeloma	*PAX5* (*BSAP*)	Paired box 5; B-cell lineage-specific activator protein, transcription factor
t(10;14)(q24;q32)	DLBCL	*NFKB2* (*LYT10, H2TF1*)	49-kD DNA-binding subunit (p52/p100) of heterodimeric NFκB transcription factor; complex regulates the expression of inflammatory and immune genes
t(11;14)(q13;q32)	Mantle cell lymphoma, B-PLL, SLVL, myeloma	*CCND1* (*BCL1, PRAD1*)	Cyclin D1; essential for control of the cell cycle at the G1/S (start) transition; interacts with the cdk4 and cdk6 protein kinases
t(11;14)(q23;q32)	Mediastinal B-NHL	*PAFAHα2*	Platelet-activating factor acetylhydrolase
t(11;14)(q23;q32)	DLBCL	*RCK* (*HLR2, p54, DEAD/H BOX 6*)	DEAD/H box ATP-dependent RNA helicase
t(12;22)(p13;q11)	B-CLL	*CCND2*	Cyclin D2; essential for control of the cell cycle at the G1/S (start) transition; interacts with the CDC2 protein kinase
t(12;14)(q23;q32)	DLBCL, B-CLL	*C4ST-1*	Chrondroitin-4-O-sulfotransferase 1
t(12;14)(q24;q32)	BL, myeloma	*BCL7A*	Unknown
t(14;15)(q32;q11-13)	DLBCL	*BCL8*	Unknown
t(14;16)(q32;q23)	Myeloma	*MAF*	Transcription factor; contains a leucine zipper motif
t(14;18)(q32;q21)	Follicular lymphoma, DLBCL	*BCL2*	Apoptosis inhibitor
t(14;19)(q32;q13)	B-CLL	*BCL3* (*BCL4*)	Transcriptional activating factor subunit-specific inhibitor of the transcription factor NF-κB; contains seven tandem copies of the SWI6/cdd10 motif
t(14;20)(q32;q11)	Myeloma	*MAFB* (*KRML*)	Transcription factor; contains a leucine zipper motif

*Variant translocations affecting other *Ig* loci than the one shown do occur for many of the translocations. †Functional data were obtained from the Source database (http://genome-www4.stanford.edu/cgi-bin/SMD/source/sourceSearch), as far as available. B-CLL, B-cell chronic lymphocytic leukemia; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; BL, Burkitt lymphoma; B-NHL, B-cell non-Hodgkin lymphoma; B-PLL, B-cell prolymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; IC, immunocytoma; MALT, mucosa-associated lymphoid tissue; MZL, marginal zone lymphoma; SLVL, splenic lymphoma with villous lymphocytes. Data adapted from Willis and Dyer [3••].

ease have come from recent comparative genomic hybridization studies of microdissected tumor cells, showing recurrent gain of chromosomes 1, 2q, 3, 4q, 5q, 6, 8q, 11q, 12q, and X, and loss of chromosome 17 in lymphocyte predominance Hodgkin disease [9•]. Moreover, FISH analysis detected recurrent rearrangement of the *BCL6* gene at 3q27 in lymphocyte predominance Hodgkin disease [9•]. By contrast, in the CD30-positive cells of classic Hodgkin disease, gains in 2p, 9p, and 12q and distinct high-level amplifications at 4p16, 4q23-q24, and 9p23-p24 have been observed, resembling the pattern of aberrations found in primary mediastinal B-cell NHLs [10•,11]. In Hodgkin/Reed-Sternberg tumor cells with 9p23-p24 amplification, an increased copy number of the *JAK2* tyrosine kinase gene was observed [10•]. The target gene for the gains observed at 2p might be the *REL* gene, as activation of the REL/nuclear factor (NF)–κB pathway by another mechanism, namely IκB inactivation, has been previously demonstrated in Hodgkin disease [12,13]. Studies of Hodgkin disease cell lines have also revealed marked karyotypic instability, leading to multiple jumping translocations that affect the *c-MYC* and *IGH* loci in some cases [14,15].

Deciphering oncogenic pathways in B-cell non-Hodgkin lymphoma through molecular characterization of recurrent translocation breakpoints

The introduction of advanced long-distance inverse polymerase chain reaction–based DNA cloning techniques [16–19] has greatly facilitated the identification of new oncogenes from recurrent translocations. Use of long-distance inverse polymerase chain reaction methods has led to the identification of several new genes thought to be important in non-Hodgkin lymphomagenesis, several of which are described in the sections that follow. Here we put emphasis on the more recently identified lymphomagenic genes; the interested reader will find a number of excellent reviews describing those genes identified in earlier studies to be important in the development of NHL (Table 1) [3••,20–25].

*BCL9, MUC1, FcγRII*B, and other 1q21-q22 genes

Abnormalities of the long arm of chromosome 1, in particular the 1q21-q22 region, occur in approximately 10 to 15% of B-cell NHLs, are usually secondary, and are associated with a poor prognosis, especially in diffuse large cell lymphomas [26,27]. Breakpoints located at 1q21-q22 show surprising heterogeneity and involve several target genes.

In 1998, Willis and colleagues reported the cloning of a t(1;14)(q21;q32) in a pre-B acute lymphoblastic leukemia cell line, identifying the novel *BCL9* gene [28]. Increased transcript levels of *BCL9*, which encodes a 1394-amino

acid protein of unknown function that contains several pentapeptide repeats and a potential nuclear localization signal, were detectable in the cell line. Southern hybridization and FISH analyses of a panel of 39 B-cell malignancies with 1q21 abnormalities revealed the *BCL9* locus to be affected in only two cases (one mantle cell lymphoma and follicular lymphoma each), however.

Recently two independent reports described the characterization of a t(1;14)(q21;q32) in the same case of large cell lymphoma, identifying dysregulation of the *MUC1* gene [29,30]. MUC1, also called EMA (epithelial membrane antigen), is a glycoprotein that contains multiple copies of a tandemly repeated mucin-like domain. This glycoprotein was previously shown to be expressed in several lymphoid malignancies (75% of lymphocyte predominance Hodgkin disease cases, 75% of plasmacytomas, and 50% of T-cell lymphomas, including essentially all anaplastic large cell lymphomas) as a result of unknown mechanisms other than 1q21-q22 rearrangements, and was shown to be involved in the progression of solid tumors [31,32]. The t(1;14) results in the dramatic upregulation of expression of an intact MUC1 protein; none of six other genes located in an 85-kb region immediately centromeric to the *MUC1* locus (*CLK2, propin, COTE1, GBA, metaxin,* or *thrombospondin-3*) were found to be overexpressed because of the translocation [30]. Southern blot analysis of 72 B-cell NHLs containing a 1q21 rearrangement revealed *MUC1* rearrangement in 4 cases (6%). In addition, increased copy number (four to six copies) of the *MUC1* locus was identified in 18 (10%) of 178 B-cell NHLs [29]. More recently three *b*ody-*c*avity-*b*ased-*l*ymphoma (BCBL) cell lines have been reported to contain rearrangements near the *MUC1* and the physically linked *MDC15* (*m*etalloproteinase-like, *d*isintegrin-like, and *c*ysteine-rich protein; also known as *ADAM15*, for *a d*isintegrin *a*nd *m*etalloproteinase) gene loci, and to result in *MDC15* overexpression in two of the three cell lines [33]. Thus, rearrangements at this particular 1q21 region appear to be capable of producing overexpression of either *MUC1* or *MDC15*, both of which may contribute to the extranodal presentation of certain B-cell lymphomas because of the involvement of these proteins normally in cell–cell or cell–matrix interactions [32,34].

By cloning the t(1;22)(q22;q11) in three follicular lymphomas also containing t(14;18), Callanan *et al.* [35] showed *FCGR2B*, which encodes the *i*mmunoreceptor *t*yrosine-based *i*nhibition *m*otif (ITIM)–containing low-affinity IgG Fc receptor FcγRIIB, to be the 1q22 target of this rearrangement. FcγRIIB is an inhibitory coreceptor that effects negative regulation of immune responses mediated by activating receptors such as B-cell antigen receptors [36]. High levels of the FcγRIIB receptor alternative splice isoform FcγRIIb2 were specifically overexpressed in t(1;22)-positive cases, whereas b1 isoform

levels were not elevated above normal. How high-level constitutive FcγRIIb2 expression might contribute to B-cell tumorigenesis is not clear, but Fcγ receptors can clearly affect B-cell growth; for example, activation of these receptors enhances the growth and differentiation of murine B-lineage progenitors *in vitro*, and FcγRII-deficient mice have an increased B-cell compartment [37].

The chromosomal region 1q21-22 is remarkably rich in *FCGR* genes. Three *FcγRII* genes and two *FcγRIIIB* genes are located in an approximately 200-kb region in 1q22. Moreover, cloning of yet another t(1;14)(q21;q32) from the FR4 myeloma cell line has revealed an additional group of highly related Fc receptor-related genes that are involved in the pathogenesis of B-lineage malignancies. Hatzivassiliou *et al.* [38••] reported the presence of five adjacent genes (named *IRTA*s, for *i*mmunoglobulin superfamily *r*eceptor *t*ranslocation-*a*ssociated genes) from a 300-kb region spanning the breakpoint in this cell line, all of which encode surface receptor molecules that are members of the immunoglobulin gene superfamily. All *IRTA* genes are expressed normally in the B-cell lineage with distinct developmental stage-specific patterns; for example, *IRTA1* is expressed in a marginal zone B-cell pattern and *IRTA2* is found in centrocytes, marginal zone B cells, and immunoblasts. As a result of the translocation in the FR4 cell line, *IRTA1* is interrupted and fused to the immunoglobulin *C*α locus, producing a chimeric IRTA1/Cα protein. The *IRTA2* gene, normally silent in centroblasts (the presumed normal cellular counterparts of Burkitt lymphoma tumor cells), is overexpressed in Burkitt lymphoma as well as multiple myeloma cell lines carrying 1q21 abnormalities. The pathologic mechanisms by which deregulation of the *IRTA* genes contributes to lymphocyte proliferation are not yet clear but presumably involve in part a disturbance of the physiologic homeostasis between activating and inhibitory antigen receptors.

BCL6 and BCL11A/EVI9

BCL6 is a highly conserved POZ/zinc finger transcription factor gene implicated in germinal center B-cell differentiation and control of inflammation [24,39]. Deregulation of *BCL6* either by *Ig* gene rearrangements or rearrangements that substitute promoters of a variety of heterologous genes upstream of the *BCL6* coding sequences plays a pathogenic role in B-cell NHLs, particularly diffuse large B-cell lymphomas [18,40]. Early reports that suggested a favorable prognosis for NHLs that contained *BCL6* dysregulation have not been confirmed by numerous later studies; a recently published study has suggested that the prognostic impact of *BCL6* translocations might differ depending on whether the gene is fused to *Ig* loci or other genes, the cases with non-*Ig* rearrangements having a worse outcome [40]. The path-

ways by which *BCL6* deregulation leads to lymphomagenesis are poorly understood, although recent microarray studies suggest that malignant transformation by *BCL6* involves the inhibition of differentiation by downregulation of *Blimp-1*, which encodes a transcriptional repressor that plays a key function in the differentiation of B cells to mature plasma cells, together with enhanced proliferation caused by increased *c-MYC* and decreased *p27/Kip1* expression [41•].

New insights into *BCL6* function may come from the recent cloning of the t(2;14)(p13;q32.3), a rare but recurrent event in B-cell malignancies, which led to identification of a new zinc finger gene termed *BCL11A* [42]. The *BCL11A* gene is the human homologue of mouse *Evi-9*, which is deregulated in murine myeloid leukemias after proviral integration [43,44]. The gene is highly conserved, with mouse, chicken, and *Xenopus* homologues, and is identical within its zinc finger motifs to another human gene (*BCL11B*) located on chromosome 14q32.1. As with *Evi-9*, three major isoforms of *BCL11A* exist, which differ in the number of their carboxy-terminal zinc fingers. *BCL11A* physically interacts with *BCL6* and, like *BCL6*, represses transcription [42,45•]. Of interest, *BCL6* also interacts with another POZ/zinc finger oncoprotein, PLZF [46]. It is intriguing to speculate that deregulated expression of BCL11A by translocation or amplification might transform B cells because of transcriptional repression of the same set of genes involved in the control of B-cell differentiation and proliferation as *BCL6*.

Mucosa-associated lymphoid tissue lymphomagenesis: what are the roles of BCL10 and API2-MLT/MALT1?

In 1999, two recurrent chromosomal changes in mucosa-associated lymphoid tissue (MALT) lymphoma were characterized at the molecular level. Three groups of investigators independently showed the t(11;18)(q21;q21) to produce a fusion of *API2* (also known as *cIAP2*, *HIAP1*, or *MIHC*) at 11q21, which encodes an inhibitor of apoptosis protein (IAP), to a gene at 18q21 named *MLT* or *MALT1* (for *MA*LT *l*ymphoma-associated *t*ranslocation) [47–49]. A more recent report indicates that *MLT/MALT1* encodes a caspase-related protein, leading to the alternative designation, human paracaspase (hParacaspase) [50•]. The t(11;18) breakpoints are heterogeneous among MALT lymphoma cases, and further complexity is introduced by alternative *MLT/MALT1* splicing, resulting in different *API2-MLT/MALT1* fusion transcripts [51–53]. The incidence of *API2-MLT/MALT1* in all extranodal lymphomas of the MALT type in various anatomic locations ranges from 19 to 36%, but the fusion is especially frequent in gastric MALT lymphomas that lack a high-grade (large cell) component, being found in roughly 50% of such tumors [51]. Interestingly, those gastric MALT lymphomas that

express *API2-MLT/MALT1* appear capable of *Helicobacter pylori*-independent growth, and therefore do not regress upon eradication of this infection with antibiotic therapy, as many gastric MALT tumors do [54].

The other recurrent translocation found in MALT lymphomas is the t(1;14)(p22;q32) [55], which is considerably less frequent and is also thought to be associated with a more aggressive tumor biology, including *H. pylori*-independent growth of gastric MALT lymphomas. Willis *et al.* [56] and Zhang *et al.* [57] independently characterized t(1;14), identifying an apoptosis-modulating gene, *BCL10*, as the 1p22 target. Intriguingly, like the *API2* gene altered in the t(11;18), *BCL10* encodes a caspase recruitment domain (CARD)-containing protein. CARD proteins are known to be essential for transducing either death or survival signals [58], and initial functional studies provided evidence for a proapoptotic and NF-κB–activating function of *BCL10* [56,57,59–63].

Given the data showing *BCL10* to be proapoptotic in most cell types (although stable *BCL10* expression could be achieved in lymphoid cell lines without inducing an apparent propensity to apoptotic death), overexpression of the gene because of t(1;14) appeared paradoxical. To address this issue, *BCL10* transcripts from t(1;14)-positive MALT lymphomas [56,57], as well as other NHLs and solid tumors lacking the translocation [56], were characterized and reported to contain a variety of mutations, suggesting that loss-of-function, or overexpression of gain-of-function, mutant BCL10 proteins might contribute to tumorigenesis rather than overexpression of the normal protein. These reports prompted a number of investigators to examine various tumor types for mutations in *BCL10* [64–70]. Based on these studies, *BCL10* mutations appear to occur very rarely in nonlymphoid hematopoietic malignancies and solid tumors of all types and are therefore unlikely to play a significant role in oncogenesis. The combined data suggest that at most 5 to 10% of B-cell NHLs may contain mutations, whereas mutation in T-lineage disease is rare. Interestingly, most mutations reported so far have been detected only in cloned cDNA fragments, and RNA editing has been suggested as a mechanism for this hitherto unknown pattern of mutations [71]. Thus, the initially reported high frequency of *BCL10* mutation in a variety of hematopoietic and solid tumors [56] has not been borne out by additional data from a large number of studies. Nonetheless, *BCL10* mutation may play a role in the development of a small subset of lymphoid and possibly other malignancies. However, the relative contributions of *BCL10* mutants to MALT lymphomas and other tumors are not currently clear and await further study, although a classic tumor suppressor model can be regarded as unlikely. The situation is even more complicated by the fact that MALT lymphomas with either the t(1;14) or

the t(11;18) display an aberrant nuclear localization of BCL10 protein by immunohistochemistry, the significance of which is not clear [72,73].

Functional evidence regarding the importance of *BCL10* in lymphocyte development and MALT lymphomagenesis has come from recently described mouse models. Transgenic mice in which *BCL10* is linked to an immunoglobulin enhancer construct that directs expression only to T and B cells develop splenomegaly because of a dramatic and specific expansion of marginal zone B cells reminiscent of human splenic marginal zone lymphoma [74]. Interestingly, these studies also revealed that mice overexpressing *BCL10* mutants in their lymphocytes have no apparent abnormalities of lymphoid development or function, suggesting that deregulated expression of the normal protein by the t(1;14) rather than BCL10 mutants contributes to MALT tumorigenesis. Moreover, studies from *Bcl10*-deficient mice indicate that in addition to being involved in neural tube closure, *Bcl10* functions as a positive regulator of lymphocyte proliferation that specifically connects antigen receptor (B- and T-cell antigen receptors) signaling in B and T cells to NF-κB activation [75••].

Intriguingly, recent studies indicate that the two independent targets of chromosomal rearrangements in MALT lymphoma, *API2-MLT/MALT1* and *BCL10*, converge functionally in a novel NF-κB activation pathway [50•,76•]. The normal MLT/MALT1/hParacaspase protein and BCL10 have been shown to physically interact and cooperate in the activation of NF-κB [50•,76•]. Although not yet fully clarified, a mechanism whereby BCL10 mediates oligomerization and activation of the MLT/MALT1/hParacaspase caspase-like domain, which in turn activates the IKK complex and eventually NF-κB, seems likely. A number of additional CARD proteins (CARD9, 10, 11, 14) as well as the TRAF2 protein interact with BCL10 also, each presumably modulating this NF-κB activation pathway [77–80]. The API2-MLT/MALT1/hParacaspase fusion, as well as BCL10, independently can activate NF-κB when expressed in cells but markedly synergize when coexpressed [50•,76•]. Thus, the expression of either the chimeric API2-MLT/MALT1/hPararcaspase protein or excess BCL10 in lymphocytes would be predicted to significantly enhance NF-κB function. NF-κB target genes such as *TRAF*-1 and -2, *cIAP*-1 and -2, *c-MYC*, and *IL6* should then be upregulated, resulting in enhanced MALT B-cell growth [23]. Enhancement of NF-κB activity because of either API2-MLT/MALT1 fusion or BCL10 overexpression presumably substitutes for the requirement of early low-grade MALT tumors for the sustained B-cell antigen receptor stimulation that occurs with *H. pylori* gastritis or in the setting of chronic autoimmune diseases.

Gene expression profiling in B-cell lymphoma

Genomic scale gene expression profiling using cDNA microarrays is a promising new approach to gain deeper insights into cancer pathobiology and the mechanisms underlying treatment resistance and susceptibility. In B-cell malignancies, a recent study [81••] identified large sets of genes that showed a characteristic coordinate expression within each of the diagnostic subtypes of B-cell chronic lymphocytic leukemia (B-CLL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL). For example, CLL and FL, in contrast to DLBCL, shared a low expression of genes that are involved in cellular proliferation, which reflects their relatively indolent clinical behavior. In contrast, FLs were discernible from CLL by their expression of genes that are characteristic of B cells at the germinal center stage of B-cell differentiation. This finding supports the view that FLs originate from this stage of B-cell differentiation and maintain a gene expression profile of germinal center B cells even after malignant transformation.

Interestingly, these microarray data also revealed considerable variation in gene expression between samples within one diagnostic category. This finding was especially true in DLBCL, in which the structure of the hierarchical clustering dendrogram indicated an inhomogeneous gene expression pattern. It has long been accepted that lymphomas classified as DLBCL might include more than one disease entity, because DLBCLs display a heterogeneous morphology and a highly variable clinical course. Based on gene expression profiling, two molecularly distinct subtypes of DLBCL could be defined (Fig. 1): The "germinal center B-like" DLBCL expressed genes are characteristic of germinal center B cells (*eg*, *CD10*, *CD38*, *JAW1*, and *A-myb*), whereas the "activated B-like" DLBCL were characterized by the expression of genes that are normally induced during *in vitro* activation of peripheral blood B cells (*eg*, *BCL2*, *cyclin D2*, and *IRF4*). Moreover, the molecularly different subtypes of DLBCL also defined prognostic categories: 76% of germinal center B-like DLBCL patients were alive after 5 years, in contrast to only 16% of the patients with activated B-like DLBCL. Although the clinical relevance of this proposed classification scheme has to be confirmed in a larger cohort of patients, further support for the existence of at least two biologically distinct subtypes of DLBCL comes from a recent study by Lossos *et al.* [82]. In this study, seven DLBCL that were previously classified as germinal center B-like DLBCL [81••] were shown to have ongoing somatic mutations of the immunoglobulin (Ig) heavy chain gene. Thus, the transforming event of this subtype of DLBCL may occur while the B cell is in the germinal center microenvironment and does not significantly alter the gene expression program characteristic of this stage of B-cell differentiation, including the ability to mutate *Ig* genes. In contrast,

Figure 1. Two types of diffuse large B-cell lymphoma defined by gene expression profiling
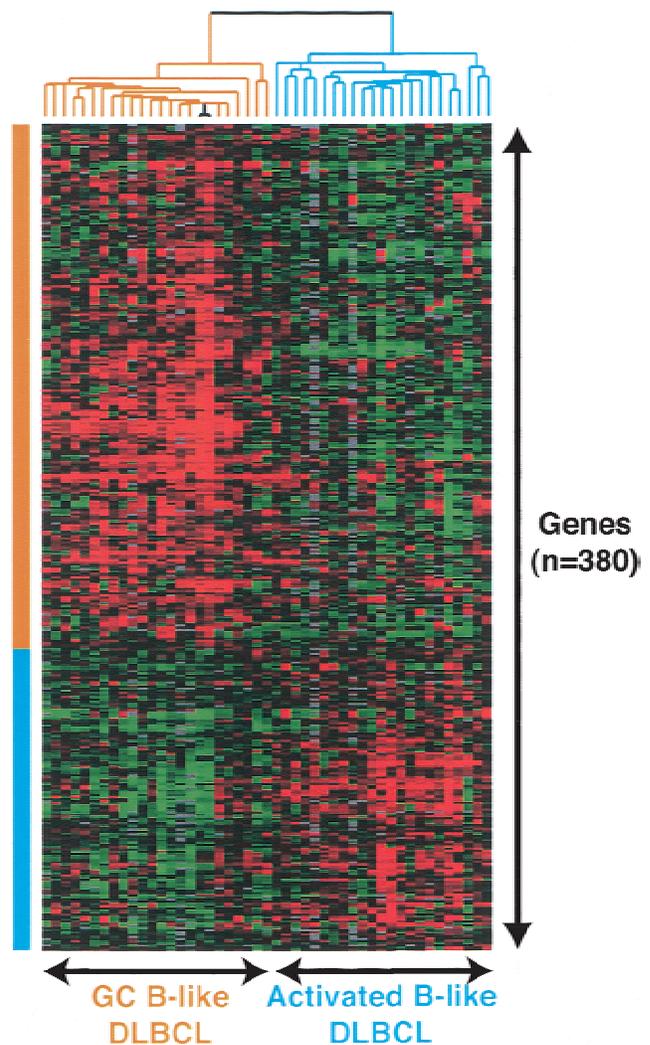
Gene expression profiling reveals two subtypes of diffuse large B-cell lymphoma (DLBCL). Global gene expression of tumor biopsies and cell lines derived from DLBCL, and germinal center (GC) B cells (black), were compared using Lymphochip microarrays. The hierarchical clustering algorithm was used to define two distinct subtypes of DLBCL based on data from 380 Lymphochip array elements. Each column represents data from a single mRNA sample, and each row represents data from a single Lymphochip array element (gene) measured across all samples. Shades of red indicate high gene expression, shades of green indicate low gene expression, and black indicates median gene expression. Genes marked by the orange bar are highly expressed in both normal germinal center B cells and in germinal center B-like DLBCL. Genes marked by the blue bar are characteristically expressed in activated B-like DLBCL.

five of seven tumor samples from the activated B-like DLBCL group did not show evidence of ongoing mutations and, in the remaining two cases, single point mutations were detected in only 4 of 36 molecular clones examined. Accordingly, the tumorigenic event in the activated B-like DLBCL might take place in a postgerminal center B cell or, alternatively, it could occur in a germinal center B cell, where it dramatically alters the gene expression profile and shuts off the *Ig* mutational machinery.

These initial studies suggest that the determination of detailed molecular pictures of gene expression in B-cell malignancies might be helpful in improving current classification schemes by defining biologically and clinically more homogeneous populations of patients. Gene expression profiling should also be useful in the identification of pathogenetically relevant pathways in malignant B cells, permitting the development of alternative therapeutic strategies targeted at these pathways.

Conclusions

Dramatic progress has been made in our understanding of B-cell lymphomagenesis through the characterization of recurrent chromosomal translocations. However, despite identification of many critical oncogenes, their functional consequences and exact mechanisms of lymphoid cell transformation remain to be fully elucidated. In the future, focus will shift toward these functional questions and to the use of microarray analysis to obtain a more global insight into gene dysregulation in lymphomagenesis.

Acknowledgments

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Papers of particular interest, published within the annual period of review, have been highlighted as:
* •   Of special interest
* ••   Of outstanding interest

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