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MOLECULAR PATHOGENESIS OF NON-HODGKIN LYMPHOMA: A CLINICAL PERSPECTIVE

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ABSTRACT

Despite a common origin from mature lymphoid cells, non-Hodgkin lymphomas (NHL) represent a surprisingly heterogeneous group of lymphoid malignancies whose classification is continuously being remodeled. The most recent proposal, the Revised European-American classification, introduces pathogenetic features among the classification criteria. In this respect, knowledge of the molecular pathogenesis of NHL, which is based upon genetic lesions leading to activation of proto-oncogenes (e.g. *BCL*-1, *BCL*-2, *BCL*-6, c-*MYC*) or disruption of tumor suppressor genes (e.g. *p53*), is becoming increasingly relevant for the clinician. These lesions combine into multiple molecular pathways which are selectively associated with distinct NHL types. Thus, for example, rearrangements of *BCL*-1, *BCL*-2, *BCL*-6, and c-*MYC* are the genetic hallmarks of mantle cell, follicular, diffuse large cell, and Burkitt's lymphoma, respectively. Overall, from a clinical perspective, NHL genetic lesions serve three purposes: a) they assist and complement histologic diagnosis; b) they provide a molecular marker with prognostic relevance; c) they allow evaluation of minimal residual disease through highly specific and highly sensitive technologies.

Key words: non-Hodgkin lymphoma, proto-oncogene, tumor suppressor gene, chromosomal translocation

The definition of non-Hodgkin lymphoma includes a variety of lymphoid neoplasms sharing common origin from mature (i.e. peripheral) lymphoid cells. The striking clinico-pathologic heterogeneity of non-Hodgkin lymphomas (NHL) is best illustrated by the number of classification systems that have been utilized in clinical practice during the last two-three decades.[1-7] The first classifications were generally guided by a single prominent criterion, which the formulator considered essential for a correct nosologic interpretation of NHL. These criteria were represented by the morphologic pattern of the tumor (follicular versus diffuse) in the case of Rappaport's classification or by the lymphoma immunophenotypic features (B-cell versus T-cell) in the case of Luke's proposal.[2,4] Later classifications, namely the *Working Formulation for Clinical Usage* and the updated Kiel classification, tried to integrate distinct clinical and biologic aspects of NHL, such as morphotype, lineage derivation and prognosis.[5,6] However, it was not until the advent of the *Revised European-American* (REA) classification that histogenetic and pathogenetic features were included among classification criteria.[7] This latest development, on the one hand, has brought further distinctions between NHL categories previously defined as a single uniform group; on the other hand, it has combined NHL subgroups previously thought to be nosologically distinct. In

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this context, the aim of the present review is to summarize the pathogenetic relevance of the molecular lesions involved in NHL and to clarify their current and potential usefulness as molecular markers for better diagnosis and prognostic assessment.

The first section of this review will summarize present knowledge regarding the pathobiology of oncogenes and tumor suppressor genes involved in NHL. The second section will outline the distinct molecular pathways associated with each NHL category. The third section will focus on how the study of NHL genetic lesions may assist clinical management of NHL.

Genetic lesions of non-Hodgkin's lymphoma

General concepts

As occurs in human neoplasia in general, genetic lesions of NHL involve proto-oncogenes, tumor suppressor genes, and – to a lesser extent – viral infection.[8-10]

Proto-oncogenes are highly conserved genes which, in normal conditions, promote cell growth and/or cell survival under the close control of mitogenic stimuli.[10] Structural lesions of proto-oncogenes convert the physiologic version of these genes into constitutively active pathologic variants: oncogenes.[10]

Proto-oncogenes and oncogenes are grouped into different categories according to their function and include *a)* growth factors; *b)* growth factor receptors; *c)* cytoplasmic signal tranducers; *d)* transcription factors, and *e)* anti-apoptotic genes. With one exception, all the oncogenes involved in NHL are transcription factors or anti-apoptotic genes (Table 1).

The function of tumor suppressor genes under normal conditions is to inhibit cell growth.[9] Since a single copy of the gene is usually sufficient to exert its physiologic role, inactivation of tumor suppressor genes in human tumors occurs biallelically, most commonly through a deletion of one allele and an inactivating mutation of the other.[9] At present, the only tumor suppressor gene known to be involved in NHL is represented by p53.

Lastly, evidence for a viral role in the pathogenesis of NHL in the Western world is virtually restricted to the Epstein-Barr virus (EBV; ref. 8).

Mechanisms of genetic lesions

In human neoplasia, proto-oncogenes can be

Table 1. Chromosomal translocations and proto-oncogenes involved in non-Hodgkin's lymphoma (NHL).

Structural abnormality	*Proto-oncogene*	*Biologic function*	*Mechanism of lesion*	*NHL type*
t(14;18)(q32;q11) t(2;18)(p11;q11) t(18;22)(q11;q11)	*BCL*-2	*BCL*-2 prolongs cellular lifespan by inhibiting apoptosis	Transcriptional deregulation	Follicular lymphoma; a subset of B cell diffuse large cell lymphoma
t(11;14)(q13;q32)	*BCL*-1/*CCND*1 (cyclin D1)	Transcription factor, cell cycle regulator	Transcriptional deregulation	Mantle cell lymphoma
t(3;?)(q27;?)	*BCL*-6	Transcription factor	Transcriptional deregulation	B-cell diffuse large cell lymphoma
t(8;14)(q24;q32) t(2;8)(p11;q24) t(8;22)(q24;q11)	c-*MYC*	Transcription factor	Transcriptional deregulation	Burkitt lymphoma; AIDS-related NHL
t(10;14)(q24;q11)	*LYT*-10	Transcription factor	Removal of regulatory region	Rare cases of small lymphocytic lymphoma
t(2;5)(p23;q35)	*NPM*/*ALK*	*NPM* is a nucleolar phosphoprotein; *ALK* is a tyrosine kinase	Fusion protein; heterotypic deregulation	$CD30^+$ T-cell anaplastic large cell lymphoma
t(9;14)(p13;q32)	?	?	?	Lymphoplasmacytoid lymphoma

activated by *a)* amplification; *b)* point mutation, and *c)* chromosomal translocation. In hematopoietic tumors, the prevalent mechanism of proto-oncogene activation is represented by chromosomal translocation (Figure 1). In NHL, virtually all known proto-oncogenes are activated by chromosomal translocations involving the site of the proto-oncogene on one chromosome and an antigen receptor locus on the partner chromosome, most commonly the immunoglobulin heavy chain locus (IgH) at 14q32, but immunoglobulin κ chain (Igk) and λ chain (Igλ) loci, at 2p11 and 22q11, respectively, have also been documented.

Chromosomal translocations in hematopoietic tumors may lead to one of the following functional consequences (Figure 1): a) *generation of a fusion protein*: the genes located at the breakpoint site of the two partner chromosomes are fused together, generating a hybrid protein with biologic properties distinct from the wild type protein; b) *homotopic deregulation*: the proto-oncogene is removed from the physiologic regulatory regions that coordinate its proper expression and is relocated in the proximity of enhancer elements which are constitutively active in the target tissue, leading to constitutive expression of the proto-oncogene; c) *heterotopic expression*: the proto-oncogene is not expressed in normal tissue, whereas in the tumor it becomes activated through juxtaposition with novel regulatory elements constitutively active in the target tissue. In NHL, the most common mechanism of proto-oncogene activation is represented by homotopic deregulation driven by regulatory elements derived from juxtaposed Ig gene loci.

Proto-oncogene activation by chromosomal translocations in NHL

Several NHL chromosomal translocations have been clarified at the molecular level and have been found to activate proto-oncogenes.[11] The most frequent translocations and the corresponding genes involved are listed in Table 1.

t(14;18) and BCL-2. The t(14;18)(q32;q21) translocation is the chromosomal hallmark of follicular lymphoma (Table 2; refs. 12-16). This translocation joins the JH locus at 14q32 with a

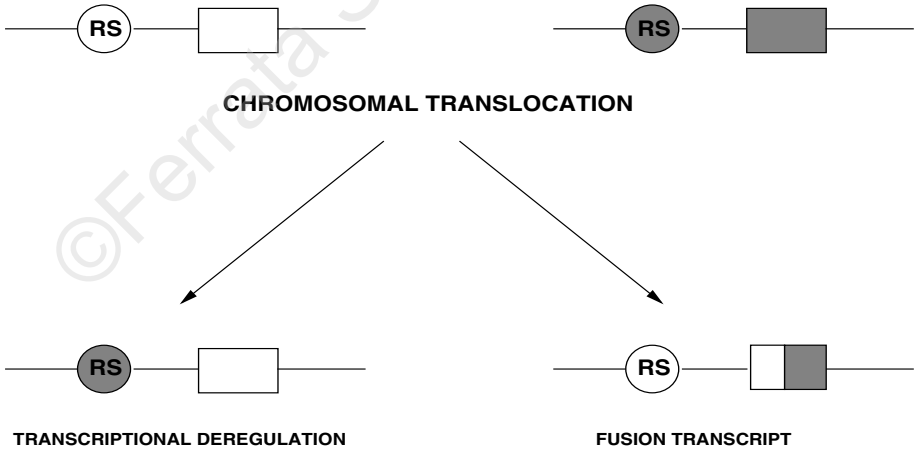

Figure 1. Models of chromosomal translocations in non-Hodgkin lymphoma. The two genes involved in the translocation event are represented by the relative coding sequences, identified by the rectangle, and by regulatory sequences (RS). The two genes are identified by different colors (white boxes and hatched boxes). Top panel: germline configuration of the two genes involved in the translocation. The coding sequence of each of the two genes is proximal to its physiologic regulatory sequences, which coordinate the normal expression of the gene. Bottom panel: As an effect of the chromosomal translocation, two main functional consequences may be observed. In the case of transcriptional deregulation, the normal regulatory sequences of the proto-oncogene are removed and substituted by regulatory sequences derived from the partner chromosome. In NHL, the novel regulatory regions are most commonly derived from the immunoglobulin gene loci, which are consistently expressed at high levels in mature B cells. Examples of translocations leading to transcriptional deregulation in NHL are the translocations involving c-MYC, BCL-1, BCL-2, and BCL-6. In the case of fusion transcript formation, part of the coding sequence of the two genes involved is fused together generating a novel fusion protein with biochemical properties distinct from the native proteins. The t(2;5) of $CD30^+$ T-cell anaplastic NHL is the only NHL translocation known to cause the formation of a fusion transcript.

transcriptional unit located at 18q21 and termed BCL-2 (Figure 2). In normal conditions BCL-2 is expressed in a variety of tissues, including B and T cells; in these settings, BCL-2 expression is strictly modulated by cellular and microenvironmental stimuli.[17] In contrast, in the setting of NHL carrying a t(14;18), deregulation of BCL-2 expression occurs as a consequence of enhanced BCL-2 transcription.[17,18] This is most likely due to a relocation of BCL-2 in the proximity of enhancer elements within the juxtaposed IgH locus. BCL-2 gene breakpoints in NHL translocations cluster into three main regions: the major breakpoint region (MBR), the minor cluster region (mcr), and the 5' breakpoint.[12-16]

The BCL-2 gene codes for a mitochondrial protein that is part of a distinct category of proto-oncogenes involved in the regulation of programmed cell death (apoptosis; ref. 19). In contrast to most other oncoproteins involved in lymphoid neoplasia, which act as regulators of cell growth and proliferation, BCL-2 prevents apoptosis and promotes cell survival.[19-21] In other words, BCL-2 activation *per se* does not lead to cell expansion by refurnishing the tumor with newly replicated cells, but rather by prolonging the lifespan and enhancing the accumulation of the neoplastic population. Indeed, transgenic animals carrying an activated BCL-2 gene develop a follicular lymphoid hyperplasia that involves the accumulation of long-lived resting B cells similar to human follicular lymphoma.[22] In further analogy to what seen in humans, where a fraction of follicular lymphomas evolve histologically into a diffuse morphology, BCL-2 transgenic mice also develop over time a more aggressive B cell lymphoma related to additional genetic alterations in a cell that already carries an activated BCL-2.[23]

The distribution of BCL-2 rearrangements among B cell NHL is selective. They are found in the overwhelming majority of follicular NHL and in a fraction of diffuse large cell lymphomas (DLCL; Table 2 and ref. 11). It is postulated that DLCL carrying BCL-2 translocations represent the histologically transformed phase of a previously follicular lymphoma[24] (and see below).

The clinical relevance of BCL-2 activation in NHL is multifold. First of all, it represents an invaluable diagnostic tool for a clear-cut definition of follicular lymphoma. Second, BCL-2 rearrangements represent an unfavorable prognostic marker for diffuse large cell lymphomas.[25-27] Third, the availability of a polymerase chain reaction (PCR)-based assay for the detection of BCL-2 rearrangements provides a powerful genetic marker for minimal residual disease evaluation.[28-31]

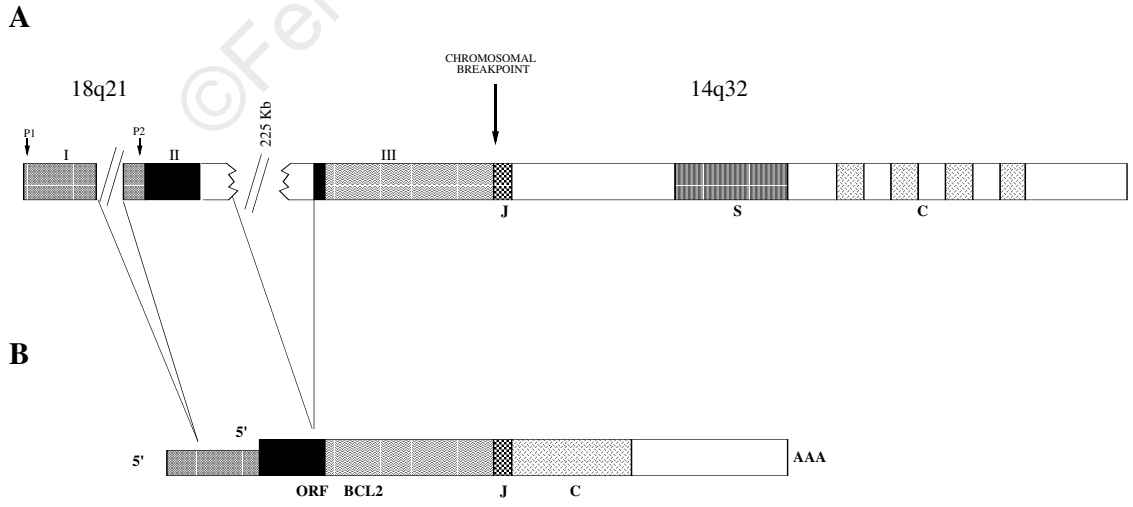

Figure 2. BCL-2-Ig fusion gene. The t(14;18) translocation introduces BCL-2 into the Ig locus, placing it in the same transcriptional orientation as the Ig and generating chimeric BCL-2/Ig mRNA. The genomic breakpoint of the der14 is shown in panel A and the fusion transcript in panel B. The BCL-2 exons (I, II, III) and promoters (P1, P2) are shown, as well as the J, S, and C regions of the Ig locus. For details, see text.

Table 2. Frequency of genetic lesions in B-cell NHL categories.

Histology	*BCL-1*	*BCL-2*	*BCL-6*	*c-MYC*	*p53*	*EBV*
Small lymphocytic lymphoma/B-CLL	–	–	–	–	10%	–
Lymphoplasmacytoid lymphoma	–	–	–	–	–	–
Mantle cell lymphoma	70%	–	–	–	–	–
Follicular lymphoma	–	90%	–	–	–	–
Marginal zone lymphoma, MALT-associated	–	–	NA°	–	rare	–
Marginal zone lymphoma, nodal monocytoid	–	–	NA°	–	NA°	–
Splenic marginal zone lymphoma	–	–	–	–	40%	–
Diffuse large cell lymphoma	–	–	40%	–	–	–
Burkitt's lymphoma	–	–	–	100%	40%	30-100%
Transformed lymphoma *	–	100%	–	rare	90%	–

**B-NHL which have undergone histological progression from a follicular to a diffuse pattern. °NA, not assessed. #30% in sporadic Burkitt lymphoma, 100% in endemic Burkitt lymphoma.*

t(11;14) and BCL-1/PRAD-1/CCND1. t(11;14)(q13;q32) juxtaposes the immunoglobulin heavy chain gene with the BCL-1 locus on chromosome 11q13 (Table 1; refs. 32-34). The association of t(11;14)(q13;q32) and BCL-1 rearrangements with the different subsets of B-cell NHL has been a matter of controversy for a number of years. By applying strict phenotypic criteria to the diagnosis of B cell lymphoproliferations, it has become evident that t(11;14) is virtually restricted to mantle cell lymphomas (MCL), in which BCL-1 rearrangements occur in 70% of the cases (Table 2; refs. 35-37). Structurally, t(11;14) resembles the better characterized t(14;18) (see above) and t(8;14) (see below), in which a proto-oncogene on chromosome 18 (i.e. BCL-2) or 8 (c-MYC) undergoes transcriptional deregulation as a consequence of its juxtaposition with an immunoglobulin gene locus. In contast to t(14;18) and t(8;14), however, no transcriptional unit was initially found in the proximity of the BCL-1 locus. Only recently has it been shown that the postulated BCL-1 oncogene lies approximately 110 Kb telomeric of the major translocation cluster (MTC; refs. 38-42). This gene was originally termed PRAD1 since it was first cloned from parathyroid adenomas, but it is now designated cyclin D1 (CCND1). CCND1 is one of several recently identified cyclins which regulate G1 cell cycle progression in various cell types.[43] CCND1 is consistently overexpressed in MCL carrying BCL-1 rearrangements putatively leading to deregulated cell cycle progression from G1 to S phase and to altered proliferation.[37-42] The clinical usefulness of BCL-1 as a marker for discriminating MCL from other lymphoproliferations is well established. Notably, a correct diagnosis of MCL is mandatory for therapeutic purposes since failure-free survival and overall survival of MCL are significantly worse than for other NHL generally assigned to the same Working Formulation categories.[44,45] Since at present the large majority of BCL-1 breakpoints can also be detected by PCR, BCL-1 may also serve as a tool for evaluating minimal residual disease and follow-up of these NHL.[46]

Breakpoints at 3q27 and BCL-6. Chromosomal translocations involving band 3q27 and several other chromosomal sites, including the immunoglobulin genes, represent recurrent aberrations in B-cell NHL of diffuse large cell histology.[47,48] Conventional cytogenetic techniques detect 3q27 alterations in approximately 15% of diffuse large cell lymphoma (DLCL). Cloning of the 3q27 breakpoint has led to identification of the involved proto-oncogene, termed BCL-6

or, alternatively, LAZ-3 (Table 1; refs. 49-53). The clinico-pathologic relevance of BCL-6 lesions in B-cell NHL is strengthened by the virtually selective association of BCL-6 rearrangements with B-cell DLCL, a type of NHL for which no other specific genetic lesion has yet been identified (Table 2 and refs. 24, 54, 55). Screening of large B-cell NHL panels has revealed BCL-6 rearrangements in approximately 40% of DLCL of the immunocompetent host and in 20% of DLCL associated with AIDS.[54,55] Rearrangements of BCL-6 occur in both DLCL variants, including large non cleaved cell lymphoma and immunoblastic plasmacytoid lymphoma. Since the frequency of molecular rearrangements in DLCL (40%) significantly exceeds that reported by conventional cytogenetics (15%), it is plausible that some of the observed rearrangements involve submicroscopic chromosomal alterations undetectable at the cytogenetic level. The potential usefulness of BCL-6 rearrangements in clinical practice lies in the fact that DLCL represent a heterogeneous group of neoplasms that are treated homogeneously despite the fact that only 50% of patients experience long-term disease-free survival. The presence of a genetic marker such as BCL-6 rearrangements identifies a sizable subset of cases with a distinct pathogenesis and, possibly, distinct clinico-biologic behavior. In this respect, a large study on DLCL established that BCL-6 rearrangements correlate with a favorable clinical outcome and may thus serve as a prognostic marker in patients with this type of lymphoma.[26] In concert with other biologic and clinical features, the identification of genetic markers with prognostic value may offer the potential to stratify DLCL patients for novel therapeutic approaches. In addition to favorable prognosis, BCL-6 rearrangements in DLCL are also associated with extranodal presentation of the lymphoma (i.e. stages IE, IIE, IIIE; ref. 26).

The biology of BCL-6 and its pathogenetic role in DLCL development are being progressively clarified. BCL-6 codes for a zinc-finger protein with structural similarities to a class of transcription factors participating in the control of cell proliferation and differentiation.[50-53] In normal conditions, BCL-6 expression is restricted to mature B cells, suggesting that its physiologic role is to control normal B cell differentiation and lymphoid organ development.[50] As a result of the translocation, the coding domain of BCL-6 is left intact, whereas the 5' regulatory region, containing the promoter sequences, is either completely removed or truncated. Consequently, the coding domain of BCL-6 is linked downstream to heterologous sequences which can originate from different chromosomes in different cases.[51-53] It is predicted that the functional consequence of these truncations is the expression of a normal BCL-6 protein under the control of heterologous regulatory sequences that lead to loss of the normal regulation pattern. As with BCL-2 and c-MYC, BCL-6 activation is thought to lead to homotopic deregulation.

Breakpoints at 8q24 and c-MYC. Historically, molecular analysis of c-MYC translocations provided the first example of oncogene involvement in tumor-associated chromosomal translocations.[56] This proto-oncogene, c-MYC, is a nuclear phosphoprotein which functionally regulates DNA synthesis (Table 1 and ref. 56). The c-MYC locus may be involved in three distinct translocations with one of the immunoglobulin loci, i.e. IgH, Igκ or Igλ.[56-61] IgH is implicated in 80% of the cases leading to t(8;14)(q24;q32). The remaining 20% are composed of t(2;8)(p11;q24) involving Igκ (15%), and t(8;22)(q24;q11) involving Igλ (5%). Throughout the pathologic spectrum of B cell NHL, breakpoints at 8q24 associate with 100% of Burkitt's lymphoma and with a fraction of AIDS-related NHL, namely those with small non cleaved cell morphology (SNCCL). In addition, breakpoints at 8q24 are also detected in 100% of L3 ALL, which is considered to be the leukemic counterpart of Burkitt's lymphoma. Lastly, the involvement of c-MYC rearrangements in a fraction (10-20%) of DLCL is controversial. In our own experience, B cell DLCL of the immunocompetent host are consistently devoid of c-MYC translocations (unpublished observation).

Although fairly homogeneous at the cytoge-

netic level, translocations involving c-MYC are remarkably heterogeneous when observed at the molecular level. In particular, the translocation breakpoints are located 5' and centromeric to c-MYC in t(8;14), whereas they are situated 3' and telomeric to c-MYC in t(2;8) and t(8;22).[56-61] Additional molecular heterogeneity is found among t(8;14), of which there are two distinct types that selectively associate with sporadic and endemic Burkitt's lymphoma, respectively.[62,63] The first type, found in sporadic Burkitt's lymphoma and in the majority of AIDS-SNCCL, involves sequences within or immediately 5' to c-MYC (< 3 kb) on chromosome 8 and sequences within the IgH switch region on chromosome 14. The second molecular type of t(8;14), associated with endemic Burkitt's lymphoma, involves sequences on chromosome 8 at an undefined distance (> 100 kb) 5' to the c-MYC locus and sequences on chromosome 14 corresponding to the IgH J (joining) region. In addition to gross truncations within or around the c-MYC locus, translocated c-MYC alleles also display small mutations within the first exon/first intron border corresponding to c-MYC regulatory regions, as well as mutations in c-MYC exon 2, where the phosphorylation and transactivation domains are located.[64-66]

Overall, breakpoints at 8q24 may contribute to c-MYC deregulation through a combination of the following mechanisms: *a)* constitutive activation of c-MYC transcription due to the juxtaposition of cis-acting enhancers within the immunoglobulin loci;[56-61] *b)* truncations within the c-MYC gene that remove its coding exons from intragenic regulatory regions;[56-61] *c)* small mutations in c-MYC regulatory regions located at the junction between the first exon and the first intron, which alter their responsiveness to factors regulating c-MYC expression levels;[64] *d)* amino acid substitutions in c-MYC functional domains that affect their transactivation potential or their availability to phosphorylation.[65,66] Whatever the exact molecular mechanism, translocated c-MYC alleles in NHL are constitutively transcribed at high levels, similar to those found in normal cells after mitogenic stimulation.[67-69]

The pathogenetic role of c-MYC activation in the development of Burkitt's lymphoma is substantiated by several lines of evidence. *In vitro*, transfection of activated c-MYC oncogenes into Epstein-Barr virus-infected B cells, a natural target for c-MYC activation in EBV-positive Burkitt's lymphoma, potentiates their full malignant transformation.[70] Furthermore, antisense oligonucleotides directed against translocated c-MYC alleles are able to revert the tumorigenicity of Burkitt's lymphoma cells.[71] *In vivo*, transgenic mice carrying an activated c-MYC oncogene in their B-cell lineage frequently develop B-cell malignancies.[72] Novel insights into the biologic role of c-MYC deregulation in NHL pathogenesis have emerged with the identification of c-MYC functional partners, such as Max and p107.[73-75]

Although c-MYC translocation is a *sine qua non* cytogenetic and molecular marker for a diagnosis of Burkitt's lymphoma and AIDS-related small non cleaved cell lymphoma, the scattered location of its breakpoints has thus far hampered the construction of a PCR assay for selective detection of translocated c-MYC alleles. Thus, at present, c-MYC rearrangements cannot be used as a tool for high sensitivity minimal residual disease detection in NHL carrying 8q24 breakpoints.

t(2;5) and NPM/ALK. The t(2;5)(p23;q35) chromosomal translocation occurs in most $CD30^+$ anaplastic large cell lymphomas (ALCL) arising from activated T cells, which predominantly develop in children and young adults.[76] Recently, this translocation has been cloned and the proto-oncogenes involved have been identified (Table 1 and ref. 77). This rearrangement causes the fusion of the NPM (for nucleophosmin) gene, coding for a nucleolar phosphoprotein and mapping to 5q35, with a previously unidentified protein tyrosine kinase gene, ALK (for anaplastic lymphoma kinase), on chromosome 2p23.[77,78] As a consequence of the translocation, a hybrid protein is formed in which the amino terminus of NPM is linked to the catalytic domain of ALK. The oncogenic effect of t(2;5) is supposed to be two-fold.[77]

First of all, NPM most likely contributes an active promoter to drive ALK expression in

lymphoma cells containing the translocation. Since ALK is not expressed in normal lymphoid cells, this is an example of oncogene activation through heterotopic expression. Second, a consistent feature of tyrosine kinase oncogenes is that much of their potency is due to mutations or gene fusions leading to constitutively active catalytic domains.[10] Thus, in the case of NPM/ALK, one would predict that the truncated ALK constitutively phosphorylates intracellular targets to trigger malignant transformation. The case of NPM/ALK is unique among NHL since at present it is the sole example of oncogene activation by formation of a fusion protein in these disorders.

In a clinical perspective, cytogenetic and/or molecular detection of NPM/ALK rearrangements may assist both correct diagnosis and clinical follow-up.

A morphologic diagnosis of ALCL was traditionally difficult before the systematic use of immunohistochemical techniques.[76] Indeed, in one study cases initially classified as malignant histiocytosis were rediagnosed as ALCL based on the presence of t(2;5) and CD30 expression.[79] Given the relatively favorable prognosis of t(2;5)-positive T-cell ALCL among $CD30^+$ NHL, a correct diagnosis might influence therapeutic choices and prognostic assessment.[76] In addition, the availability of a PCR-based assay for detection of NPM/ALK fusion transcripts is an invaluable tool for monitoring minimal residual disease in NHL carrying the t(2;5) translocation.[77]

Disruption of tumor suppressor loci in NHL

At present, the molecular characterization of tumor suppressor loci in NHL is restricted to the case of p53 and 6q deletions.

Inactivation of p53. p53 represents the prototype of tumor suppressor genes.[80,81] The product of p53 is a transcription factor which acts as a checkpoint in cell cycle control and regulation. Biallelic inactivation occurs most frequently through deletion of one allele at 17p13 and point mutation of the other allele. Typically, mutations cluster in four hotspots corresponding to exons 5 through 8, which harbor functionally active domains of the p53 protein. As a consequence of mutation, p53 mutants fail to bind DNA and thus fail to exert their normal transactivating function.[9]

Inactivation of p53 is a frequent event in NHL and is present in approximately 30% of Burkitt's lymphoma and in 60% of AIDS-related SNCCL (Table 2 and refs. 82, 83). In both disorders, p53 inactivation occurs in association with c-MYC activation in 100% of the cases. In addition to Burkitt's lymphoma and AIDS-related SNCCL, p53 mutations are also associated with follicular NHL that have transformed to diffuse morphology and with a fraction of splenic NHL.[84,85] Other NHL types are consistently devoid of p53 mutations.[82] For clinico-diagnostic purposes, p53 mutations are useful in distinguishing the transformed (i.e. from a previously follicular phase) from the *de novo* origin of DLCL (24 and see below).

Deletions of 6q. The long arm of chromosome 6 represents the site of one or more putative tumor suppressor loci relevant to B-cell lymphomagenesis.[86] Evidence from various cytogenetic studies has long suggested that deletions of 6q represent recurrent abnormalities in various lymphoid malignancies, including NHL.[87] The pathogenetic role of these deletions is strongly supported by the observation that 6q deletions may occur as the sole cytogenetic abnormality in lymphoid tumors.[88] The relatively limited power of resolution of conventional cytogenetics, however, has hampered identification of the specific regions involved. A detailed molecular analysis of 6q deletions in B-cell NHL demonstrated the existence of two discrete, non overlapping regions of minimal deletion (RMD) along 6q, suggesting the presence of two distinct tumor suppressor genes.[86] These two regions map to 6q27 and 6q21-q23 and have been termed RMD-1 and RMD-2, respectively. An active hunt for the involved tumor suppressor genes is currently in progress. Interestingly, the two types of 6q deletions define distinct clinico-pathologic NHL subsets.[89] In particular, RMD-1 is associated with intermediate grade NHL whereas RMD-2 is preferentially associated with high grade NHL. Lastly, the clinical relevance of 6q deletions in

NHL is underlined by the unfavorable prognosis of cases carrying this abnormality.[90]

Other chromosomal abnormalities

Several other structural abnormalities have been reported in NHL, although only a small fraction of them have been characterized at the molecular level (Table 1). Breaks at 10q24 are a recurrent abnormality in 5-10% of low grade B-cell NHL and in some cases may involve the LYT-10 gene, a member of the NF-κB family of transcription factors.[91] Chromosomal translocations involving 9p13 identify a subset of low grade B cell NHL with plasmacytoid features (i.e. lymphoplasmocytoid lymphoma), whereas breaks at 1p32-36 and at 1q21-q23 are detected in a variety of NHL and are associated with shorter median survival.[87,90,92] Lastly, t(11;18) has been described as a chromosomal alteration specific for MALT NHL.[93] None of the genes implicated in these alterations have been identified. Apart from structural abnormalities, trisomy 12 represents the most common numerical alteration in NHL; it occurrs in more than 30% of both follicular and diffuse lymphomas.[87,90,94] Its actual contribution to lymphomagenesis is currently unknown.

The role of viral infection

The role of viral infection in NHL is virtually restricted to EBV, a DNA virus of the herpesvirus family.[95] EBV was first identified in cases of endemic Burkitt's lymphoma from Africa; subsequently, it was also detected in a portion of sporadic forms of Burkitt's lymphoma and of AIDS-related lymphomas.[83,95-99] Upon infection of a B lymphocyte the EBV genome is transported into the nucleus, where it exists predominantly as a circular extrachromosomal molecule (episome). The formation of circular episomes is mediated by cohesive terminal repeats, which are composed of a variable number of tandem repeats (VNTR) sequence. Because of this terminal heterogeneity, the number of VNTR sequences enclosed in newly formed episomes may differ considerably, thus representing a constant clonal marker of the episome and, consequently, of a single infected cell.[100]

Evidence for a pathogenetic role of this virus in EBV-infected NHL is at least twofold. On one hand, it is well recognized that EBV is able to alter the growth of B cells significantly.[95] On the other hand, EBV-infected lymphomas usually display a single form of fused EBV termini, suggesting that the lymphoma cell population represents the clonally expanded progeny of a single infected cell.[83,97,98]

Other viruses involved in the pathogenesis of NHL include HTLV-I in adult T-cell leukemia/lymphoma of the Caribbean and Japan, and the newly identified KSHV virus (limited to a small subset of AIDS-associated lymphomas).[8,101]

Clinico-pathologic correlations

As previously stated, the REA classification is the only NHL classification system to include pathogenetic features among classification criteria.[7] From the standpoint of molecular pathology, the REA classification represents the first attempt to present the clinician with the concept that differences in the genotypic features of NHL may define distinct nosologic entities. The best application of this concept is probably represented by B-cell DLCL, which the REA classification itself recognizes as a histogenetically and clinically heterogeneous group.[7] Indeed, molecular pathology has identified at least three distinct subsets of B-cell DLCL associated with distinct clinical features.[24]

Another group of B-cell NHL likely to benefit in the near future from a detailed histogenetic and pathogenetic revision is the poorly defined category of non-follicular chronic lymphoproliferations. Efforts toward a genetic classification of NHL should not be viewed merely as a cultural achievement, but rather should be constantly confronted with their potential application in clinical practice.

In this respect, we have only recently realized that application of strict genetic criteria to therapeutic stratification of ALL patients has been instrumental in improving the life expectancy of these patients.[102,103] In this perspective, the following section will outline the genetic pathways associated with the main NHL types recognized by the REA classification.

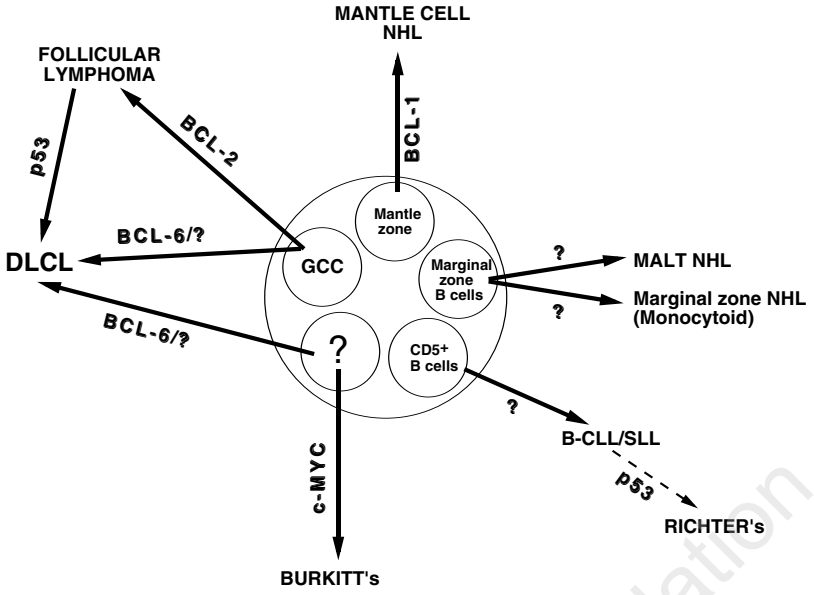

Figure 3. Molecular pathways in B-cell lymphoma pathogenesis. Based on histogenetic and molecular pathology studies, distinct NHL types are thought to derive from distinct subpopulations of mature B cells whose neoplastic conversion is associated with specific genetic lesions. According to this model, follicular lymphomas and a fraction of DLCL derive from germinal center cells, whereas mantle cell lymphoma originates from the mantle zone. The $CD5^+$ B-cell lineage would be the normal counterpart of SLL/B-CLL, whereas MALT NHL and monocytoid NHL would derive from extranodal and nodal marginal zone B cells, respectively. Lastly, the histogenesis of Burkitt's lymphoma and a putative subset of DLCL is still under discussion. The main genetic lesions identified so far include BCL-1 activation in mantle cell lymphoma, c-MYC in Burkitt's lymphoma, BCL-2 in follicular lymphoma, and BCL-6 in DLCL. Notably, a fraction of follicular lymphomas accumulate p53 mutations and histologically transform into DLCL, which may be morphologically undistinguishable from *de novo* DLCL. Since $BCL\text{-}2^+p53^+$ DLCL represent a DLCL group with unfavorable prognosis, their identification at the molecular level bears clinical relevance (For details, see text).
Abbreviations: GCC, germinal center cells; DLCL, diffuse large cell lymphoma; SLL, small lymphocytic lymphoma; B-CLL, B-cell chronic lymphocytic leukemia; MALT, mucosa-associated lymphoid tissue; NHL, non-Hodgkin lymphoma.

B-cell non-Hodgkin lymphoma

The following B-cell NHL categories will be considered: small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia, lymphoplasmacytoid lymphoma, mantle cell lymphoma, follicular lymphoma, MALT (*mucosa associated lymphoid tissue*) lymphomas, splenic marginal zone lymphomas, diffuse large cell lymphomas, Burkitt's lymphomas, and AIDS-associated lymphomas. The main genetic pathways active in B-cell lymphomagenesis are presented in Figure 3.

Small lymphocytic lymphoma and B-cell chronic lymphocytic leukemia. According to the REA classification, the term small lymphocytic lymphoma (SLL) should be restricted to cases that show the characteristic morphology and immunophenotype of B-cell chronic lymphocytic leukemia (B-CLL), i.e. faint surface IgM expression coupled with coexpression of CD19, CD5, and CD23.[7] Although most patients whose lymph nodes contain the characteristic infiltrate associated with B-CLL prove to have BM and PB involvement at the time of diagnosis or shortly thereafter, it is possible that some may not develop leukemia. In this respect, the relationship between SLL and B-CLL would mirror the one that exists between Burkitt's lymphoma and L3 ALL (see below). A few genetic lesions are known to be involved in SLL/B-CLL, including p53 mutations in 10 % of the cases (Table 2 and Figure 3; refs. 82, 104). The frequency of p53 inactivation increases substantially in cases that evolve to Richter's syndrome, suggesting that p53 may be at least partially responsible for this aggressive clinical transformation (Figure 3 and

ref. 82). Despite initial suggestions from several institutions, it is now well established that *true* cases of SLL/B-CLL (i.e. $CD5^+$, $CD23^+$ according to the Fifth Workshop on B-CLL and the REA classification) are consistently devoid of BCL-1 and BCL-2 rearrangements.[104] Intriguingly, however, B-CLL cells tend to express high levels of BCL-2 in the absence of detectable structural alterations of the gene, suggesting that BCL-2 deregulation in B-CLL is the result of epigenetic mechanisms whose nature is currently unknown.[105] Other chromosomal abnormalities associated with SLL/B-CLL are mainly represented by trisomy 12 and deletion of 13q14.[106] By using FISH analysis, it has been shown that trisomy 12 occurs in 30-40 % of cases and that it correlates with poor survival.[107] Deletion of 13q14, the most frequent structural abnormality of SLL/B-CLL, does not involve the RB1 gene and thus is the putative site of a novel tumor suppressor gene.[108] Lastly, SLL/B-CLL with prolymphocytoid forms have been associated with deletions of chromosome 6 at bands q21-q23.[88]

Lymphoplasmocytoid lymphoma. Lymphoplasmocytoid lymphomas are typically CD5 negative and associated in a large percentage of cases with a monoclonal serum paraprotein of the IgM type, causing the clinical syndrome known as Waldenström's macroglobulinemia.[7] The molecular pathway associated with this NHL category is presently obscure, although t(9;14)(p13;q32) has been proposed as a specific genetic marker (Table 1 and ref. 92).

Mantle cell lymphoma. Mantle cell lymphomas (MCL) constitute 25% of non follicular small B-cell lymphomas.[44,45] The precise identification of MCL among non follicular small B-cell lymphomas is clinically relevant since MCL is a far more aggressive disease and displays a significantly shorter survival than other histologically related forms.[44,45] Morphologic diagnosis of MCL is assisted by immunophenotypic as well as genetic criteria. The former include coexpression of CD19 and CD5 in the absence of CD23, and strong expression of surface IgM.[7] The latter are represented by t(11;14), which is found in the majority of MCL cases and is specific for this type of lymphoma, since it is absent in other lymphoproliferations (Table 2 and Figure 3; refs. 35-42). t(11;14) is detectable by PCR, in addition to cytogenetic techniques and Southern blot, and thus constitutes a convenient disease marker for minimal residual disease evaluation.[46]

Follicular lymphoma. Chromosomal translocations involving BCL-2 are the hallmark of follicular lymphoma; they are found in 80 to 90% of cases, independently of the cytologic subtype (Tables 2 and 3 and Figure 3; refs. 12-16). Their detection rate depends on the method utilized: cytogenetic techniques reveal the highest proportion of cases, while Southern blot and/or PCR-based assays do not generally uncover more than 70% of the abnormalities partially due to the presence of BCL-2 translocations with Igκ and Igλ, which cannot be detected by available PCR assays. It was recently proposed that BCL-2 activation interacts with antigenic stimulation in the pathogenesis of follicular lymphoma.[109,110] According to this model, BCL-2 translocation would occur at an early stage of B-cell development in a germinal center centrocyte/centroblast.[109] Subsequent antigen stimulation of the centrocyte/centroblast carrying a BCL-2 rearrangement would drive deregulated clonal expansion and tumor development. While this model is quite original, its validity is still under investigation.

Other oncogenes involved in lymphomagenesis, such as c-MYC, BCL-6, p53 and BCL-1, are not involved in follicular lymphomas. Deletions of chromosome 6 at 6q27 occur in approximately 20% of cases.[86-89] Over time, a significant fraction of follicular lymphomas evolve into an aggressive lymphoma with a diffuse large cell architecture.[111-117] This histologic shift is almost always accompanied by p53 mutation and deletion in addition to the pre-existing BCL-2 lesion (Figure 3 and ref. 84). Deletions of 6q and/or rearrangements of c-MYC may also occur occasionally in this setting.[84,118]

Marginal zone B cell lymphoma. Marginal zone lymphomas (MZL) include extranodal lymphomas, represented by MALT lymphomas, as

well as cases of nodal derivation, which are usually referred to as monocytoid lymphomas. The molecular pathogenesis of this latter group is virtually unknown, whereas the current efforts of several institutions are directed at elucidating MALT-associated lymphomagenesis.

Identification of MALT lymphomas as a single nosologic entity is recent.[7,119,120] They are thought to derive from marginal zone lymphoid cells associated with the epithelia in different organs, most commonly the gastrointestinal tract, but also the lung, thyroid, salivary glands and testis. At present there is no unifying genetic lesion for MALT NHL. BCL-1, BCL-2 and c-MYC lesions are consistently absent in these tumors.[121]

Recent data from our group suggest that rearrangements of BCL-6 and mutations of p53 are implicated in a fraction of cases, particularly those constitued of both small and large cells (unpublished observation). For small cell gastric MALT NHL, a major role is attributed to chronic antigen stimulation since these lymphomas are always associated with *Helicobacter pylori* and apparently regress upon eradication of this infection with antibiotic therapy.[122-125] Large trials comparing the effect of conventional chemotherapy versus antibiotic therapy in the treatment of gastric MALT NHL are currently in progress. Given the clinical relevance and therapeutic implications of *H. pylori* infection in gastric MALT NHL, studies aimed at a molecular definition of the exact role of chronic antigen stimulation are urged.

Splenic marginal zone lymphoma. Splenic marginal zone lymphoma represents a provisional category in the REA classification.[7, 126] The molecular pathway associated with this lymphoma type is virtually unknown, with the exception of p53 mutations in a subset of cases (Table 2 and ref. 85).

Diffuse large cell lymphoma (DLCL). B-cell DLCL, which account for up to 40% of NHL in the adult, are aggressive but potentially curable lymphomas.[7,76,127-129] The REA definition of DLCL has been expanded to include three morphologic variants, namely centroblastic, immunoblastic and anaplastic B-cell lymphoma.[7] Pathologists generally agree that with current knowledge and methods it is impractical to subdivide DLCL histologically, and that the three morphologic variants should all be designated as DLCL.[7,128,129] However, both clinicians and pathologists accept the fact that DLCL is indeed a heterogeneous disease.[7,128,129] Most likely, diversity does not depend upon cytologic features but rather upon the biologic characteristics of the tumor.[24] Finally, the inclusion of diffuse mixed, small and large cell lymphoma under the heading DLCL, as suggested by the REA classification,[7] further adds to the heterogeneity of this NHL category.

The biologic diversity of DLCL has been validated by the identification of at least three distinct genetic types of DLCL (Table 2 and Figures 3 and 4; refs. 24, 26). The first type is associated with rearrangements of BCL-6 in the absence of other known genetic lesions.[24,26,48-54] BCL-6-positive DLCL are *de novo* lymphomas, presenting without a previous history of follicular lymphoma. The second genetic type of DLCL combines BCL-2 activation and p53 mutation and derives from histologic transformation of a previously follicular lymphoma.[24-27,84] The third genetic group of DLCL displays germline BCL-2 and BCL-6 genes.[24,26] The DLCL genotypic configuration bears prognostic relevance since cases associated with BCL-6 rearrangements display the best prognosis, whereas the ones carrying BCL-2 translocations have the poorest outcome.[25-27] The third group, i.e. cases without BCL-6 and BCL-2 rearrangements, have an intermediate prognosis.[26]

Several open issues remain to be investigated in the field of DLCL pathogenesis and heterogeneity, including the relationship between the ALCL subtype and Hodgkin's disease as well as the genetic pathway leading to DLCL in the context of immunodeficiency (ref. 130 and see below).

Burkitt's lymphoma. Burkitt's lymphoma, otherwise known as small non cleaved cell lymphoma (SNCCL), includes three clinical variants: sporadic Burkitt's lymphoma, endemic Burkitt's lymphoma, and AIDS-related SNCCL.[7] Some cases may present as acute leukemias with

Burkitt's tumor cells and are termed L3 ALL.[7] Despite this clinical heterogeneity all Burkitt's lymphomas share a common pathogenetic pathway, represented by c-MYC activation due to chromosomal translocation in 100% of the cases and p53 disruption 40-60% of the time (Table 2 and Figure 3; refs. 56-67, 82, 83). Another lesion that contributes to the development of this malignancy is EBV infection, which is found in virtually all cases of endemic Burkitt's lymphoma and in approximately 30% of the sporadic and AIDS-related types.[83,95-99] Several molecular and immunologic features, including secretion of IgM and location of the chromosomal breakpoint within the IgH locus, suggest that sporadic Burkitt's lymphomas and AIDS-SNCCL derive from a slightly more mature B cell than endemic Burkitt's lymphoma.[7]

AIDS-associated lymphomas. The association between an immunodeficiency state and the development of lymphoma is well established.[131-133] In recent years the increasing frequency of HIV infection has emerged as a major risk factor for lymphomagenesis.

At present 10% of all newly diagnosed NHL in the United States are thought to be related to AIDS.[133] AIDS-related lymphomas are invariably derived from B cells and are classified into two main groups, AIDS-SNCCL and AIDS-DLCL. When compared to NHL of similar histology in immunocompetent patients, AIDS-related NHL are characterized by presentation in advanced stages, poor prognosis, and frequent involvement of extranodal sites. The repertoire of genetic lesions in AIDS-NHL differs substantially according to histology and clinical features.[55,66,83,131-133] AIDS-related DLCL is consistently associated with low CD4 counts, long persistent HIV infection and a poor prognosis. AIDS-related DLCL harbors EBV infection in 80% of cases; in addition, mutually exclusive rearrangements of BCL-6 and c-MYC are found 40% of the time. AIDS-related SNCCL may develop in the presence of relatively well-preserved CD4 counts, may be the presenting clinical feature of AIDS, and responds better to treatment. AIDS-related SNCCL is associated with c-MYC activation, p53 mutation, and EBV infection in 100%, 60%, and 30% of patients, respectively. Chronic antigen stimulation by self antigens also contributes to the development of AIDS-related SNCCL.[134]

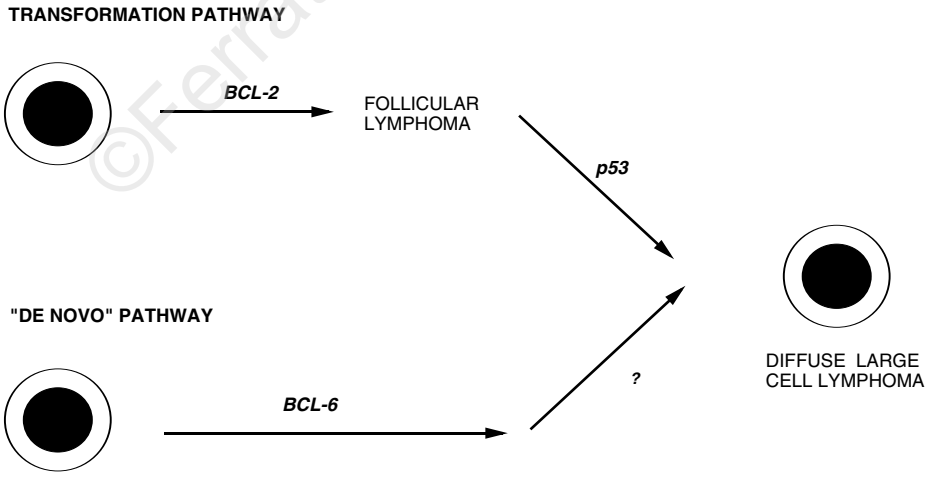

Figure 4. Genetic lesions involved in the pathogenesis of *de novo* and *transformed* B-cell DLCL. B-cell DLCL is thought to be a heterogeneous category of NHL that originate from germinal center cells in at least a portion of cases. Two molecular pathways have been characterized in detail. The first pathway, which accounts for approximately 20-30% of cases, is associated with cases originating from the histologic transformation of a follicular lymphoma and involves BCL-2 activation and p53 mutations. The second pathway, which accounts for 40% of cases, is associates with DLCL presenting as *de novo* lymphoma and involves BCL-6 rearrangements. A third molecular pathway (not shown in the figure) is postulated based on the detection of cases showing only germline BCL-2 and BCL-6 alleles. Identification of the genetic lesion(s) involved in this third pathway is currently lacking.

T-cell non-Hodgkin lymphoma. Peripheral (i.e. mature) T-cell malignancies are relatively rare in the Western world and mainly include T-cell chronic lymphoproliferations, mycosis fungoides/Sézary syndrome, peripheral T-cell lymphoma, and CD30$^+$ anaplastic large cell lymphoma (ALCL; ref. 7). Other peripheral T-cell tumors, namely adult T-cell leukemia/lymphoma, are seen only occasionally in Europe and the U.S.A. With the exception of CD30$^+$ ALCL, the molecular pathogenesis of peripheral T-cell malignancies is poorly understood and few, if any, genetic lesions have been found with significant frequency in these tumors. CD30$^+$ ALCL associates with the t(2;5) translocation, which leads to NPM/ALK activation (Table 1 and ref. 77). It is notable that of all the phenotypic variants of CD30$^+$ ALCL, t(2;5) is selectively associated with cases of T-cell origin.[76] The availability of a genetic marker for T-cell CD30$^+$ ALCL is of exteme practical relevance since CD30$^+$ ALCL, which are potentially curable with aggressive therapy, have frequently been misdiagnosed as histiocytic tumors, metastatic carcinomas, melanomas or sarcomas.[79] Furthermore, the recently identified NPM/ALK fusion gene can be detected by PCR, thus providing molecular evaluation of minimal residual disease in the follow-up after treatment.[77]

The clinical value of genetic lesions in non-Hodgkin lymphoma

From a clinical standpoint, NHL genetic lesions represent molecular markers of disease that serve three purposes: *a)* they assist morphologic diagnosis; *b)* they provide a prognostic indicator in some cases; *c)* they allow evaluation of minimal residual disease by highly specific and highly sensitive technologies.

The use of genetic lesions as markers for NHL diagnosis is justified by the selective association between a given genetic alteration and a specific NHL category (Table 2). Several examples of these associations have been described in the previous sections, including BCL-1 and MCL, BCL-2 and follicular NHL, BCL-6 and DLCL, as well as c-MYC and Burkitt's lymphoma. With respect to diagnosis, the use of genetic markers is particularly useful in classifying cases which do not display peculiar morphologic features or morphologically mimick other NHL types. Thus, for example, the detection of BCL-1 activation in non-follicular small cell NHL is considered the most specific clue for a diagnosis of MCL.[35] On the other hand, many cases of AIDS-related Burkitt's lymphoma display unusual features resembling DLCL and may be correctly diagnosed only upon definition of their molecular characteristics.[131-133] In both of these examples correct diagnosis is essential for therapy and prognosis. The widespread use of molecular diagnostics for NHL is likely to substantially refine the way we classify NHL. Ideally, genetically distinct groups of NHL should be considered as distinct diseases requiring distinct therapeutic options. Indeed, as already stated above, molecular classification of other hematologic malignancies, namely acute leukemia, has been instrumental in revising clinical attitudes toward the disease.[102,103]

The second potential application of NHL genetic lesions in clinical practice lies in the fact that the prognosis of NHL belonging to the same histologic category depends upon the molecular pathway activated in each individual case. In this respect DLCL is paradigmatic, since marked differences in survival are observed in DLCL patients with BCL-6 rearrangements compared to those with BCL-2 rearrangements.[25-27] The use of molecular prognostic indicators may thus complement clinical parameters in the therapeutic stratification of patients affected by the same morphologic category of NHL.

Lastly, genetic lesions of NHL represent the most specific and the most sensitive marker for evaluation of minimal residual disease by PCR. In contrast to acute leukemia, for which the study of minimal residual disease is well codified, routine evaluation of minimal residual disease in NHL is still in its infancy. At present, the application of minimal residual disease analysis by PCR is restricted to follicular or diffuse NHL carrying BCL-2 rearrangements, MCL associated with BCL-1 translocations, and CD30$^+$ ALCL with NPM/ALK fusion transcripts. The development of PCR assays for rearrangements of

BCL-6 and c-MYC is currently in progress. Several reasons account for the difficulties in setting up PCR assays for minimal residual disease evaluation in NHL as compared to acute leukemias, including a lack of chromosomal clustering in many NHL translocations (e.g. c-MYC translocations) and/or the heterogeneity of chromosomal partners juxtaposed to the involved oncogene (e.g. BCL-6 translocations).

In the case of BCL-1 and BCL-2 lesions, the relative consistency of the nucleotide regions involved in the translocation has made it possible to design PCR technology for the BCL-1 and BCL-2 breakpoint clusters by taking advantage of the J_H consensus sequence on chromosome 14.[28-31,46] Knowledge about the clinical relevance of minimal residual disease evaluation in NHL is particularly expanded in the case of BCL-2. In particular, PCR studies have demonstrated that morphologically normal peripheral blood and bone marrow from all patients with advanced stage follicular NHL are generally infiltrated by lymphoma cells after induction or salvage therapy, suggesting that conventional treatment, while inducing complete clinical remission, does not eradicate BCL-2-positive cells.[135,139] Intriguingly, analysis of multiple samples obtained from different bone marrow sites after conventional therapy for follicular lymphoma showed that not all the samples were PCR positive for BCL-2.[138] This finding indicates that there is a patchy infiltration of the BM in follicular NHL even when it is assessed by a highly sensitive technique. Consequently a single negative PCR result must be interpreted with caution, and bilateral BM biopsies/aspirates should be mandatory for minimal residual disease assessement in NHL. After high-dose myeloablative therapy and autologous bone marrow (BM) transplantation for follicular lymphoma, the persistence or reappearance of PCR-detectable residual lymphoma in the BM is associated with an increased risk of subsequent disease relapse.[139] After high-dose therapy, but before infusion of autologous BM, the overwhelming majority of patients display BCL-2 PCR negativity; however, a significant fraction becomes PCR positive within two hours after infusion of autologous BM or peripheral blood stem cell harvests.[138] These observations indicate that malignant cells harbored within the BM or peripheral blood stem cell harvests are infused in the patient and contribute to subsequent relapse.

Besides making a fundamental contribution to the diagnosis and prognostic stratification of non-Hodgkin lymphomas, the study of the molecular pathogenesis of these disorders has led us to a deeper understanding of how current chemotherapeutic regimens act on lymphoma cells. Perhaps the best example comes from apoptosis manipulation in the therapy of low grade lymphoid malignancies such as follicular lymphoma and B-CLL.[140,141] A common feature of both follicular lymphoma and B-CLL cells is the overexpression of BCL-2, resulting from either chromosomal rearrangements (in follicular lymphoma) or putative epigenetic mechanisms (in B-CLL).[17,18,105] High levels of BCL-2 prevent apoptosis and are thought to be the main reason for the characteristically long-lived cells of these lymphoproliferations.

Interestingly, a very effective treatment for both these diseases is based upon the use of nucleoside analogs, including fludarabine and 2-chlorodeoxyadenosine (2-CdA), which are well-known inducers of apoptosis.[142-144] The *in vivo* pro-apoptotic activity of fludarabine and 2-CdA is supported by *in vitro* experiments, which have shown that both these compounds are in fact able to cause apoptosis of malignant B cells.[145-148] Notably, the therapeutic action of fludarabine and 2-CdA against lymphoid malignancies is virtually confined to indolent lymphoproliferative disorders associated with BCL-2 deregulation and prolonged viability of the neoplastic population, whereas both drugs are ineffective against DLCL or other high grade NHL types.

In addition to the current applications of genetic lesions in NHL management, other uses may be developed in the future. In this respect, of particular appeal is the possibility that therapy might be targeted to the very genetic lesions that are responsible for the pathogenesis of lymphomas. Such therapy should, by definition, be largely specific for the lymphoma cells and hence devoid of the major side effects

presently encountered. Preliminary reports from *in vitro* studies do substantiate the validity of gene targeting in controlling the tumorigenic behavior of NHL.[71,149-151] As with all human cancers in general, however, many issues must be resolved before therapeutic use of gene targeting can be attempted in clinical practice.

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