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Pathogenetic mechanisms in B-cell lymphomagenesis

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The pathogenesis of B-cell non Hodgkin lymphoma (NHL) is a highly complex process involving genetic alterations in the tumor clone as well as biologic alterations in the host. Three main mechanisms of lymphomagenesis are recognized: *i)* accumulation of gene alterations in the tumor genome; *ii)* infection of the tumor clone by an oncogenic virus; *iii)* stimulation and selection of tumor cells by an antigen.

As in most human cancers, the genetic lesions involved in B-cell NHL include the activation of proto-oncogenes and the disruption of tumor suppressor genes. In contrast to solid tumors, the pathogenesis of B-cell NHL involves one or few genetic lesions generally represented by chromosomal translocation. These translocations are frequently characteristic of a specific clinicopathologic category of B-cell NHL and represent a molecular marker useful for the diagnosis and for the assessment of response to therapy. Generally, chromosomal translocations of B-cell NHL share a common feature, i.e. the presence of a proto-oncogene mapping to the vicinity of one of the two chromosomal recombination sites. In contrast to neoplasms of precursor lymphoid cells, chromosomal translocations associated with B-cell NHL do not generally lead to coding fusions between two genes. Rather, chromosomal translocations of B-cell NHL juxtapose the proto-oncogene to heterologous regulatory sequences derived from the partner chromosome. The heterologous regulatory regions implicated in B-cell NHL translocations may be derived from antigen receptor loci as well as from other loci. The common consequence of the translocation is the deregulated expression of the proto-oncogene. The precise genetic mechanisms driving the chromosomal recombination in B-cell NHL are poorly understood. It has been proposed that translocations involving antigen receptor loci may constitute errors of the machinery involved in antigen receptor gene rearrangement as well as somatic hypermutation in normal lymphoid cells.

In addition to chromosomal translocations, other mechanisms of proto-oncogene activation can also occur in B-cell NHL. Proto-oncogene amplification in NHL is substantially less common than in epithelial cancers, but it occurs in some cases of aggressive B-cell NHL, as exemplified by the instance of *REL* amplifications in diffuse large B-cell lymphoma (DLBCL). Recently, a novel mechanism of genomic instability has been shown in over 50% of DLBCL. In these cases, the somatic hypermutation process appears to malfunction and aberrantly targets the 5' sequences of multiple non-immunoglobulin genes, including well-known proto-oncogenes such as *PIM-1*, c-*MYC, PAX-5*, and *RhoH/TTF*. Point mutations due to aberrant hypermutation can alter the coding sequence of the proto-oncogene and thus alter the biologic properties of the proto-oncogene product. Alternatively, mutations may affect the proto-oncogene regulatory sequences, thus altering their sensitivity to factors normally regulating the expression of the proto-oncogene.

Disruption of tumor suppressor loci in B-cell NHL occurs through deletion of one allele and mutation of the other, although variable combinations of allelic deletion, mutation, and methylation may occur in some cases. The tumor suppressor gene most frequently involved in the pathogenesis of B-cell NHL is *p53*. Other tumor suppressor genes involved in B-cell NHL pathogenesis include *p16*, a regulator of cell cycle, and *ATM*, a gene involved in apoptosis and DNA repair. In addition to tumor suppressor gene inactivation, B-cell NHL frequently carry specific chromosomal deletions, which conceivably are the site of tumor suppressor loci that have not yet been identified. The most frequent of these deletions involve chromosomes 6 (6q) and 13 (13q).

A second molecular mechanism implicated in B-cell NHL development is infection of the tumor clone by oncogenic viruses. Two oncogenic herpesviruses have been associated with B-cell NHL development, including Epstein-Barr virus (EBV) and human her-

pesvirus type-8 (HHV-8). Apart from EBV and HHV-8, other viruses contribute to lymphomagenesis. These include human immunodeficiency virus (HIV) and hepatitis virus C (HCV). In contrast to EBV and HHV-8, HIV and HCV do not infect the lymphoma clone, and thus do not contribute directly to lymphomagenesis. Rather, they may be responsible for host's conditions, such as immunodeficiency in the case of HIV, which predispose the patient to develop lymphoma through indirect mechanisms.

Given the fundamental role played by antigen stimulation in B-cell development and mitogenesis, it is generally agreed that challenging surface immunoglobulin molecules by antigenic stimuli may play a role in the development of B-cell NHL. This notion is documented in B-cell NHL by the following experimental evidence: *i)* presence of somatic mutations of immunoglobulin genes, typical of the antigen-driven process; *ii)* biased usage of specific families of immunoglobulin genes, consistent with stimulation and selection by specific antigens; *iii)* identification of antigenic specificity of the antigen receptors expressed by B-cell NHL cells.

The mechanism of immunoglobulin gene hypermutation leads to introduction of somatic mutations in immunoglobulin genes. Because contact with an antigen is mediated primarily by amino acid residues in the complementarity determining region (CDR), mutations replacing the amino acids in the CDR (defined as R mutations) may potentially alter antigen binding and be positively selected in this fashion. Somatic hypermutation of immunoglobulin genes characterizes several types of B-cell NHL, including follicular lymphoma (FL), DLBCL, Burkitt lymphoma (BL), MALT lymphoma, and lymphoplasmacytic lymphoma (LPL). Biased usage of immunoglobulin genes in B-cell NHL has also been taken to reinforce the concept of antigen stimulation and selection in B-cell NHL pathogenesis. B-cell NHL frequently express immunoglobulin genes containing V segments that are implicated in the generation of autoantibodies, indirectly suggesting that selection by autoantigens may have been involved in lymphoma development. In the case of FL and BL, the demonstration of expression of immunoglobulins with autoantibody reactivity has reinforced the possibility that self-antigens may play a role in B-cell NHL growth.

Although it appears that, in some B-cell NHL, immunoglobulin gene hypermutation is an ongoing phenomenon persisting after full neoplastic transformation, other B-cell NHL associated with immunoglobulin hypermutation no longer express surface antigen receptor molecules. Thus, for some B-cell NHL, antigen selection may be required for the maintenance of the tumor clone, whereas, in other cases, antigen selection may play a role in the early phases of tumor development. The clinicopathologic heterogeneity of B-cell NHL is reflected by a high degree of heterogeneity in the molecular pathogenesis of these disorders.). The molecular pathogenesis of small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/B-CLL) has been elucidated only partially. Based on karyotypic and deletion mapping studies, it is likely that the 13q14 chromosomal region may harbor a novel tumor suppressor gene that is involved in the pathogenesis of 60% SLL/B-CLL cases. Other recurrent chromosomal aberrations include +12, del 17p and del 11q. Among known cancer-related genes, mutations of *p53* occur in 10% of the cases of SLL/B-CLL.

Approximately 50% of LPL are associated with a t(9;14)(p13;q32) translocation. The chromosomal breakpoints of t(9;14)(p13;q32) involve the IgH locus on chromosome 14q32, and, on chromosome 9p13, a genomic region containing the *PAX-5* gene. *PAX-5* encodes a B-cell-specific transcription factor involved in the control of B-cell proliferation and differentiation. Presumably, the juxtaposition of *PAX-5* to the IgH locus leads to deregulated expression of the gene, thus contributing to tumor development. Aside from t(9;14)(p13;q32), no other genetic lesion has been detected at significant frequencies in LPL.

Mantle cell lymphoma (MCL) is typically associated with t(11;14)(q13;q32). The translocation juxtaposes the *BCL-1* locus at 11q13 with the IgH locus at 14q32. The t(11;14)(q13;q32) translocation consistently leads to deregulation of cyclin D1, a member of the D-type G1 cyclins, which regulate the early phases of cell cycle. Other genetic alterations may also be involved in MCL. Inactivation of *p53* occurs in a subset of cases and is a marker of poor prognosis. Inactivation of *p16*, by deletion, mutation, or hypermethylation, is detectable in approximately half of the cases belonging to an aggressive MCL variant characterized by a blastoid cell morphology.

The genetic hallmark of FL is represented by chromosomal translocations involving 18q21, which are detected in 80% to 90% of the cases. Typically, these translocations juxtapose 18q21 to IgH [t(14;18)(q32;q21)], although occasionally 18q21 is translocated to Igκ [t(2;18)(p11;q21)] or Igλ [t(18;22)(q21;q11)]. In t(14;18), the rearrangement joins the BCL-2 gene at its 3' untranslated region to an IgH J segment. Each of these translocations lead to deregulated expression of *BCL-2*, and, consequently, constitutively high levels of *Bcl-2* protein. Two main breakpoint cluster sites are known in t(14;18)(q32;q21). Approximately 70% of the chromosome 18 breakpoints cluster within the major breakpoint region (MBR). The remaining cases usually break in the more 3' distant minor cluster region (mcr). Over time, a significant fraction of FL evolves into DLBCL. This histologic shift is almost consistently accom-

panied by the accumulation of *p53* mutation and deletion in addition to the pre-existent BCL-2 lesion. In some cases, transformation is accompanied by inactivation of *p16*, *c-MYC* rearrangements or 6q deletions.

The molecular pathogenesis of MALT lymphoma involves antigen stimulation and the accumulation of genetic lesions. The potential role of antigen stimulation is supported by the example of gastric MALT lymphoma, that is strictly associated to *Helicobacter pylori* infection. Several genetic lesions have been associated with MALT lymphoma development. The translocation t(11;18)(q21;q21) occurs in approximately 50% of cases independent of the site of origin. The t(11;18) involves the *API2* gene, mapping at 11q21, and the *MLT* gene, mapping at 18q21, and results in the *API2/MLT* fusion protein. The oncogenic activity of *API2/MLT* remains elusive, and seems to involve both enhanced anti-apoptotic activity and gain of function of the NF-kB transcription factor pathway. Alternatively, MLT may be activated by the translocation t(14;18)(q32;q21), causing juxtaposition of MLT to the IgH locus. The translocation t(1;14)(p22;q32) is an uncommon recurrent chromosomal aberration associated with gastric and lung MALT lymphoma and affects the *BCL-10* gene.

Approximately 40% DLBCL carry chromosomal translocations affecting band 3q27, leading to the activation of the *BCL-6* proto-oncogene. In addition to rearrangements, the *BCL-6* gene may be altered by mutations that selectively cluster within the 5' noncoding regions of the gene. Mutations cause deregulated *BCL-6* transcription, since they affect two adjacent *Bcl-6* binding sites located in the first non-coding exon of the gene, and prevent Bcl-6 from binding its own promoter, thereby disrupting the negative autoregulatory circuit that normally controls its expression. The BCL-6 gene is a transcriptional factor, whose role in lymphomagenesis involves the suppression of p53 expression and the modulation of DNA damage-induced responses in germinal center B-cells. Several other genetic lesions have been detected in DLBCL. Activation of REL occurs through gene amplification and appears to be a relatively frequent event, occurring in 20% of cases. Translocation of BCL-2 occur in a subset of de novo DLBCL and in all cases of DLBCL transformed from a previous follicular phase. Among tumor suppressor genes, inactivation of *p53* and *p16* and deletion of 6q are frequently associates with DLBCL transformation from a previous follicular lymphoma.

Chromosomal breaks at 8q24 are found in 100% of BL, and lead to deregulated expression of the *c-MYC* proto-oncogene. In 80% of cases the translocation involves IgH, leading to t(8;14)(q24;q32). In the remaining 20% of cases, *c-MYC* juxtaposes either to Igκ, leading to t(2;8)(p11;q24) (15% of cases), or to Igλ, leading to t(8;22) (q24;q11) (5% of cases). The product of the *c-MYC* proto-oncogene is a ubiquitously expressed transcriptional regulator controlling cell proliferation, differentiation, and apoptosis. The common functional effect of t(8;14), t(2;8), and t(8;22) is that *c-MYC* undergoes constitutive expression in tumor cells. In addition to transcriptional deregulation, c-myc function may be altered by mutations affecting the amino-terminal transcriptional activation domain in *c-MYC* exon 2. Although under normal conditions the activity of the *c-MYC* transactivation domain is suppressed by protein-protein interactions with the pRB-related protein p107, c-myc proteins carrying exon 2 mutations can escape the p107-mediated modulation. In addition to *c-MYC* translocations, the molecular pathogenesis of BL also involves infection of the tumor clone by EBV, inactivation of the *p53* and *p16* tumor suppressor genes, mutations of the 5' noncoding regions of *BCL-6*, and deletions of 6q.

The use of genetic lesions as clinical tools may be envisioned in at least three settings. First, genetic lesions integrate morphologic and immunohistochemical diagnosis of lymphoma. Second, genetic lesions may allow follow-up of minimal residual disease by highly specific and highly sensitive technologies. Third, genetic lesions may provide the rationale for designing novel therapeutic strategies directly aimed at correcting the molecular defect of the lymphoma.

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