Molecular pathophysiology of indolent lymphoma

DANIELA CAPELLO, GIANLUCA GAIDANO
Division of Internal Medicine, Department of Medical Sciences, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

ABSTRACT

Indolent lymphomas are a markedly heterogeneous group of lymphoproliferative disorders including B cell chronic lymphocytic leukemia/small lymphocytic lymphoma, lymphoplasmacytoid lymphoma, follicular lymphoma, mantle cell lymphoma and mucosa-associated lymphoid tissue (MALT) lymphoma. The molecular pathophysiology of indolent lymphoma is characterized by distinct genetic pathways which selectively associate with different clinico-pathologic categories of the disease. At diagnosis, B-cell chronic lymphocytic leukemia frequently display deletions of 13q14, trisomy 12 and alterations of the ATM gene, whereas evolution to Richter’s syndrome is associated with disruption of p53. Lymphoplasmacytoid lymphoma carries t(9;14) (p13;q32) in approximately 50% of cases, leading to the deregulated expression of the PAX-5 gene. Follicular lymphoma consistently harbors rearrangement of BCL-2. With time, a fraction of follicular lymphoma accumulates mutations of p53 and of p16 and evolves into a high grade lymphoma. MALT-lymphoma frequently associates with alterations of API2/MLT and, in some cases, of p53, BCL-6 and BCL-10. Studies of genotypic and phenotypic markers of histogenesis have shown that mantle cell lymphoma and a fraction of B-CLL/SLL derive from naive B-cells, whereas follicular lymphoma, lymphoplasmacytoid lymphoma and MALT-lymphoma originate from germinal center (GC) or post-GC B-cells. The identification of distinct genetic categories of indolent lymphoma may help in the therapeutic stratification of these disorders. In addition, genetic lesions of indolent lymphoma provide useful molecular markers for disease monitoring by high sensitivity techniques.

Key words: indolent lymphomas

The term indolent lymphoma refers to a group of B-cell lymphoproliferative disorders characterized by an indolent clinical course and inability to obtain disease eradication by most conventional treatments. Early studies of indolent lymphoma revealed the marked clinical and pathologic heterogeneity of these disorders. During the last decade, it has been realized that the clinico-pathologic heterogeneity of indolent lymphoma reflects heterogeneity in the molecular pathophysiology of these diseases. In the past, most molecular investigations of indolent lymphoma have focused on the pathogenetic heterogeneity of these disorders. Recently, pathogenetic studies have been paralleled by analysis of the molecular histogenesis of these malignancies. This review aims at covering current knowledge of the molecular pathogenesis and histogenesis of indolent lymphoma and at providing clues for the clinical relevance of the genotypic features of these malignancies.

Pathogenesis of indolent lymphoma

Analogous to most human cancers, the genetic lesions of indolent lymphoma include the activation of proto-oncogenes and disruption of tumor suppressor genes. In contrast to many types of epithelial cancers, the genome of indolent lymphoma cells tends to be relatively stable and is not subject to the generalized random instability which characterizes many types of solid cancers.

Chromosomal translocation is the main mechanism of proto-oncogene activation in indolent lymphoma. Analogous to most types of hematopoietic neoplasms, chromosomal translocations of indolent lymphomas are constituted by reciprocal and balanced recombination events between two specific chromosomal sites. These translocations recur within a specific clinico-pathologic category of indolent lymphoma and are clonally represented in each tumor case. All chromosomal translocations of indolent lymphomas share a common feature, i.e., the presence of a proto-oncogene mapping to the vicinity of one of the two chromosomal recombination sites. As a consequence of the translocation, the proto-oncogene is juxtaposed to heterologous regulatory sequences which are derived from the partner chromosome and are invariably represented by antigen receptor loci. Because antigen receptor genes are expressed at sustained levels in normal B-cells corresponding to the differentiation stage of the lymphoma, the common consequence of the translocation is the deregulated expression of the proto-oncogene. Disruption of tumor suppressor genes in indolent lymphoma occurs through mechanisms similar to those associated with other human cancers and generally lead to biallelic inactivation of the gene, most frequently achieved through a combination of...
deletion, mutation and methylation.

The clinicopathologic heterogeneity of indolent lymphoma is reflected by a high degree of heterogeneity in the molecular pathophysiology of the disorders. The following pages will summarize the molecular pathways associated with the main categories of indolent lymphoma recognized by the REAL classification.4

B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma

The molecular pathogenesis of B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (B-CLL/SLL) is still largely unknown. Mutations of the p53 gene and loss of heterozygosity in 17p, the p53 site, are found in a small fraction (10 to 15%) of B-CLL/SLL cases.5,6 A higher frequency of p53 alterations is observed after transformation of B-CLL/SLL to Richter’s syndrome, a highly aggressive lymphoma with a poor clinical outcome,5 suggesting that p53 may be involved in the genetic mechanisms underlying B-CLL/SLL progression.

A number of dominant oncogenes, including c-MYC, BCL-1, BCL-2, and of tumor suppressor genes, namely RB1 and BRCA2, have been widely investigated in B-CLL/SLL; however, none of these genes has shown clear associations with the disease. Since high levels of BCL-2 expression are consistently seen in B-CLL/SLL,7 it is conceivable that they result from mechanisms other than chromosomal translocation. Abnormalities in 11q22-q23, the mapping site of the ataxia telangectasia mutated (ATM) gene, have been described in a fraction of B-CLL/SLL.8,9 These abnormalities associate with absence of ATM protein and have been linked to poor prognosis.8 Because the ATM gene is thought to have a role in programmed cell death, its loss of function may contribute to the prolonged cell survival characteristically observed in B-CLL/SLL. Mutations of ATM gene occur in the germline of a fraction of B-CLL/SLL patients, suggesting that carriers of ATM mutations may be at a particular risk of developing the disease.10 On these grounds, germline mutations of ATM may account, at least in part, for the familial cases of B-CLL/SLL.

Despite the paucity of information regarding the molecular lesions associated with B-CLL/SLL, cytogenetic studies have revealed several recurrent chromosomal abnormalities.2,11 Trisomy 12 is found in approximately 35% of B-CLL/SLL cases evaluated by interphase fluorescent in situ hybridization and correlates with a poor survival.12 Based on karyotypic and deletion mapping studies, it is likely that the 13q14 chromosomal region harbors a novel tumor suppressor gene that is involved at high frequency in B-CLL/SLL.15,16 In fact, deletions of 13q14 occur in approximately 60% of cases analyzed by sensitive molecular tools, but the relevant gene has not been identified.15

Lymphoplasmacytoid lymphoma

The t(9;14)(p13;q32) translocation associates with approximately 50% of lymphoplasmacytoid lymphomas (Figure 1).17 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 (paired homeobox-5) gene.17 Pax-5 encodes a B-cell specific transcription factor (BSAP) expressed throughout B-cell

![Figure 1. Pathogenesis and histogenesis of indolent lymphoma. The major compartments of mature B cells are schematically represented as a secondary follicle containing the germinal center and the mantle zone. The marginal zone and the CD5+ B cell compartment are also shown. At the time of germinal center transit, B-cells acquire mutations of Ig and BCL-6 genes. The putative histogenetic derivation of each lymphoma category is indicated by an arrow originating from the relevant B-cell compartment. Arrows are flanked by the cancer related gene which is selectively involved in the pathogenesis of each specific lymphoma category and, based on current knowledge, represents the genetic hallmark of the disease. Abbreviations: B-CLL/SLL (for B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma), LPL (for lymphoplasmacytoid lymphoma), MCL (for mantle cell lymphoma), FL (for follicular lymphoma), DLCL (for diffuse large cell lymphoma), MALT (for MALT lymphomas).]
development and involved in the control of B-cell proliferation and differentiation. In particular, PAX-5/BSPAP coordinated isotype class switching and Ig gene transcription are necessary for the expression of the B-cell antigen CD19. The juxtaposition of PAX-5 to the IgH locus in lymphomas carrying t(9;14) (p13;q32) causes the deregulated expression of the gene, thus contributing to tumor development through presently unclarified mechanisms. Translocations involving PAX-5 are virtually specific for lymphoplasmacytoid lymphoma.

Follicular lymphoma

Follicular lymphoma derives from germinal center (GC) B-cells. Chromosomal translocations that involve BCL-2 are the hallmark of follicular lymphoma, being detected in 80% to 90% of cases (Figure 1). The BCL-2 gene was identified by molecular cloning of the t(14;18)(q32;q21) translocation, which is present in virtually all cases of follicular lymphomas as well as in a proportion of B-lineage diffuse large cell lymphomas. The translocation joins the BCL-2 gene at its 3' untranslated region to IgH sequences from chromosome 14, resulting in deregulation of BCL-2 expression because of the nearby presence of Ig transcriptional regulatory elements. Approximately 70% of the breakpoints on chromosome 18 are clustered within a major breakpoint region (MBR), while the remaining cases usually break in the more distant minor cluster region (mcr).

The BCL-2 gene encodes a 26-kDa integral membrane protein that has been localized to mitochondria, smooth endoplasmic reticulum and perinuclear membrane. Whereas most proto-oncogenes of lymphoid neoplasia directly enhance cell growth, BCL-2 has no ability to promote cell cycle progression or cell proliferation but rather controls the cellular apoptotic threshold by preventing programmed cell death. BCL-2 is only one member of a family of apoptotic regulators, which also includes BAX and BCL-X. It is now clear that BCL-2 exists as part of a high molecular weight complex generated through heterodimerization with BAX. The inherent ratio of BCL-2 to BAX determines the functional activity of BCL-2. When BAX is in excess, BAX homodimers dominate and cell death is accelerated; conversely, when BCL-2 is in excess, as in lymphomas carrying BCL-2 rearrangements, BCL-2/BAX heterodimers are the prevalent species and cell death is prevented.

The precise contribution of BCL-2 deregulation to follicular lymphoma development is complex. The pathogenicity of BCL-2 lesions in the context of follicular lymphoma is substantiated by the ability of BCL-2 specific antisense oligonucleotides to inhibit the growth of human B-cell lymphomas bearing BCL-2 translocations. In vivo, however, the BCL-2 transgene leads to a pattern of polyclonal hyperplasia of mature, long lived B-cells resting in G0, which, despite morphologic similarities, contrasts with the consistent monoclonality of human follicular lymphoma.

Hence the view that BCL-2 activation is not sufficient for follicular lymphoma development, and that other genetic lesions or host factors are required. A strong candidate is chronic antigen stimulation and selection which would synergize with BCL-2 in driving follicular lymphoma expansion. With time, and analogous to the evolution of the human disease, a fraction of BCL-2 transgenic mice progresses to develop aggressive, clonal diffuse large cell lymphomas which have acquired additional genetic lesions.

Other cancer related genes involved in lymphomagenesis, such as c-MYC and p53, do not appear to be involved in follicular lymphoma. Deletions of chromosome 6 are present in 20% of cases. Over time, follicular lymphomas tend to convert into an aggressive lymphoma with a diffuse large cell architecture. This histologic shift is generally accompanied by the accumulation of p53 mutations and, in a fraction of cases, by mutations of BCL-6 and/or inactivation of p16. Rearrangements of c-MYC may also accompany the histologic transformation of follicular lymphoma in rare cases.

Mantle cell lymphoma

Mantle cell lymphoma is a relatively rare lymphoma of CD5- B-cells originating from the mantle zone surrounding reactive follicular centers. The t(11;14) (q13;q32) translocation and BCL-1 rearrangement are the characteristic abnormalities of mantle cell lymphoma (Figure 1). The BCL-1 locus was originally identified as a breakpoint site on chromosome 11 in B-cell malignancies carrying the t(11;14) (q13;q32) translocation. Translocations involving BCL-1 are characteristically detected in 70% of mantle cell lymphomas. BCL-1 translocations are selective for mantle cell lymphoma and, despite initial suggestions, are not found in B-CLL/SLL.

The BCL-1 translocation results in the juxtaposition of the IgH locus on chromosome 14 to sequences from chromosome 11. The relevant oncogene, i.e. cyclin D1, lies 200 kb apart from the BCL-1 locus. The cyclin D1 gene, also known as PRAD1 or CCND1, is a member of a family of proteins that regulate cell cycle progression. As for other D-type cyclins, cyclin D1 is thought to act primarily as a growth factor sensor integrating extracellular signals with the cell cycle machinery. The pathogenic role of BCL-1 activation in human neoplasia is suggested by the ability of cyclin D1 overexpression to transform cells in vitro and contribute to B-cell lymphomagenesis in transgenic mice. Since the morphology of mantle cell lymphoma frequently simulates that of other low grade lymphoproliferative dis-
Histogenesis of indolent lymphomas

The histogenesis of indolent lymphoma can be assessed by identifying the precise cellular subset from which a given lymphoma category derives. This is achieved by defining the precise differentiation stage of the various types of lymphoma and by comparing it with the features proper of the maturation stage of normal lymphocytes.

B-lymphocytes are generated in the bone marrow as a result of a multistep differentiation process, which can be divided into two phases: i) an antigen-independent phase, and ii) an antigen-dependent phase. The antigen-independent phase occurs in the bone marrow and leads to rearrangement of unmutated Ig genes. Naïve B-cells migrate from the bone marrow to the peripheral lymphoid organs, where they encounter (and are selected by) antigen and form germinal centers (GC). Subsequently, B-cells exit from the GC and differentiate into plasma cells or memory B-cells. Within the GC, antigen-activated B-cells accumulate somatic point mutations within their rearranged Ig genes (a phenomenon known as somatic hypermutation) which modify the affinity of their surface antibody to the antigen. In addition to Ig gene mutations, it has been recently shown that also mutations of the BCL-6 proto-oncogene are accumulated by B-cells at the time of GC transit. Because mutations of both Ig and BCL-6 genes are maintained during further B-cell maturation, they represent a histogenetic marker of GC and post-GC B-cells.

Until not long ago, only a few markers were available for histogenetic studies of B-cell neoplasia. More recently, the use of Ig and BCL-6 somatic hypermutation as specific markers of B-cell transit through the GC has allowed the definition of two broad histogenetic categories of indolent lymphoma: i) lymphomas devoid of somatic Ig and BCL-6 hypermutation, which derive from naive, pre-GC B-cells; ii) lymphomas associated with somatic Ig and/or BCL-6 hypermutation and thus derived from either GC or post-GC B-cells. This model may be complemented by the use of phenotypic markers of histogenesis which further refine B-cell differentiation stages. In particular, expression of the BCL-6 protein selectively clusters with B-cells residing within the GC, whereas CD138/syndecan-1 expression identifies post-GC B-cells which are differentiating toward plasmacells.

Mantle cell lymphoma (MCL) and a fraction of B-CLL/SLL are devoid of Ig gene and BCL-6 mutations, do not express BCL-6 or CD138/syndecan-1 and are therefore considered as lymphomas deriving from naive B-cells (Figure 1). Conversely, follicular lymphomas and lymphoplasmacytoid lymphomas associate with mutations of Ig and BCL-6 genes, indicating their derivation from GC-related B-cells. Follicular lymphomas express the BCL-6/CD138− phenotype and thus closely reflect B-cells residing in the GC. Lymphoplasmacytoid lymphomas express the BCL-6/CD138+ phenotype and thus reflect post-GC B-cells. MALT-lymphomas associated with somatic Ig and/or BCL-6 hypermutation are postulated to originate from B-cells which have undergone a GC-like reaction and subsequently migrated to the involved extranodal site.

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Recent studies of B-CLL/SLL have yielded novel insights into the disease histogenesis. It is now apparent that the clinical heterogeneity of B-CLL/SLL might be related to heterogeneity in the disease histogenesis. Although B-CLL/SLL has been traditionally viewed as a tumor of naive, pre-GC B-cells, recent data have suggested that a fraction of B-CLL/SLL derives from GC-related B-cells. In fact, the malignant cells of approximately 50% of B-CLL/SLL harbor mutations of Ig and/or BCL-6 genes. The molecular spectrum of Ig and BCL-6 mutations in B-CLL/SLL is superimposable to that of GC-derived lymphomas. Intriguingly, the histogenetic heterogeneity of B-CLL/SLL appears to carry prognostic relevance, since cases with mutations of Ig genes are associated with a significantly longer survival.

Minimal residual disease in indolent lymphomas

Although patients with indolent lymphoma often achieve a clinical complete response, the majority of these patients eventually relapse. Traditionally, the source of relapse of indolent lymphomas has been thought to be a reservoir of residual neoplastic cells not detectable by standard diagnostic techniques. The development of high sensitivity molecular techniques, namely nested polymerase chain reaction (PCR), has allowed the formal demonstration of the presence of these residual malignant cells in the peripheral blood and bone marrow of patients with indolent lymphoma. On these grounds, the study of minimal residual disease (MRD) has assumed a pivotal role in monitoring patients with complete remission and, more recently, in assessing the curative potential of high-dose chemotherapy and bone marrow transplantation. In addition, PCR monitoring of MRD is also essential to evaluate the contamination of the autograft harvest by residual tumor cells.

In B-cell malignancies, the rearrangement of variable, diversity and joining segments of IgH genes generates unique DNA sequences that are specific for each B-cell clone and represent a tumor specific marker which is amenable to PCR analysis. Knowledge of chromosomal abnormalities associated with indolent lymphomas provides an alternative strategy for MRD detection, based on PCR analysis of the genes involved by the translocation. Examples are the PCR assay for BCL-2 in follicular lymphoma and for BCL-1 in mantle cell lymphoma. The combination of the two MRD strategies, i.e. PCR of Ig genes and PCR of chromosomal translocations, provides a molecular marker in approximately 90% of cases of indolent lymphoma.

Overall, the sensitivity of PCR analysis is several fold higher than that of standard diagnostic techniques and allows the detection of one tumor cell among 10^6 normal cells. Most MRD studies of indolent lymphoma have been conducted on follicular lymphoma. These studies have shown that all patients with advanced stage follicular lymphoma carry tumor cells in their peripheral blood and bone marrow which cannot be eradicated after treatment with conventional chemotherapy regimens. Eradication of MRD, however, can be achieved with intensive therapy involving autologous stem cell transplantation. The prerequisite for MRD eradication is tumor purging which has been performed with both ex vivo and in vivo purging procedures. Achievement and sustainment of MRD eradication in follicular lymphoma has been shown to be predictive of long term survival. Cursory, however, a minority of patients with persistent MRD have also enjoyed long-term clinical remission of their disease. During the last few years, non-myeloablative therapeutic protocols of follicular lymphoma, such as the FND regimen, have been suggested to lead to MRD eradication, although these results need to be confirmed by multicenter trials. Very recently, vaccine immunotherapy directed against the lymphoma idiotypic has been succesful in eradicating MRD in follicular lymphoma after chemotherapy-induced first clinical complete remission. Vaccination could, therefore, represent a novel strategy for MRD achievement both as first line treatment and in those patients who have failed to get benefit from other treatment modalities.

Future clinical studies of MRD should establish whether MRD predicts relapse uniformly and, therefore, justifies intensification of therapy when found, or whether in some cases it simply represents leukemic cell populations whose proliferative potential has been altered by chemotherapy. The availability of novel drugs, highly effective at eliminating residual lymphoma cells, namely anti-CD20 antibodies, should prompt large, multicenter studies aimed at defining the clinical significance of MRD in such a context.

Conclusions and perspectives

A number of general considerations can be drawn from the extensive body of studies of the molecular genetics of indolent lymphoma. First, the well recognized clinical and histologic heterogeneity of indolent lymphoma reflects, and possibly is due to, the extreme degree of diversity among the molecular lesions associated with these disorders (Figure 1). Second, it may be presumed that improved molecular diagnosis will soon translate into differential therapeutic protocols according to the tumor genotype, thus leading to therapeutic stratification and, hopefully, improved patients' survival. Also, the molecular evaluation of MRD in the patient's blood/bone marrow as well as in grafts (bone marrow or peripheral blood stem cells) for autologous transplantation procedures is likely to influence therapeutic decisions and clinical follow-up. Third, lymphoid neoplasia results from the accumulation of multiple genetic lesions interplaying in the same clone. In this respect, follicular lymphoma is the best characterized example of multitstep tumorigenesis among indolent lymphomas.

In addition to the current applications of genetic lesions in the management of indolent lymphomas, other uses may be developed in the future. Of particular appeal is the possibility that therapy might be directed at correcting the precise genetic lesion responsible for the development of each single type of indolent lymphoma. Such therapeutic strategy should, by definition, be largely specific for the lymphomatous cells and hence devoid of the major side effects presently encountered with standard therapeutic regimens.
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