MYC Rearrangements in Histologically Progressed Follicular Lymphomas

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Histologic transformation of low-grade follicular lymphoma to an aggressive-grade lymphoma occurs in 60% to 80% of patients during their clinical course. The events that drive the transformation process are poorly understood. Deregression of the MYC gene has been implicated in a small number of cases. This observation led us to examine the molecular organization of the MYC oncogene in 38 cases of histologically transformed lymphomas that arose from follicular lymphomas, and in 18 of the initial pretransformation follicular lymphomas. In addition, we examined 58 "control" low-grade follicular lymphomas that had not yet shown evidence of histologic progression. Immunoglobulin heavy chain and light chain gene rearrangements were detected in all biopsies and rearrangements of the BCL-2 locus were seen in 38 of 38 of the transformed lymphomas (consistent with their origin from follicular lymphomas), in 18 of 18 of the pretransformation follicular lymphomas, and in 51 of 58 of the control follicular lymphomas. All 18 pretransformation follicular lymphoma specimens displayed at least one immunoglobulin gene and BCL-2 rearrangement in common with the corresponding histologically progressed lymphomas, indicating a clonal relationship between the original follicular lymphoma and the histologically transformed lymphoma. MYC rearrangements were detected in 3 of 38 (8%) transformed lymphomas and in 1 of 58 (2%) control follicular lymphomas. The latter MYC rearranged follicular lymphoma was clinically aggressive and transformed to a high-grade lymphoma that led to the death of the patient within 20 months. None of the 18 pretransformation follicular lymphomas showed MYC rearrangement, including two from patients who later demonstrated MYC rearrangement in the progressed aggressive lymphoma. In all mutational analysis failed to identify additional MYC gene abnormalities in the progressed lymphomas. Because the Epstein-Barr virus (EBV) is associated with a fraction of high-grade lymphomas and is known to upregulate BCL-2, we looked for a potential role for this agent in our progressed lymphomas. We did not detect viral sequences in any case indicating that EBV does not play a major role in progression. The presence of MYC rearrangements in a small fraction of progressed aggressive lymphomas, and not in the corresponding antecedent follicular lymphomas, suggests that acquisition of a MYC rearrangement is in some cases associated with the transformation event.

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FOLLCULAR LYMPHOMA accounts for approximately 50% of all non-Hodgkin's lymphomas occurring in adults in the United States. Despite advanced-stage disease, patients with follicular lymphoma have a relatively favorable prognosis. However, histologic conversion to a more aggressive diffuse lymphoma occurs in a high percentage of patients (59% to 78%) during their clinical course.14 This transformation is associated with increased clinical aggressiveness of the lymphoma and a poor prognosis even with intensive treatment.5-7 The underlying genetic basis for this histologic transformation is not known.

The t(14;18)(q32;q21) translocation is detected in up to 90% of follicular lymphomas.8 This event juxtaposes the bcl-2 proto-oncogene to the Ig heavy chain joining region and leads to its overexpression.9,10 Overexpression of bcl-2 is believed to promote lymphomagenesis, as the protein has been implicated as an inhibitor of programmed cell death.11 Cyogenetic studies indicate that follicular lymphomas which undergo histologic transformation retain their t(14;18) translocation and generally acquire multiple, complex secondary chromosomal abnormalities.12-15 Although some of these secondary chromosomal aberrations tend to recur, none accounts for an appreciable fraction of the total abnormalities, and the cytogenetic data to date do not suggest a predominant genetic locus responsible for histologic transformation.

One candidate gene that may play a role in the transformation process is the MYC oncogene on chromosome 8q24. The MYC protein has been shown to be a member of the leucine zipper family of DNA-binding proteins and has been implicated as a potent regulator of cellular proliferation.16,17 Experimentally, the MYC gene has been shown to act synergistically with the BCL-2 gene both in transfection studies18 and in transgenic mouse models, where it has been shown to be altered in 50% of the high-grade tumors arising in mice with BCL-2 transgenes.19,20 Clonal rearrangements of the MYC oncogene, the hallmark of sporadic Burkitt's lymphoma, have also been reported in a fraction of diffuse large-cell lymphomas.21-24 More interestingly, there have been several cases of high-grade lymphomas or lymphoid leukemias carrying both a t(14;18) translocation and a t(8;14), t(2;8) or t(8;22) translocation that have involved the MYC and BCL-2 loci.25-35 Some of these patients had documented evidence of a prior or coexisting low-grade follicular lymphoma. This observation suggests that in these cases the low-grade lymphoma containing the BCL-2 rearrangement acquired a second genetic alteration involving the MYC oncogene, resulting in its transformation to a diffuse high-grade lymphoma or an acute leukemia. Chromosomal translocations involving 8q24 and chromosomal loci other than those associated with the Ig genes have also been reported36-38 in aggressive lymphoproliferative diseases and include the t(8;17) translocation, the site of the recently described BCL-3 gene.39 In this latter case, MYC involvement was not suggested by cytogenetic studies.
and was only identified using molecular techniques. Observations such as these have led a number of investigators to postulate a potential role for the MYC gene in tumor progression.30

Thus, to assess the role of the MYC oncogene in histologic progression, we examined its molecular organization in 38 cases of histologically transformed lymphomas, in 18 matched pretransformation follicular lymphomas, and in 58 “control” cases that consisted of follicular lymphomas that had not yet shown evidence of progression. We identified MYC rearrangements in three transformed lymphomas and in one unusually aggressive control follicular lymphoma, and describe in detail the pattern of involvement of the MYC gene in these cases.

MATERIALS AND METHODS

Materials Studied and Diagnostic Criteria for Histologic Transformation

Thirty-eight histologically transformed follicular lymphomas from patients observed at the National Institutes of Health were selected for this study based on the availability of frozen tissue for the molecular analyses and a pretransformation biopsy documenting a prior diagnosis of low-grade follicular lymphoma. In 18 of the 38 patients, frozen tissue was also available on the matched pretransformation follicular lymphoma. An additional 58 randomly selected low-grade follicular lymphomas that had not yet undergone histologic transformation were also studied as controls. All patients in this study are part of ongoing clinical studies and have provided informed consent according to the guidelines of the Institutional Review Board of the National Institutes of Health.

All lymphoma diagnoses were classified according to the International Working Formulation for Clinical Usage.40 A follicular lymphoma was considered low grade if it was diagnosed as a follicular small cleaved cell lymphoma or a follicular mixed small cleaved and large cell lymphoma. It was considered to be histologically transformed if on subsequent biopsy any of the following histologies were seen: follicular large cell (FL), diffuse mixed (small cleaved and large cell) (DM), diffuse large cell (DL), large cell immunoblastic (IBL), or small noncleaved cell (SNC) lymphoma. The histology of the transformed tumors included two FL, eight DM, 26 DL, and two SNC (Table 1).

Immunohistochemistry for Surface Igs and Terminal Transferase Activity of the Tumor Cells

Neoplastic cells from biopsies of involved lymph nodes or other tissues, ascitic or pericardial fluid were prepared and stained with a panel of monoclonal antibodies that included anti-λ, anti-μ, anti-κ, anti-λ, and anti-μ Ig (Becton Dickinson, monoclonal Antibodies, Mountain View, CA) and anti-μ Ig (Bethesda Research Laboratories [BRL], Gaithersburg, MD) for analysis by flow cytometry and immunohistochemistry as previously described.41 The presence of immunoreactive terminal transferase (Tdt) was assessed on frozen sections of MYC rearranged cases using a cross-reacting rabbit anti-bovine Tdt antibody (BRL) by an avidin-biotin-peroxidase method.42

Molecular Analysis

Southern blot hybridization. High-molecular weight DNA was isolated directly from involved frozen tissue samples or cell suspensions as described previously.43 After restriction enzyme digestion with HindIII, EcoRI, BamHI, PstI, XhoI, KpnI, PvuII, SstI, Smal, or HaeIII (BRL), the DNA was size-fractionated by agarose gel electrophoresis and transferred to nylon membranes (Gene Screen Plus, New England Nuclear Research Products, Boston, MA; or Zeta-Probe, Bio-Rad, Los Angeles, CA). The filters were serially hybridized with random primed 32P-labeled DNA probes.

Table 1. Histologic Diagnoses and Summary of Molecular Results

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Cases*</th>
<th>mbr</th>
<th>mcr</th>
<th>5 BCL-2 †</th>
<th>MYC Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed lymphomas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular large cell</td>
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<td>0 (0)</td>
<td>2 (0)</td>
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<tr>
<td>Diffuse mixed cell</td>
<td>8 (4)</td>
<td>5 (2)</td>
<td>3 (2)</td>
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<td>0 (0)</td>
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<tr>
<td>Diffuse large cell</td>
<td>26 (13)</td>
<td>19 (10)</td>
<td>5 (3)</td>
<td>0 (0)</td>
<td>2 (0)</td>
</tr>
<tr>
<td>Small noncleaved cell</td>
<td>2 (1)</td>
<td>1 (0)</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>38 (18)</td>
<td>25 (12)</td>
<td>11 (6)</td>
<td>0 (0)</td>
<td>2 (0)</td>
</tr>
</tbody>
</table>

| Nontransformed lymphomas         |               |     |     |          |                   |
| Low-grade follicular lymphomas    | 58            | 37  | 13  | 1        | 7                 |
|                                 |               |     |     |          | 1                 |

*Numbers in parentheses are the results for the available antecedent low-grade follicular lymphomas.
†Included in the 18 antecedent low-grade follicular lymphomas studied for MYC rearrangement were two of the three cases in which the progressed lymphomas showed MYC rearrangement. Of the three cases not available for molecular analysis.
‡Despite its low-grade histology, this lymphoma pursued an aggressive clinical course, resulting in the patient's death in 20 months. Autopsy showed progression to a diffuse large cell lymphoma (not available for molecular analysis). See case 4 in Results and Table 2.

DNA probes. Four different probes were used to assess the configuration of the MYC gene and are shown in Fig 1. The third exon probe, a 1.4-kb ClaI/EcoRI fragment, was used for screening of MYC rearrangements. The other three probes, the 5' first exon probe (1.1-kb HindIII/ClaI fragment), the first exon probe (0.9-kb PvuII fragment), and the second exon probe (0.4-kb PstI fragment), were used for mapping chromosome 8 breakpoints in cases with MYC rearrangements. The first exon probe was also used to detect potential PvuII site mutations in the first exon (discussed below).

BCL-2 gene probes included bcl-2 (BCL-2 mbr), pFL2 (BCL-2 mcr), and pB16 (5' BCL-2). The two Epstein-Barr virus (EBV) terminal repeat probes were used with each probe (9.5-kb PvuII fragment) and the second exon probe (0.4-kb PstI fragment), were used for mapping chromosome 8 breakpoints in cases with MYC rearrangements. The first exon probe was also used to detect potential PvuII site mutations in the first exon (discussed below).

Mapping of chromosome 8 breakpoints. The location of chromosome 8 breakpoints was mapped by sequential hybridizations of two or more digests with the each of the three MYC probes. If the rearranged bands detected with any two of the MYC gene probes differed in size in the same restriction enzyme digest, the break-point was considered to lie between the two probes. A breakpoint was considered to lie within a probe sequence if two MYC rearrangements were identified in two or more digests. If all three MYC probes showed the same rearrangement in HindIII digest and no rearrangement in EcoRI digest, the chromosome 8 breakpoint was assigned 3' to the MYC gene (EcoRI-HindIII fragment). The absence of the rearrangement with the 5' first exon probe despite the presence of the same rearrangement with the other two probes...
in more than two enzyme digests was attributed to a deletion of sequences located 5' to the chromosome 8 breakpoint including at least the 5' first exon probe sequence.

Evaluation of restriction enzyme site mutations in the MYC gene. In cases with chromosome 8 breakpoints occurring far 5' or 3' to the MYC locus, it is not possible to detect their breakpoints directly using conventional probes. However, most Burkitt's lymphomas that have these classes of breakpoints also have mutations near the 3' boundary of the MYC first exon. These are particularly common at the PvuII site. Therefore, by evaluating the status of this PvuII site, it is possible to look indirectly for evidence of MYC involvement. For this purpose, PvuII-digested DNA was probed with the first exon probe. Tumors were assessed positive for the PvuII site mutations if a novel 1.8-kb fragment appeared with a corresponding decrease in intensity of the normal 0.9-kb germline fragment. PstI, SstI, or HaeIII digests were also used to look for possible mutations in the MYC locus of some tumors in combination with our MYC probes.

Assignment of the breakpoints in the Ig gene loci. Breakpoints in the JH locus were identified by comigration of a JH rearrangement with our MYC probes. Additional comigration studies using specific constant region probes indicated the break occurred within or near any one of several switch loci including Sµ, Sα, Sε, and, under some conditions, Sγ, because of the homology of the Sµ probe sequence to the other switch loci. Additional comigration studies using specific constant region probes (Cy, Cγ, Cε, or Cα) with the appropriate enzyme digests showed which switch loci were involved in the translocations. In those cases where we could not demonstrate comigration with an Ig heavy-chain gene fragment, potential light-chain gene comigration was studied using the appropriate enzyme and probe combinations.

RESULTS

Molecular Organization of Ig Gene and 18q21 Loci

Thirty-eight histologically transformed lymphomas from patients with a prior diagnosis of low-grade follicular lymphoma were studied (Table 1). Ig heavy and light chain gene rearrangements were detected in all 38 biopsies. Thirty-six of the 38 (95%) showed rearrangement of the BCL-2 locus, consistent with their derivation from follicular lymphoma. Chromosome 18 breakpoints occurred within the major breakpoint region (mbr) in 25 tumors and within the minor cluster region (mcr) in 11 tumors. All 18q21 rearrangements except for two mcr rearrangements conformed with a JH rearrangement, indicating t(14;18) translocation.

In 18 of the 38 progressed cases, DNA samples from the original low-grade follicular lymphoma were also available for study. All 18 displayed at least one JH rearrangement in common with the corresponding progressed tumor, as well as an identical BCL-2 rearrangement consistent with the expected clonal relationship between the two. However, nine of the 18 exhibited a monoallelic alteration of the size of the JH rearrangement. This has been previously observed by us and others and is believed to be due to ongoing mutations in the Ig heavy chain locus.

An additional 58 control follicular lymphomas that had not yet undergone histologic transformation were also studied. All showed JH rearrangements, and 51 of the 58 (88%) had BCL-2 locus rearrangements. The incidence of BCL-2 rearrangement in the control group is not statistically different from that found in the progressed follicular lymphomas (P > .5). Fisher's exact test. Chromosome 18 breakpoints occurred in the mbr in 37 cases, in the mcr in 13 cases, and in the 5' BCL-2 region in one case. All 18q21 rearrangements with the exception of three mcr rearrangements conformed with a JH rearrangement.

The frequencies of mbr and mcr BCL-2 breakpoints is similar to that previously reported in the literature by several groups for follicular lymphomas.

Involvement of the MYC Oncogene

MYC gene rearrangement was observed in 3 of the 38 (8%) transformed lymphoma specimens (cases 1, 2, and 3; Tables 1 and 2) using a combination of the three described MYC gene probes covering exons 1 through 3. None of the 18 matched pretransformation low-grade follicular lymphomas showed MYC gene rearrangement, including two of the three cases that subsequently displayed a MYC rearrangement in the corresponding progressed lymphoma sample.

Somewhat unexpectedly, 1 of the 58 control follicular lymphomas was found to have a MYC gene rearrangement. However, this case proved to be clinically aggressive and unresponsive to treatment, and the patient died 20 months later with disseminated diffuse large cell lymphoma. Tissue from the autopsy was not available for study.

Because the PvuII site mutations in the first exon are often associated with MYC translocations that lie outside of the regions covered by our probes (ie, far 5' or 3' to the MYC gene exons), we also probed for the presence of this mutation. None of the 38 cases showed PvuII site mutation.

Role of EBV in Tumor Progression

EBV is associated with high-grade lymphoproliferative disorders, including virtually all cases of endemic Burkitt's
A high fraction of lymphomas occurring in immunodeficiency settings, and a smaller fraction of nonimmunodeficiency-related sporadic Burkitt’s lymphomas and diffuse large-cell lymphomas. It has also been shown to alter the regulation of the follicular lymphoma-associated oncogene, BCL-2. We looked for viral sequences in 37 of the 38 progressed cases to determine if this lymphotropic virus might play a role in tumor progression. Epstein-Barr viral DNA was not detected in any of the progressed lymphomas, excluding this agent from playing a significant role in the transformation process.

**Detailed Analysis of the Four MYC Translocated Tumors**

**Case 1.** (Fig 2A, Table 2) This case was diagnosed as a follicular lymphoma of small cleaved cell type in 1973 and progressed to a diffuse large cell lymphoma in 12 years. The patient died 6 months after histologic progression. Biopsies from both time points were available for study. The original follicular lymphoma showed biallelic JH rearrangements, one of which comigrates with a BCL-2 mbr rearrangement. No MYC rearrangement was present at diagnosis. The transformed tumor showed the same BCL-2 mbr/JH rearrangement pattern as seen in the original follicular lymphoma. The second JH allele that was present in the original lymphoma had been lost at transformation and in its place was a new JH rearrangement that shows comigration with rearrangements of BCL-2 and MYC. Immunodetectable Tdt was not present.

**Case 2.** (Fig 2B, Table 2) This case was diagnosed as a follicular lymphoma of small cleaved cell type and progressed after 12 years to a composite tumor composed of low-grade follicular lymphoma and high-grade diffuse lymphoma of small-nonecleaved cell type. The patient died 13 months after histologic progression. Biopsies from both specimens were available for study. The original follicular lymphoma showed biallelic JH rearrangements, one of which comigrated with a BCL-2 mcr rearrangement. MYC rearrangement was not observed in this specimen. The transformed tumor showed the same BCL-2 mcr/JH rearrangement pattern as seen in the original follicular lymphoma. Rearrangement of MYC was present and showed comigration with rearrangements of Sp, Ce, and Ca, suggesting that the translocation took place during an abortive attempt at switch recombination into the JH heavy chain constant region. The 5' first exon MYC probe detected a fragment that comigrated with JH in BamHI and EcoRI digests (not shown). The chromosome 8 breakpoint mapped to the ClaI-PvuII fragment, which corresponds to the 5' flanking region of the first exon of the MYC gene.

Immunophenotypic analysis of the progressed lymphoma showed an absence of surface Ig, as would be expected with both immunoglobulin heavy chain gene alleles involved in interchromosomal rearrangements (BCL-2 and MYC). Immunodetectable Tdt was not present.

**Case 3.** This case was diagnosed as a follicular lymphoma to have surface expression of IgM, while no surface Ig could be detected on the high-grade component of the progressed lymphoma. Immunodetectable Tdt was absent from the progressed tumor.
Figure 2
MYC rearrangements in progressed lymphomas

Case 3. (Fig 2C, Table 2) This case was initially diagnosed as a follicular lymphoma of small cleaved cell type and progressed in 15 years to a diffuse lymphoma of large cell type. The patient died 16 months after histologic progression. Tissue from only the progressed sample was available for study. The progressed tumor showed two JH rearrangements. One showed comigration with a rearranged BCL-2 mbr fragment, while the other (presumably the functional allele) showed migration with the Ig Cμ heavy chain gene segment. Rearrangement of the MYC oncogene was present, but there was no comigration with any Ig heavy or light chain gene segment examined, including JH, Sp, Cμ, Cy4, Cox1, Ce, Jk, Ck, and Ca. This raises the possibility that non-Ig locus may be involved. The chromosome 8 breakpoint mapped 3' to the MYC coding exons (PvuII-XbaI fragment). This location eliminates truncation of the MYC gene as a potential mechanism for deregulation and suggested that the mechanism of MYC deregulation may be mutation of the 5' regulatory sites in the first exon. However, with a battery of enzymes, including PvuII, PstI, SsrI, and HaeIII, failed to identify loss of these restriction sites (data not shown). Thus, a potential mechanism of MYC deregulation could not be implicated.

Immunophenotypic analysis showed expression of IgMκ; immunodetectable Tdt was absent.

Case 4. (Fig 2D, Table 2) This case was the nontransformed follicular lymphoma in which a MYC rearrangement was identified. Although morphologically this follicular lymphoma did not appear unusual, the patient had an unusually aggressive course and died 20 months following his initial biopsy with disseminated, histologically transformed lymphoma. Only the initial follicular lymphoma biopsy was available for study. This biopsy displayed two JH rearrangements. Rearrangements of both the BCL-2 mcr and the MYC gene were present; however, neither showed comigration with JH nor any of the other Ig gene fragments examined (Sp, Cμ, Cy4, Cox1, Ce, Jk, Ck, and Ca). This finding, similar to that in case 3, again raises the possibility that a non-Ig gene may be involved. The chromosome 8 breakpoint mapped to the Smal-Pst1 fragment, which corresponds to the first intron of the MYC gene. Interestingly, and similar to the pattern found in case 1, both the 5' first exon probe and the first exon probe failed to demonstrate rearrangements in any digest, suggesting that an unknown stretch of genetic material located 5' to the breakpoint was deleted in association with the translocation event.

Immunophenotypic analysis showed expression of IgMκ; immunodetectable Tdt was absent.

DISCUSSION

We have identified MYC rearrangements in 3 of 38 (8%) transformed follicular lymphomas. None of the 18 available pretransformation biopsies from the corresponding cases demonstrated a rearranged MYC gene, including two of the three cases that acquired a MYC rearrangement at histologic progression. Only 1 of 58 control low-grade follicular lymphomas (case 4) demonstrated rearrangement of the MYC oncogene, and this case proved to be unusually aggressive, resulting in the death of the patient 20 months later. These data support the concept that rearrangements involving the MYC oncogene are associated with clinically aggressive disease and are generally acquired at the time of histologic transformation in a small proportion of follicular lymphomas that undergo progression.

The incidence of MYC rearrangement that we report in our progressed follicular lymphomas may underestimate the total contribution of the MYC gene to the transformation process. Our analysis assumes that MYC involvement in the high-grade progressed lymphomas will follow the same rules that have been delineated for the prototypic Burkitt's lymphoma, where deregulation of the MYC gene is believed to occur primarily through the uncoupling of the MYC gene from its 5' regulatory elements or through mutation of regulatory sites in the first exon (PvuII site and surrounding sequences). A recent study22 raises the possibility that in other types of lymphomas other additional mechanisms of activation may also be important. These investigators studied MYC involvement in cases of large cell lymphomas with known 8q24 abnormalities. If one eliminates from consideration their cases of acquired immunodeficiency syndrome (AIDS)-associated lymphomas, they were able to identify MYC rearrangements in two of eight cases. Of the remaining six (which presumably had breakpoints far 5' or 3' to the MYC gene), PvuII site mutations were present in only one additional case. Thus, of the eight cases with cytogenetic evidence of 8q24 abnormalities, five (62%) showed none of the usual molecular abnormalities of the MYC gene that have been previously associated with deregulation of the oncogene in the Burkitt's lymphomas. These data raise the possibility that alternative mechanisms...

Fig 2. Southern blot analysis of four MYC rearranged cases. Loci depicted are BCL-2, the immunoglobulin heavy chain joining region, and MYC. Solid lines represent germline restriction fragments; open and closed arrows denote noncomigrating and comigrating rearranged restriction fragments, respectively. (A) Southern blot analysis of case 1. HindIII digested probed with BCL-2 mbr, JH, and MYC third exon probes. One JH rearrangement, common to both pretransformation (F) and transformed (T) lymphoma biopsies, shows comigration with identical BCL-2 mbr fragments. (Faint bands in the pretransformation follicular lymphoma are due to the small number of tumor cells in this specimen.) A MYC rearrangement is detected in the transformed lymphoma, but not in the antecedent follicular lymphoma. (B) Southern blot analysis of case 2. HindIII digested probed with BCL-2 mcr, JH, and 5' first exon MYC probes. One JH rearrangement, common to both pretransformation (F) and transformed (T) lymphoma biopsies, shows comigration with identical BCL-2 mcr fragments. A MYC rearrangement is identified in the transformed lymphoma, but not in the antecedent follicular lymphoma. (A 5' first exon probe was used to better demonstrate the MYC rearrangement in this case.) (C) Southern blot analysis of case 3. HindIII digested probed with BCL-2 mbr, JH, and MYC third exon probes. Only the transformed lymphoma (T) was available for study. One JH restriction fragment shows comigration with a BCL-2 mbr rearranged fragment. A rearranged MYC fragment is also present. (D) Southern blot analysis of case 4. EcoRI digested probed with BCL-2 mcr, JH, and MYC third exon probes. This case was a clinically aggressive follicular lymphoma (F*) (see text for more detailed discussion). Rearrangements of both BCL-2 mcr and MYC are present. Neither show comigration with JH.

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of deregulation exist and/or that PvuII site mutations are not associated in high frequency with far 5′ or 3′ rearrangements in lymphomas other than Burkitt’s, leaving this class of rearrangements undercounted in our results. Unfortunately, we do not have cytogenetic data for correlation with our molecular data.

The pattern of MYC involvement in our progressed lymphomas is of interest. Each case was unique, and three of the four displayed some unusual features different from the ordinary recurring patterns that occur in Burkitt’s lymphomas.

In case 1, the translocation appears to have occurred in or just 5′ to the joining region of the functionally rearranged allele. This would explain our inability to detect surface Ig in the progressed lymphoma. Although we are not aware of a similar case of MYC translocating 5′ to or within a productively rearranged Ig gene, replacement of a rearranged VH region with another has been demonstrated experimentally,57,58 and there is one report of a Line-1 element inserting itself into the JH segment of a productively rearranged Ig allele.59 In both of the above settings, residual or persistent recombinase activity was implicated by sequence analysis and this is a likely mechanism in our case as well.

Cases 3 and 4 failed to demonstrate rearrangement of MYC into any of the Ig loci, including both light chains. Although these studies do not eliminate the possibility of rearrangement into an unidentified Ig locus, they raise the interesting prospect of a non-Ig locus being involved in the rearrangement. Several cases have been reported of 3′ rearrangements involving MYC and T-cell receptor loci.60,61 Although most of these have occurred in T-cell lymphomas or leukemias, there is one report of this occurrence in a B-cell lymphoma,57 MYC rearrangement to a locus that has been named BCL-3 on chromosome 17q22 has also been reported in a case of aggressive prolymphocytic leukemia.58 Both cases 3 and 4 were examined for involvement of the BCL-3 locus. However, neither case displayed comigrations with the rearranged MYC fragment (data not shown).

Although cases 1 and 4 had breakpoints in the first exon or intron of the MYC gene, typical sites for the majority of sporadic Burkitt’s lymphomas, both of these cases showed deletion of an unknown amount of genetic material immediately 5′ to the breakpoint. This is distinctly unusual in the Burkitt’s lymphomas, where the translocations involving the MYC gene are typically reciprocal, and suggests that the mechanism of translocation in these cases may be more complex. Thus, three of the four cases had some unusual features, suggesting that the rearrangement process in these cases might be different from that which occurs in the sporadic Burkitt’s lymphomas.

Case 4 was the single low-grade follicular lymphoma in which we identified a MYC rearrangement. Despite the fact that this case behaved aggressively, it was somewhat surprising that a rearranged, truncated, and presumably deregulated MYC gene was found in a histologically low-grade follicular lymphoma. We know of only a single additional case in which a rearranged, truncated MYC gene has been reported in a low-grade follicular lymphoma.23 No clinical follow-up data were reported for that patient. Until recently, even cytogenetic evidence that might implicate MYC, ie, the presence of 8q24 involvement, in low-grade follicular lymphomas has been limited to rare cases.62,63 Furthermore, with one exception,27 all of the lymphoid neoplasms reported to contain both the t(14;18) translocation and a translocation involving 8q24 have been aggressive lymphoid leukemias or lymphomas.25,26,28,35 However, as part of a large series, Offit et al recently reported t(8;14)(q24;q32) translocation in 7 of 86 (8.1%) low-grade lymphomas (defined to include both follicular and small lymphocytic lymphomas).34 It was not stated whether these cases also possessed the t(14;18) translocation. Median follow-up data of 14.6 months were available for five of the patients, and all were alive without apparent evidence of disease progression. This is still a relatively short follow-up period, and it will be extremely interesting to see if these lymphomas behave more aggressively over time. Others have reported rare or no cases of t(8q24) involvement in their series of follicular lymphomas. Notably, in two large series with a combined total of 111 follicular lymphomas, no cases with 8q24 involvement were observed, although both groups reported other abnormalities of chromosome 8 (trisomy and duplication 8q).12,14 In our series, we were able to detect MYC alterations in only one of the 76 (1%) low-grade follicular lymphomas studied (case 4). Because we do not have cytogenetic correlation, it is possible that there exists a class of translocations which breaks far 5′ or 3′ to the MYC gene locus and which is not associated with the mutations in the first exon. If these kinds of translocations occur with any frequency in low-grade follicular lymphomas, we would not expect them to be associated with deregulation of the MYC gene based on the biologic behavior of these lymphomas.

Despite suggestions that the MYC gene may play an important role in histologic progression of low-grade lymphomas, there have been few studies designed to test this hypothesis directly. The clinical evidence supporting this proposition has been limited to scattered case reports. De Jong et al29 reported a composite lymphoma in which they showed that the high-grade lymphoblastic component contained both a BCL-2 and MYC rearrangement, while the clonally related low-grade follicular lymphoma component contained only the BCL-2 rearrangement. Lee et al31 reported a case of small noncleaved cell lymphoma that evolved from a follicular lymphoma. The follicular lymphoma showed only a BCL-2 rearrangement, while the clonally related small noncleaved cell lymphoma displayed rearrangements of both BCL-2 and MYC. Gauwerky et al30 reported MYC and BCL-2 involvement in a B-cell lymphoblastic leukemia (B-ALL) from a patient who had had a 5-year history of follicular lymphoma and progressed to diffuse large cell lymphoma and finally to B-ALL. Brito-Babapulle et al33 reported an additional case of B-ALL that showed both BCL-2 and MYC rearrangements. This case was thought to have evolved from a follicular lymphoma due to the presence of small cleaved cells in the bone marrow. The MYC gene in the absence of BCL-2 rearrangement has also been implicated in progression in one case of
"blastic transformation" of a chronic lymphocytic leukemia. Additional cases with coexistent BCL-2 and MYC abnormalities have been reported in four high-grade lymphomas, in several cases of de novo B-ALL, and in an aggressive prolymphocytic leukemia. However, none of these latter cases were reported to have a history of antecedent low-grade follicular lymphoma, suggesting that MYC may not act as a progression gene in all cases with double rearrangements.

There are surprisingly few well-documented cases of transformed follicular lymphomas that have cytogenetics reported. Of four large series totaling more than 600 cases, there were only 13 cases of aggressive lymphoma that had a clear history of an antecedent follicular lymphoma. None of those cases were reported to have an 8q24 abnormality. On the other hand, double translocations involving 18q21 and 8q24 have been reported in several cases of intermediate- and high-grade lymphoma/leukemias. Although many of these appear to be de novo B-ALLs, there have been a few cases that evolved from an antecedent follicular lymphoma. Changevelu et al reviewed the University of Chicago series and identified six high-grade lymphoid leukemias or lymphomas with translocations involving both 8q24 and 18q21. Two of the six had a history of follicular lymphoma. Three additional patients with B-ALL and an antecedent history of follicular lymphoma were recently reported by Fiedler et al. Thus, the molecular genetic literature and, to a lesser extent, the cytogenetic literature have implicated MYC as a candidate gene for driving the transformation process. However, because of the small number of progressed cases examined and the design of earlier studies, it had not been possible to estimate the relative importance and frequency of MYC involvement in histologic transformation. The current study suggests that the MYC gene is involved in a small fraction of progressed follicular lymphomas (~10%).

As discussed, some of the previously reported double translocation or double rearrangement–bearing tumors generally presented as acute lymphoblastic leukemias with an immature Tdt-positive phenotype and without a clear history of a preceding follicular lymphoma. Cases that have had well-documented histories of antecedent follicular lymphoma seem to be able to differentiate (or dedifferentiate) along two pathways. Some have displayed lymphoblastic morphology and an immature phenotype as indicated by the presence of Tdt, similar to the de novo B-ALLs with double translocations/rearrangements. Others, like the cases we have reported, have progressed to a phenotypically mature, Tdt-negative, intermediate- or high-grade lymphoma.

It was of interest to study our 38 progressed lymphomas for evidence of EBV infection for several reasons. First, EBV is associated with a considerable fraction of intermediate- and high-grade lymphomas. Second, EBV has been shown to upregulate the follicular lymphoma-associated protooncogene, BCL-2. Nevertheless, none of the 37 cases examined contained EBV sequences, eliminating this agent from having an important role in the transformation process.

All 18 pretransformation tumors with matched biopsies had at least one Ig gene and BCL-2 gene rearrangement in common between the two biopsies. Thus, it is apparent that most transformed lymphomas do not represent new second tumors, but rather are derived from the original follicular lymphoma clone. This is consistent with the notion that follicular lymphoma is an acquired, slowly evolving disease that, over time, accumulates additional molecular lesions that drive the low-grade neoplasm to transformation. It remains to be determined what additional molecular loci, other than MYC, will be implicated in the majority of follicular lymphomas that undergo histologic progression.

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