MYC Rearrangement and Translocations Involving Band 8q24 in Diffuse Large Cell Lymphomas

By Marc Ladanyi, Kenneth Offit, Suresh C. Jhanwar, Daniel A. Filippa, and R.S.K. Chaganti

The configuration of the MYC gene in diffuse large cell lymphomas (DLCL) with translocations involving band 8q24 \( [t(8q24)] \) has not been systematically studied. We collected cytogenetic and clinical data on 171 consecutive cases of DLCL, including cleaved, noncleaved, and immunoblastic types, of which 96 had DNA available and 124 had abnormal karyotypes. The cases with DNA available were evaluated for MYC rearrangement (MYC-R) by Southern hybridization of EcoRI-digested tumor DNA using an exon-1 probe, a combination of probe and enzyme known to detect over 85% of breaks in sporadic Burkitt’s lymphoma. In cases studied at diagnosis, MYC-R, \( t(8;14)(q24;q32) \), or other \( t(8q24) \) were not prognostically significant. Among the 124 cases with karyotypic abnormalities, seropositivity for human immunodeficiency syndrome (AIDS)-associated BL with \( t(8;14) \), two had a \( t(8;14) \), one had a \( t(7;8;14)(p15;q24;q32) \), and one had a \( t(8;7)(q24;?) \) and a \( del(8)(q24). \) In the three previous cases with translocations involving 8q24 and 14q32, comigration of the rearranged MYC band with either the J region or the switch-\( \mu \) region of the Ig heavy chain gene could not be demonstrated, leaving the 14q32 breakpoint undefined at the molecular level. Among the remaining 72 cases where both an abnormal karyotype and molecular data were available, 11 had a \( t(8q24), \) either \( t(8;14) \) or \( t(8;22)(q24;q11), \) in the absence of an MYC-R. In these cases, the \( 8q24 \) break was presumably located outside of the EcoRI MYC fragment. All 15 cases with a \( t(8q24) \) were also screened for point mutations in the \( PvuII \) site in the first exon of MYC; two cases that were not MYC-R showed loss of this restriction site. These results indicate that in most DLCL with \( t(8;14) \) or other \( t(8q24), \) the \( 8q24 \) breakpoint lies away from the MYC gene; in a minority of these cases, point mutations in regulatory noncoding regions were detected.

**MATERIALS AND METHODS**

The present series of 171 cases of DLCL is a subset of the series of 434 specimens of NHL consecutively ascertained over a 5-year period (1984 through 1988) at the Memorial Sloan-Kettering Cancer Center (New York, NY), which has been defined in more detail elsewhere. In these 171 DLCL, DNA for Southern blotting analysis was available in 96, and an abnormal karyotype was obtained in 124; 76 cases had both available, 48 had only abnormal karyotypes available, 20 had only DNA results available, and 27 cases had neither type of data available. The DLCL were classified pathologically according to the International Working Formulation, and included cleaved, noncleaved, and immunoblastic cell types. There was no histologic component of BL in any of the cases. The cell surface IG determination and the cytogenetic analysis, which consisted of short-term culture followed by G- and/or Q-banding, were performed as previously described. Comparison of proportions of cases with specific cytogenetic or molecular genetic features were performed using the method of inference from proportions, and actuarial survival rates were compared using the method of Kaplan and Meier and the logrank test for significance. Immunoperoxidase studies were performed on B3-fixed (B3: 3% mercuric chloride in 4% formaldehyde) paraffin-embedded sec-

From the Laboratory of Cancer Genetics, Sloan-Kettering Institute and the Departments of Pathology (Cytogenetics and Surgical Pathology Services) and Medicine, Memorial Hospital, New York, NY.

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Address reprint requests to Marc Ladanyi, MD, Cytogenetics Service, Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

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tions using an avidin-biotin peroxidase complex method. The L26* and UCHL-1* monoclonal antibodies, specific for B cells and T cells, respectively, were obtained from Dakopatts (Santa Barbara, CA).

Gene rearrangements were studied by Southern blotting of DNA extracted from snap-frozen tissue, using an Oncor Probe-Tech vacuum blotting device, as previously described. The following combinations of probes and enzymes were used. For the \( IGH \) gene, DNA digested with HindIII or EcoRI was probed with a 5.5-kb BamHI-HindIII fragment encompassing the entire \( J \) region (\( JH \)), a 1.3-kb EcoRI fragment of the constant-\( \mu \) region (\( C\mu \)), and a 2-kb SstI fragment including all but the 3' end of the switch-\( \mu \) (\( Sp\)) region. For analysis of the configuration of the \( MYC \) gene, DNA digested with EcoRI, HindIII, PstI, HindIII, PvuII, SmaI, or XhoI restriction enzymes was probed with an XhoI-XbaI genomic fragment of a double digestion with \( PvuII \) and XhoI restriction enzymes was performed as described, followed by hybridization with the same probe as described above. The \( IGH \) and \( MYC \) gene probes used in this study were gifts from J. Ravetch and W. Hayward, respectively.

RESULTS

Cytogenetics. Abnormal karyotypes were obtained in 124 of 171 cases. Of these, 17 (14%) had a classic t(8;14)(q24;q32) translocation, four (3%) had a t(8;22)(q24;q11) translocation, and two had other 8q24 translocations, including a t(7;8;14)(p15;q24;q32) in case 408 and a t(8;?) (q24;?) and del(8)(q24) in case 230 (Fig 1). In all, 23 cases (19%) had a translocation involving 8q24.

In addition to t(8q24), other notable karyotypic abnormalities were present in two cases listed in Table 1: case 591 also showed a t(3;22)(q27;q11) and was included in a previous report of a series of cases with this translocation, while in case 143 a t(8;22)(q24;q11) and a t(14;18)(q32;q21) were present together. The latter is the only case with involvement of 18q21 and 8q24 in the same specimen in this series; in all, 24 of the 124 cases of DLCL with karyotypic data showed translocations involving 18q21, most often t(14;18)(q32;q21).

Gene rearrangements. DNA was available for Southern blot analysis in 96 cases, including 15 cases with a t(8q24) (Table 1). EcoRI-digested DNA from these specimens was probed with the \( MYC \) exon-1 probe; this combination of probe and enzyme is known to detect over 85% of breaks in sBL. Four cases (4%) displayed an \( MYC \) rearrangement (cases 230, 348, 408, and 705). Mapping of the breakpoints was performed by studying the pattern of rearrangements obtained with additional enzymes, including HindIII, PstI, \( PvuII \), \( SmaI \), and \( XbaI \) (Fig 2). A clustering of breaks was observed within exon-1/intron-1 for cases 348, 408, and 705, and a more downstream break site was detected in case 230; these results are schematically represented in Fig 3. None of the 20 cases with molecular data alone were found to be rearranged for \( MYC \).

The 15 cases with a t(8q24) were evaluated for exon-1 \( MYC \) mutations at the \( PvuII \) restriction enzyme site (Table 1). In cases 348, 408, and 705, the \( PvuII \) fragment was rearranged; in cases 540 and 591, the \( PvuII \) site at the 3' end of exon-1 was lost, indicating a small deletion or a point mutation at this site (Fig 4). The remaining cases showed no alteration of the \( PvuII \) fragments. Hence, 2 of 11 cases (18%) of DLCL with an 8q24 break remote from the \( MYC \) gene showed point mutation at this site.

Rehybridization of blots with probes for \( Sp\), \( C\mu \), \( JH \), and \( MYC \) were performed in cases 348, 408, and 705 to detect comigrating bands. Two rearranged \( JH \) bands were present in each of these cases (data not shown). Case 705 also showed a rearrangement of \( Sp\), in the presence of a germline \( C\mu \) band, whereas in cases 348 and 408 both \( Sp\) and \( C\mu \) were in germline configuration (data not shown). However, in none of the three cases was comigration of rearranged \( Sp\) or \( JH \) bands with the rearranged \( MYC \) bands demonstrated.

Immunophenotypes. Surface IG results on fresh tumor cell suspensions were available in 11 of the 15 cases in Table 1. Three cases expressed only IGM, five cases also expressed other heavy chains, while the remaining three cases expressed no surface IG. Six cases, including one studied in frozen section only (case 295), expressed IKG light chains, while three expressed IGL light chains. Case 143 had an IGK phenotype in the presence of a t(8;22). The cell
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Table 1. Clinical, Pathologic, Cytogenetic, and Molecular Data on the 15 DLCL With t(8q24)

<table>
<thead>
<tr>
<th>UNN</th>
<th>Age</th>
<th>Sex</th>
<th>Pathology</th>
<th>Surface Ig</th>
<th>MYC</th>
<th>PvuII</th>
<th>Treatment</th>
<th>Cells</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. DLCL with t(8q24) and MYC rearrangement</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>230</td>
<td>24/M</td>
<td>IMB-D</td>
<td>LN</td>
<td>None</td>
<td>R</td>
<td>G</td>
<td>Pre</td>
<td>6</td>
<td>46,XY</td>
</tr>
<tr>
<td>348</td>
<td>29/M</td>
<td>LNCC-D</td>
<td>SB</td>
<td>None</td>
<td>R</td>
<td>R</td>
<td>Pre</td>
<td>1</td>
<td>46,XY</td>
</tr>
<tr>
<td>408</td>
<td>35/M</td>
<td>IMB-D</td>
<td>LN</td>
<td>IGG-K</td>
<td>R</td>
<td>R</td>
<td>Post</td>
<td>21</td>
<td>47,XY, +5t(7;8;14)(p15;q24;q32)</td>
</tr>
<tr>
<td>705</td>
<td>38/F</td>
<td>IMB-D</td>
<td>CW</td>
<td>ND</td>
<td>R</td>
<td>R</td>
<td>Pre</td>
<td>32</td>
<td>46, X, der(X)(11)(q26,q21),t(8;14)(q24;q32),dup(11)(q13→q23)</td>
</tr>
</tbody>
</table>

| B. DLCL with t(8;14) without MYC rearrangement |       |     |           |            |     |       |           |       |           |
| 70 | 23/F | IMB-D | LN | None | G | G | Post | 28 | 50,XX, + 11, + 12, + 14, t(1;14)(p31;q32), t(8;14)(q24;q32), der(9) |
| 147 | 60/F | LNCC-D | Sp | IGG-K | G | G | Pre | 19 | 48,XX, + 1, + 7, + 9, - 20, t(1;6;11)(p31;q23;13), t(1;13)(q25;q22), t(8;14) |
| 178 | 28/M | LNCC-D | LI | IGM-L | G | G | Pre | 4 | 46,XY |
| 295 | 42/M | LNCC-D | LN | K | G | G | Post | 23 | 55-57,XX, 6, + 7, + 22, t(8;14)(q24;q32), 2Xdel(10)(p11→ter), der(13) |
| 534 | 71/F | LCC-D | SP | ND | G | G | Post | 1 | 46,XX |
| 536 | 31/F | LNCC-D | SP | IGM-K | G | G | Pre | 16 | 48,XX, - 15, + 6, t(8;14)(q24;q32), + der(X)(1;X)(q21;q27), der(2) |
| 594 | 37/M | IMB-D | LN | IGG-L | G | D | Pre | 9 | 47,XY, t(3;22)(q11;18), t(8;14)(q24;q32) |
| 626 | 60/F | LNCC-D | LN | IGM-L | G | G | Pre | 18 | 51,XX, + 3, + 18, + 19, t(8;14)(q24;q32), del(6)(q21→ter), der(3)(13;3) |
| 656 | 41/F | LNCC-D | BN | IGG | G | G | Post | 33 | 85,XX, +/−, - 2, - 4, - 6, - 10, - 13, - 15, - 16, - 20, - 21, + 7, t(8;14) |

| C. DLCL with other translocations involving 8q24, without MYC rearrangement |       |     |           |            |     |       |           |       |           |
| 143 | 58/F | IMB-D | LN | IGG-K | G | G | Post | 27 | 48, X, - X, + 7, + 8, t(8;22)(q24;q11), t(14;18)(q32;q21), del(11)(q22→ter), + der(1)(qter→q21:?:), der(9)(qter→p14;9q13→ter) |
| 540 | 74/F | IMB-D | LN | ND | G | D | Pre | 6 | 46,XX |

Abbreviations: UNN, unique tumor number; LN, lymph node; SM, small bowel; CW, chest wall; SP, spleen; LI, liver; BN, bone; IMB-D, immunoblastic diffuse; LNCC-D, large noncleaved cell diffuse; LCC-D, large cleaved cell diffuse; G, germline; R, rearranged; D, deleted; PvuII, PvuII restriction enzyme site in MYC exon-1; Pre, studies performed on pretreatment material; Post, studies performed posttreatment material; ND, not done.

Of the 144 cases that were not studied in cell suspension (cases 295, 534, 540, and 705) were determined using the L26 and UCHL1 monoclonal antibodies in paraffin sections. Case 230 was a Ki-1 positive peripheral T-cell lymphoma; its phenotype and genotype have been reported in detail elsewhere. The remaining cases were positive for L26 and negative for UCHL1, confirming their B-cell lineage.

Clinical correlations. Of the 144 cases with an abnormal karyotype or DNA available for molecular analysis, 102 were studied before cytotoxic treatment. The median survival of the 14 patients with a t(8q24) or a rearrangement of MYC was no different from the remaining 88 patients (P = .23). Serotyping for human immunodeficiency virus (HIV) was performed in 29 of the 144 cases; of the seven HIV-positive patients, five had a t(8q24) (71%), including cases 230, 408, and 591 in Table 1, compared with only two of the remaining 22 patients (9%); this difference was statistically significant (P < .05).

The salient clinical features of the final study group of 15 cases of DLCL with t(8;14) or other t(8q24) translocations and DNA available for blotting are summarized in Table 1. These 15 patients ranged in age from 23 to 76 years, and there were nine women and six men. The mean age of the MYC-rearranged group (cases 230, 348, 408, and 705) was 39.5 years compared with 48.4 years for the rest of the group (P = .10). The DLCL were classified as large non-
cleaved cell in seven cases, immunoblastic in seven cases, and cleaved cell in one case. In none of the cases was a component of BL observed. Three of the 15 specimens in Table 1 were obtained from extranodal sites: small bowel in case 348, chest wall in case 705, and bone in case 656. Of the 15 cases in Table 1, cytogenetic and molecular studies were performed before cytotoxic chemotherapy in eight cases. Among these, the median survival of the three patients with rearrangement of MYC was no different than that of the five patients with a t(8q24) but with a germline configuration of MYC (P > .50).

DISCUSSION

Up to 20% of DLCL show a t(8;14) translocation. In our series, 14% showed this translocation, and another 5% showed other translocations involving band 8q24. The incidence of t(8;14) appears to be roughly the same in the three histologic subgroups of DLCL, namely the cleaved, noncleaved, and immunoblastic. Recent long-term follow-up studies of the DLCL group have failed to demonstrate any major differences in survival between these subtypes. Hence, from both the cytogenetic and clinical viewpoint, grouping the three histologic subtypes for the purposes of this analysis appears justified.

Two of the cases in Table 1 also displayed other characteristic translocations. Case 591 showed a t(3;22)(q27;q11), a recently identified translocation associated with diffuse NHL. In case 143, the t(8;22) was present in association with a t(14;18)(q32;q21). Coexistent 18q21 and 8q24 rearrangements, involving BCL2 and MYC, have been described in rare cases of high-grade NHL or acute lymphoblastic leukemia. Our patient 143 had an immunoblastic lymphoma and died of disease 7 months after the initial diagnosis, despite aggressive chemotherapy. However, case 143 is the only case out of 24 with a t(18q21) in our series to
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Fig 4. Southern blot analysis of the MYC gene for point mutation at the PvuII restriction enzyme site in exon-1 using double digestion with PvuII and Xho I. In cases 540 and 591, the PvuII site was lost, presumably by point mutation, producing a new fragment of the combined size of the two germline fragments (1.3kb = 0.9kb + 0.4kb). Case 348 shows a rearrangement within the PvuII fragment, producing a new band larger than 1.3 kb. Cases 408 and 705 also showed rearrangement of the PvuII fragments (not illustrated). The remaining cases showed no alteration of the PvuII fragments.

also display a t(8q24), suggesting that this is not a common event in the transformation of low-grade NHL with t(18q21).

We found MYC exon-1/intron-1 breaks in cases 348, 408, and 705. This region is also typically involved in the t(8;14) sBL.21 With the exception of case 230, the lack of detectable MYC rearrangement in the remaining cases indicates that the 8q24 break in these cases must lie outside of the EcoRI fragment containing the MYC gene; this is usually the case in eBL with t(8;14) and in the variant t(2;8) and t(8;22) translocations.21 Most breaks in eBL appear to be located at least 90 kb upstream of the MYC first exon,23 whereas the breaks in the variant translocations occur at least 10 kb distal to the MYC gene,23 beyond the 3’ HindIII site (Fig 3).

Molecular studies of the MYC gene in cases of non-AIDS DLCL have been limited. Chenexiv-Trench et al29 studied 18 DLCL and found a rearrangement of MYC in one case, located immediately 5’ to exon-1; unfortunately, no karyotype data were available on this tumor, or on most of their other cases. Sozzi et al29 reported a case of DLCL with a t(8;14) and a rearrangement of MYC. Delia et al41 studied the configuration of the MYC gene in five primary cutaneous B-cell DLCL and found it to be germline in all five; unfortunately, no cytogenetic data were provided. The presence of Epstein-Barr virus (EBV) within the lymphoma cells, which is thought to be involved in the pathogenesis of at least some AIDS-associated NHL, was not studied in the present series. However, it should be pointed out that no consistent relationship between EBV infection and the type of cytogenetic or molecular lesion has so far been shown in these cases.22,33

About 75% of AIDS-associated NHL, both BL and DLCL, show MYC rearrangement, usually with breaks similar to those in sBL.22,33 Accordingly, two of the three HIV-positive cases in our group of 15 DLCL with t(8q24) had a rearrangement in MYC. On the other hand, some AIDS-associated cases show eBL-type breaks in the IGH and MYC genes. In our series of DLCL, HIV positivity was significantly more common in cases with a t(8q24) (71%) than in cases without it (9%) (P < .05).

Our case 230 was a T-cell Ki-1 positive NHL9 and had cytogenetic involvement of the 8q24 band on both homologues [del(8)(q24) and (8;?)(q24;?)], while the two chromosomes 14 appeared normal. One MYC allele, most likely the one involved in the t(8;?) (q24;?), showed a rearrangement in the 3’ region or immediately downstream; it was impossible to determine whether the other MYC allele, presumably the one in the del(8)(q24) chromosome, was deleted or germline, due to the high content of non-neoplastic cells in this specimen. However, our data do allow the exclusion of a rearrangement of this allele. Translocations involving band 8q24, typically t(8;14)(q24; q11), have been well described in other T-cell neoplasms: the break at 8q24 has been localized to the region 3’ of MYC in several T-cell leukemia cell lines.35,36

Possible mechanisms of MYC activation in these translocations include: (1) transcriptional activation by adjacent IGH enhancers; (2) transcriptional activation by distant IGH enhancers; (3) mutational inactivation of 5’ regulatory sequences by IGH-related hypermutational activity; and (4) altered half-life and kinetics of a mutated MYC protein.35,37 Whether MYC activation is manifested by absolute overexpression or merely inappropriately sustained expression at normal levels is still not entirely clear.

In relation to the third mechanism mentioned above, cases of BL with distant MYC breakpoints, most commonly endemic cases with t(8;14) or cases with variant translocations, have been found to have a high incidence of point mutation within and upstream of exon-1 and within intron-1.23,25 The most commonly affected area is the PvuII restriction site at the 3’ end of exon-1, which is mutated in over 50% of cases, eliminating the restriction site and resulting in transcriptional deregulation of MYC.20,33,38 Of the 15 cases with a t(8q24) in the present series, two cases (540 and 591) showed clear evidence of point mutation at the PvuII site; neither of these two cases was MYC-R. Hence, they appear to be analogous to eBL with distant MYC breakpoints, although the incidence of point mutation appears to be significantly lower (15%). Although the PvuII site is the most common restriction enzyme site to undergo mutation in this setting, mutations in exon-1 outside of this site are also numerous22,25 and may be present in at least some other cases in our series.

The t(8;14) breakpoints within the IGH locus differs according to the form of BL:25 in sBL and AIDS-associated BL, the breaks usually are in the Sµ region, whereas in eBL they most commonly are located in the JH region. We
studied cases 348, 408, and 705 for comigration of rearranged MYC and IGH bands. Case 705 showed rearrangement of both JH and Sµ, in the presence of a germline Cµ band, indicating that the lymphoma cells had undergone class switching with deletion of the Cµ region. In cases 348 and 408, JH was rearranged, but Sµ and Cµ were in a germline configuration. No surface IG could be demonstrated in case 348; however, monoclonal surface IGG was present in case 408, suggesting that the class switching that occurred must have involved the 3' end of the Sµ region, as previously described in some cases, with deletion of the remainder of Sµ, including the entire region covered by our Sµ probe. In none of the three cases was comigration of the rearranged Sµ or JH bands with the rearranged MYC band demonstrated. Thus, in these cases the 14q32 breakpoint remains undefined at the molecular level. Occasionally, the breakpoints in BL have been shown to occur in the DHI region or Sα or Sγ regions, which we did not study.

Cases of BL with the variant translocations t(8;22) and t(2;8) typically express IGL and IGK light chains, respectively, because of the sequential rearrangement of the light chain genes. Interestingly, however, our case 143 had an IGK phenotype in the presence of a t(8;22); this exceptional combination has also been reported in a case of BL.

Thus, of the 15 DLCL with a t(8q24), three (20%) had breaks in the 5' region of MYC, similar to sBL, and another two cases (13%) showed evidence of point mutation within exon-1, a regulatory noncoding region, analogous to some cases of eBL. One T-cell DLCL had a more 3' break in MYC, as described in other T-cell lymphomas. The molecular events affecting the MYC gene in the other nine cases (60%) remain to be clarified. It is possible that the t(8;14) in at least some of these cases is ineffective in terms of MYC deregulation, relative to its counterpart in BL, which would be consistent with its relative lack of impact on survival in DLCL in the present study, despite the over-representation of HIV-related cases in the t(8q24) group. In a separate study, we are currently assessing MYC expression in this series of DLCL by in situ hybridization. Thus, these studies show that cases of DLCL with t(8;14) provide another useful experimental setting for the analysis of the mechanisms of MYC deregulation. Finally, the localization of 8q24 breakpoints at the molecular level in cases of DLCL may also have future therapeutic implications, because rearrangements within the MYC gene result in abnormal MYC transcripts that may be targeted in a tumor-specific fashion by antisense oligonucleotides.

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MYC rearrangement and translocations involving band 8q24 in diffuse large cell lymphomas

M Ladanyi, K Offit, SC Jhanwar, DA Filippa and RS Chaganti