Intermediate- to High-Grade Histology of Lymphomas Carrying t(14;18) Is Associated With Additional Nonrandom Chromosome Changes

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We describe additional nonrandom chromosome abnormalities in 18 cases of intermediate- to high-grade non-Hodgkin's lymphoma (NHL) bearing t(14;18) that were ascertained in a prospective cytogenetic study of all lymphomas seen at Memorial Hospital during the period January 1, 1984, to December 31, 1986. These included seven cases that had histological evidence of transformation from a lower grade and 11 that lacked such evidence. The most common of the additional changes seen in both groups affected chromosomes 6 and 7 and comprised the loss of chromosome 6 or del(6q) and the presence of more than two copies of chromosome 7 or duplication of 7q. Changes affecting these two chromosomes were less frequent in low-grade lymphomas with t(14;18) as well as in lymphomas lacking the translocation. These data suggest that common cytogenetic mechanisms underlie expression of high-grade histologies by lymphomas carrying t(14;18) and may serve as indicators of lymphoma transformation and patient prognosis when encountered in low-grade lymphomas with t(14;18).

MATERIALS AND METHODS

We have been performing prospective cytogenetic, histological, cell marker, and molecular studies of all lymphoma specimens submitted to the pathology department of Memorial Hospital for diagnostic evaluation since January 1, 1984. Correlation studies involving histological, cytogenetic, and cell marker phenotypes of the tumors ascertained through April 1984 have been published elsewhere. The present report is based on all tumors ascertained between January 1984 and December 1986. This series comprised 168 specimens with a confirmed diagnosis of NHL removed from 167 patients and included lymph node, spleen, and nonlymphoid tissues (fibrous tissue, muscle, lung, testis, etc.). All tissue samples were divided, with portions submitted for cytogenetic and molecular genetic studies. The former were processed for chromosome analysis following previously described methods. The latter was snap frozen in liquid nitrogen and stored at −70°C until studied. Portions destined for histology were fixed in B3, and slides were prepared and stained with hematoxylin and eosin by conventional methods. NHL specimens were retrospectively classified according to the working formulation. Bone marrow, pleural effusion, and ascites tumors, although studied, have been excluded from this analysis because they can not be classified by the working formulation. All tissues used in this study were submitted to the laboratory for diagnostic evaluation; informed consent for diagnostic evaluation was obtained at the time of admission to Memorial Hospital.

RESULTS

From this series of lymphomas, clonal chromosome abnormalities were identified in 107 cases; the remaining either showed normal karyotypes only or were cytogenetic failures. One clonal abnormal case was excluded from the analysis because poor chromosome morphology did not permit analysis beyond detection of a t(14;18). Of the 106 remaining cases, 35 showed t(14;18), 17 of which were classified as low grade (12 small cleaved cell follicular and five mixed follicular) and 18 as intermediate to high-grade tumors. Of the latter 18, seven had evidence of histological transformation from a previous lower grade as documented by evaluation of initial and rebiopsy slides (designated here as group 1 lesions), and 11 lacked such evidence (designated here as...
group II lesions). Recurrent chromosome changes in addition to t(14;18) found in the 18 groups I and II patients were compared with those seen in the 17 low-grade tumors with t(14;18) as well as with those seen in the remaining 71 clonally abnormal NHL lacking t(14;18).

Data on recurrent chromosome changes, cell surface markers, histology, and clinical features of patients belonging to groups I and II are summarized in Table 1. All seven group I patients had received chemotherapy before the rebiopsy sample that showed transformation; of these, three died within 1 month after transformation, three have not gone into remission after 4 to 25 months of treatment, and one patient is in complete remission 15 months after transformation. In six of the seven cases, cytogenetic studies were obtained after transformation had occurred (Table 1). Three of the eleven group II patients had received treatment at the time of cytogenetic analysis; four died within 4 months of diagnosis, five are alive under treatment for recurrent disease.

### Table 1. Karyotypic, Immunologic, Clinical, and Histological Features of Intermediate- to High-Grade NHL With t(14;18)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Site</th>
<th>Surface Markers</th>
<th>Recurrent Chromosome Abnormalities*</th>
<th>Initial Biopsy</th>
<th>Repeat Biopsy</th>
<th>Survival (mo)</th>
<th>Status</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68/M</td>
<td>LN</td>
<td>ND</td>
<td>SCC-F</td>
<td>1/81</td>
<td>SCC-D</td>
<td>11/84</td>
<td>25+</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>54/F</td>
<td>LN</td>
<td>ND</td>
<td>SCC-F</td>
<td>4/79</td>
<td>LCC-D</td>
<td>8/84</td>
<td>1/2</td>
<td>Exp BLEO</td>
</tr>
<tr>
<td>3</td>
<td>40/M</td>
<td>ST</td>
<td>ND</td>
<td>SCC-F</td>
<td>10/67</td>
<td>SCC-D</td>
<td>5/84</td>
<td>RT</td>
<td>NHL-4</td>
</tr>
<tr>
<td>4</td>
<td>38/F</td>
<td>SC</td>
<td>ND</td>
<td>Mix-F&amp;D</td>
<td>5/67</td>
<td>LCC-D</td>
<td>8/86</td>
<td>4+</td>
<td>PR</td>
</tr>
<tr>
<td>5</td>
<td>61/F</td>
<td>LN</td>
<td>ND</td>
<td>SCC-F</td>
<td>12/79</td>
<td>LCC-D</td>
<td>4/85</td>
<td>20+</td>
<td>PR</td>
</tr>
<tr>
<td>6</td>
<td>57/F</td>
<td>LN</td>
<td>ND</td>
<td>LC-D</td>
<td>9/84</td>
<td>Imb-D</td>
<td>3/85</td>
<td>1</td>
<td>EXP</td>
</tr>
<tr>
<td>7</td>
<td>40/M</td>
<td>LN</td>
<td>ND</td>
<td>SCC-F</td>
<td>2/67</td>
<td>SCC-D</td>
<td>8/84</td>
<td>4</td>
<td>EXP</td>
</tr>
<tr>
<td>8</td>
<td>54/L</td>
<td>LN</td>
<td>ND</td>
<td>SCC-F</td>
<td>12/84</td>
<td>LCC-D</td>
<td>9/84</td>
<td>26+</td>
<td>PR</td>
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<tr>
<td>9</td>
<td>70/F</td>
<td>ST</td>
<td>IgGx</td>
<td>LC-D</td>
<td>2/86</td>
<td>LCC-D</td>
<td>3/86</td>
<td>4</td>
<td>EXP</td>
</tr>
<tr>
<td>10</td>
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<td>ST</td>
<td>IgMx</td>
<td>Imb-D</td>
<td>8/86</td>
<td>LCC-D</td>
<td>1/85</td>
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<tr>
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<td>IgMx</td>
<td>SCC-D</td>
<td>9/84</td>
<td>LCC-D</td>
<td>12/86</td>
<td>24</td>
<td>PR</td>
</tr>
<tr>
<td>12</td>
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<td>ST</td>
<td>Inc</td>
<td>SCC-D</td>
<td>3/86</td>
<td>LCC-D</td>
<td>12/86</td>
<td>12</td>
<td>PR</td>
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<tr>
<td>13</td>
<td>84/F</td>
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<td>ND</td>
<td>SCC-D</td>
<td>2/86</td>
<td>LCC-D</td>
<td>12/86</td>
<td>12</td>
<td>PR</td>
</tr>
<tr>
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<td>IgGx</td>
<td>SCC-D</td>
<td>9/84</td>
<td>LCC-D</td>
<td>2/86</td>
<td>10</td>
<td>PR</td>
</tr>
<tr>
<td>15</td>
<td>53/M</td>
<td>ST</td>
<td>IgMx</td>
<td>SCC-D</td>
<td>2/86</td>
<td>LCC-D</td>
<td>12/86</td>
<td>24</td>
<td>PR</td>
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<tr>
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<td>72/M</td>
<td>LN</td>
<td>ND</td>
<td>SCC-D</td>
<td>8/83</td>
<td>LCC-D</td>
<td>12/86</td>
<td>12</td>
<td>PR</td>
</tr>
<tr>
<td>17</td>
<td>73/F</td>
<td>LN</td>
<td>IgMx</td>
<td>Imb-D</td>
<td>12/85</td>
<td>LCC-D</td>
<td>12/86</td>
<td>12</td>
<td>PR</td>
</tr>
<tr>
<td>18</td>
<td>46/F</td>
<td>LN</td>
<td>IgGx</td>
<td>SCC-D</td>
<td>1/86</td>
<td>LCC-D</td>
<td>12/86</td>
<td>12</td>
<td>CR</td>
</tr>
</tbody>
</table>

Abbreviations: LN, lymph node; ST, soft tissue (fibrous, adipose, muscular); ND, not determined; Inc, inconclusive; SCC, small cleaved cell; SNCC, small noncleaved cell; LC, large cell; LCC, large cleaved cell; LNCC, large noncleaved cell; Mix, mixed small and large cell; Imb, immunoblastic; F, follicular; D, diffuse; EXP, expired; PR, partial response; CR, complete response; M2, vincristine, cyclophosphamide, BCNU, prednisone, melphalan; MACOP-B/M-BACOD, mithotrexate, doxorubicin, cyclophosphamide, vincristine, desamethasone/prednisone, bleomycin; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CVP, cyclophosphamide, vincristine, prednisone; C-MOPP, cyclophosphamide, vincristine, procarbazine, predni-sone; 1-20, vincristine, cyclophosphamide, doxorubicin, mithotrexate, daunorubicin, ara-C, l-asparagene, 5MP, prednisone, BCNU; NHL-4, thiopeta, vincristine, chlorambucil, prednisone; EDAM, deazoaminopterim; RT, radiation therapy; CYT, cyclophosphamide; ADR, doxorubicin; BLEO, bleomycin; MTX, mithotrexate.

*Nonrecurrent chromosome abnormalities are not included. Detailed karyotypes may be obtained from the authors (R.S.K.C.).
†Survival from time of final tissue diagnosis.
‡A bone marrow sample of July 16, 1984, revealed the recurrent abnormalities del(6q), t(14;18).
10 to 41 months after diagnosis, and two are alive in complete remission after chemotherapy 12 to 19 months after diagnosis (Table I).

Recurrent chromosome abnormalities in addition to t(14;18) were identified in both groups, and those affecting chromosomes 6 and 7 were the most frequent among them. Loss of chromosome 6 or del(6q) was seen in four of these 18 cases (22.2%) (nos. 3 and 5 of group I and 11 and 15 of group II). Similar abnormalities affecting chromosome 6 were seen in three of 17 low-grade NHL (17.7%) with t(14;18) and nine of 71 (12.76%) NHL without t(14;18) in our series. More than two copies of chromosome 7 or duplication of 7q were identified in 12 of the 18 (66.7%) cases (nos. 1, 5, and 6 of group I and 8, 9, 11, 12, 13, 14, 15 and 17 of group II). Similar abnormalities affecting chromosome 7 were seen in three of 17 (17.7%) of low-grade NHL with t(14;18) and six of 71 (8.5%) NHL without t(14;18) in our series.

**DISCUSSION**

Recent studies have established a strong correlation between t(14;18) and follicular small cleaved cell lymphoma. However, this translocation has been noted in lymphomas lacking both a follicular pattern and a small cleaved cell component. A previous report described two cases of nodular poorly differentiated lymphocytic lymphoma that transformed to L3 acute leukemia and exhibited t(14;18). In addition, we have identified in the published literature nine cases of lymphomas carrying the t(14;18) that showed evidence of histological grade transformation. In this report we describe seven cases of lymphoma with evidence of transformation from low grade to intermediate or high grade and an additional 11 cases that presented with a diffuse growth pattern, a large cell component, or both, all of which displayed t(14;18). These data, although underscoring the association between t(14;18) and small cleaved cell follicular histology, also demonstrate that such lymphomas retain the t(14;18) when they transform to a higher grade.

We have identified recurrent chromosome abnormalities affecting chromosomes 6 and 7 in addition to t(14;18) in association with intermediate and high-grade histology in our series of 18 cases. Examination of the published karyotype data of 11 cases of low-grade lymphomas that have undergone transformation and 18 cases of intermediate to high-grade NHL all of which carried t(14;18), revealed some of the same changes that we have described here. Thus, loss of chromosome 6 or del(6q) was seen in two cases of the former group and five cases of the latter group (7/29; 29.1%), and more than two copies of chromosome 7 or duplication of 7q were seen in five cases of the former group and eight cases of the latter group (13/29; 44.8%). Thus, these data are in agreement with our results.

The highly nonrandom nature of chromosome 6 and 7 changes that we identified here strongly suggest a role in determining the ultimate grade of t(14;18)-carrying lymphomas. Interestingly, these same abnormalities have recently been shown by Yunis et al to correlate with an aggressive course when accompanying t(14;18) in follicular lymphomas. Thus, the additional abnormalities described here can serve as indicators of grade transformation and patient prognosis.

Evidence for gene deregulation in lymphomas mediated by chromosome rearrangement comes from data on c-myc expression in t(8;14)(q24;q32) associated with Burkitt's lymphoma. The constitutive function of c-myc appears to be cell cycle regulation, and its deregulated expression under the influence of the immunoglobulin locus leads to escape of growth regulation by the cell. The gene, bcl-2, situated at 18q21 is frequently rearranged in t(14;18) and has been suggested to undergo deregulation. Its constitutive function is unknown; however, it is commonly rearranged in follicular lymphomas, and it has been suggested to undergo deregulation in t(14;18). Histologically, the follicular lymphomas express an architecture reminiscent of a normal lymph node. Therefore, it is attractive to speculate that bcl-2 also may play a role in cell cycle regulation, but with lesser efficiency than c-myc, or alternatively, that it is a locus associated with differentiation of lymph node architecture. In either case, t(14;18)-carrying tumors with a documented history of grade transformation as well as those presenting as apparent de novo intermediate or high-grade lesions represent an overriding of the deregulated function of bcl-2. Whether the basis for this is a secondary activation of c-myc, as suggested by the phenotype, or deregulation or dosage changes of genes on the additional chromosomes that we now show to exhibit nonrandom abnormalities is unknown at present.

Chromosomes 6 and 7 are of interest from the point of view of the genes that they carry. The frequent site of deletion of 6q observed in these tumors corresponds to the site of the cellular oncogene c-myb. Loss of heterozygosity at key loci have been shown to be critical steps in the origin of tumor types such as retinoblastoma and Wilms' tumor. Chromosome 7 perturbations similar to the ones that we describe here have been noted in a variety of tumors originating from diverse cell types. The epidermal growth factor receptor gene has been localized to this chromosome. Growth factors have been shown to have a synergistic effect on cellular oncogenes; for example, platelet-derived growth factor increases the level of c-myc expression in NIH3T3 cells. Dosage alteration of one or more growth-regulating genes on chromosome 7 may exert similar effects on bcl-2 or c-myc that result in expression of higher-grade phenotypes by t(14;18)-carrying lymphomas.

**REFERENCES**

4. Bloomfield CD, Arthur DC, Frizzera G, Levine EG, Peterson...
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ME Richardson, QG Chen, DA Filippa, K Offit, A Hampton, PR Koduru, SC Jhanwar, PH Lieberman, BD Clarkson and RS Chaganti