RAPID COMMUNICATION

Trisomy 3 in Low-Grade B-Cell Lymphomas of Mucosa-Associated Lymphoid Tissue

By Andrew C. Wotherspoon, Teresa M. Finn, and Peter G. Isaacson

Characteristic chromosomal aberrations have been associated with subtypes of non-Hodgkin’s lymphoma (NHL). The t(8:14) and deregulation of the c-myc oncogene is associated with Burkitt’s lymphoma, t(14;18) and rearrangements of the bcl-2 oncogene with centroblastic-centrocytic (follicle center cell) lymphoma, and the t(11:14) involving the bcl-1 (PRAD-1/CCND1) oncogene with mantle cell lymphoma. More recently, t(2;5) has been reported in CD30+ anaplastic large-cell lymphoma. In each case the lymphomas characterized by these specific genetic rearrangements have distinct pathologic appearances and clinical behavior. It is now well established that many low-grade B-cell NHL that arise at extranodal sites share characteristic clinicopathologic features that are distinct from B-cell lymphomas of nodal type. These lymphomas are thought to arise within locally acquired mucosa-associated lymphoid tissue (MALT). The cell of origin of MALT lymphoma remains controversial, but the tumors show morphologic and immunophenotypic evidence of marginal zone B-cell differentiation. Other tumors thought to arise from marginal zone B cells include nodal monocytoid B-cell lymphoma and the so-called splenic marginal zone lymphomas with or without circulating villous lymphocytes.

Few cytogenetic studies of low-grade B-cell MALT lymphomas exist because of the poor availability of fresh tissue and poor in vitro growth. The translocations t(1;14) and t(11;18) have been reported in a small proportion of cases, but the most frequent cytogenetic abnormality in these small series has been trisomy of chromosome 3, although trisomy of chromosomes 12 and 18 has also been identified.

The recent development of interphase cytogenetic techniques using chromosome-specific α-satellite probes and in situ hybridization allows for the examination of interphase nuclei for numerical chromosomal abnormalities. This technique can be applied to routinely formalin-fixed and paraffin wax-embedded material, which greatly extends its application and allows the investigation of a large series of archival cases. We report an interphase cytogenetic study of trisomy 3 in 70 cases of low-grade B-cell lymphoma of MALT type from various extranodal sites and 11 cases of splenic marginal zone cell lymphoma with and without circulating villous lymphocytes. In view of previous metaphase cytogenetic results, the cases were also examined for numerical abnormalities of chromosomes 7, 12, and 18.

MATERIALS AND METHODS

Formalin-fixed paraffin wax-embedded material from 70 cases of low-grade B-cell lymphoma of MALT (29 stomach, 4 small intestine, 13 thyroid, 11 salivary gland, 7 lung, 5 conjunctiva/orbit, and 1 tonsil) and 11 cases of splenic marginal zone cell lymphoma were retrieved from the surgical files of the Department of Histopathology of the University College London Medical School (London, UK). Ten reactive tonsils and 37 cases of low-grade B-cell lymphoma of nodal type were included as controls. In each case, hematoxylin and eosin-stained slides were reviewed to confirm the presence of tumor within the tissue blocks selected. Of the cases of splenic lymphoma, 5 were documented as having circulating villous lymphocytes, whereas for the remaining 6 cases the pathologic features were identical but the peripheral blood picture was not recorded. No cases of nodal monocytoid B-cell lymphoma, another type of NHL possibly related to MALT lymphoma, were included in this study because our surgical files only contained cases with coexisting extranodal (mucosal) disease in which spread from an MALT lymphoma could not be excluded.

Free nuclei were isolated using a modification of established techniques. Briefly, several 40-μm sections were deparaffinized in xylene, washed in 100% ethanol, and resuspended in 10 mL of a

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TRISOMY 3 IN MALT LYMPHOMA

Table 1. Interphase Cytogenetic Results for Cases of Low-Grade B-Cell MALT Lymphoma and Splenic Marginal Zone Lymphoma

<table>
<thead>
<tr>
<th>Site</th>
<th>No.</th>
<th>Trisomy 3</th>
<th>Trisomy 7</th>
<th>Trisomy 12</th>
<th>Trisomy 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>29</td>
<td>18</td>
<td>1</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Sm Int</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid</td>
<td>13</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Sal GI</td>
<td>11</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Lung</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Orb/conj</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>42 (60%)</td>
<td>2 (3%)</td>
<td>9 (13%)</td>
<td>15 (21%)</td>
</tr>
</tbody>
</table>

| Spleen     | 11  | 3 (27%)   | 0         | 1 (9%)     | 2 (18%)    |

Abbreviations: Sm Int, small intestine; Sal GI, salivary gland; Orb/conj, orbit/conjunctiva.

Freshly prepared solution of 0.5% pepsin (Sigma Ltd, Poole, UK) and 3% polyethylene glycol compound 6000, pH 1.5, in saline. Digestion proceeded at 37°C for 30 minutes with vigorous shaking. The suspension was allowed to settle briefly before the supernatant was removed. The suspension was pretreated in 3% hydrogen peroxidase. The slides were blocked in 3% hydrogen peroxidase. The slides were allowed to settle briefly before the supernatant was removed.

Slides were prepped for hybridization by soaking in xylene, immersion in 3:1 methanol:acetic acid, and air drying. Endogenous peroxidase was blocked in 3% hydrogen peroxidase. The slides were pretreated in 10 mmol/L citrate buffer in a pressure cooker at full pressure for 2 minutes. Additional treatment with pepsin (0.25% in 0.1 mol/L HCI at 37°C) was used as necessary. The slides were dehydrated through grade alcohols and air dried.

Biotinylated α-satellite probes for chromosomes 3 (D3Z1), 7 (D7Z1), 12 (D12Z1), and 18 (D18Z1) (all from ONCOR, Gaithersburg, MD) were ethanol precipitated with salmon sperm DNA and resuspended in a hybridization mix of 60% formamide, 10% dextran sulphate, and 2× SSC to a final probe concentration of 5 ng/μL. Each slide was covered with 5 μL of probe solution and covered by a coverslip. Probe and target DNA were denatured at 100°C for 5 minutes, followed by overnight incubation at 37°C. The slides were soaked in 2× SSC to remove the coverslip, washed in 50% formamide/2× SSC for 1 hour at 42°C, and finally washed in 2× SSC for 30 minutes. Probe was detected by incubation with mouse antibiotin (Dako Ltd. High Wycombe, UK) followed by the DUET system (Dako), developed with diaminobenzidine, and counterstained with hematoxylin. The slides were examined under oil immersion and the signals per nucleus were counted for a minimum of 200 intact nuclei. Overlapping or indistinct nuclei were discounted and cases with greater than 15% nuclei showing trisomy were scored as positive.

RESULTS

A summary of the results is shown in Tables 1 and 2. Nuclei obtained were well disaggregated and clear signals were seen on isolated nuclei. No trisomy was seen in the 10 cases of reactive tonsil. Trisomy 3 was seen in nuclei of 60% of low-grade MALT lymphomas arising in a variety of extranodal sites. The percentage of nuclei showing a trisomy varied from case to case with a mean of 29% (range, 15% to 60%), reflecting the amount of tumor tissue in the section analyzed and the variable reactive T- and B-cell infiltrate seen in these tumors. Trisomy 3 was also seen in 27% of splenic marginal zone cell lymphomas and in 16% of low-grade B-cell lymphomas of nodal type. In the cases of MALT lymphoma, trisomy of chromosomes 3 and 18 was seen in 6 cases, of chromosomes 3 and 12 in 3 cases, and of chromosomes 3 and 7 in 1 case. Three cases showed trisomy of chromosomes 12 and 18 without numerical abnormalities of chromosome 3 and 2 cases showed trisomy of chromosomes 3, 12, and 18. No case showed trisomy of all four chromosomes.

DISCUSSION

Characteristic recurring chromosomal abnormalities have been recognized in many specific types of lymphoma and leukemia. In each case the genetic aberration has been linked to tumors with distinct clinicopathologic features and has often provided additional information for prognosis. Low-grade B-cell lymphomas of MALT form a group of low-grade B-cell lymphoma with distinct clinicopathologic features. They have been described at many extranodal sites and are characterized by specific morphologic and immunophenotypic appearances and an indolent clinical course, usually remaining localized to the site of origin until late in the course of the disease. As such, MALT lymphomas would be expected to be characterized by a distinct genetic abnormality.

Few conventional metaphase cytogenetic studies of low-grade MALT lymphomas have been performed because these tumors are infrequently received fresh in the laboratory because the diagnosis of lymphoma is often not suspected. We have found that they generally grow poorly in culture with a very low proliferation and a low yield of analyzable metaphase spreads. Translocations t(1;14) and t(11;18) have been observed and proposed as possibly significant in the genesis of MALT lymphomas. However, trisomy 3 has been the most frequently found abnormality in these metaphase analyses, occurring in 50% to 60% of cases with abnormal karyotypes. We have confirmed this incidence by looking specifically for trisomy 3 in a large series of low-grade MALT lymphomas from diverse extranodal sites. The incidence of trisomy 3 was found to be independent of site of origin, providing further evidence for the unification of

Table 2. Interphase Cytogenetic Results for Reactive Tonsil and Nodal-Type Low-Grade B-Cell Lymphoma

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>Trisomy 3</th>
<th>Trisomy 7</th>
<th>Trisomy 12</th>
<th>Trisomy 18</th>
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</thead>
<tbody>
<tr>
<td>NHL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>13</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>MCL</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LC</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>LPC</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>PC</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>6 (16%)</td>
<td>5 (14%)</td>
<td>6 (16%)</td>
<td>6 (16%)</td>
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</tbody>
</table>

Abbreviations: N Tonsil, normal tonsil; NHL, low-grade B-cell NHL; nodal type; FL, follicular lymphoma; MCL, mantle cell lymphoma; LC, lymphocytic; LPC, lymphoplasmacytic/cytoid; PC, plasmacytic.
all of the MALT type of extranodal lymphomas into a single entity.

The high incidence of trisomy 3 in MALT lymphoma is in distinct contrast to that seen in low-grade B-cell lymphomas of nodal type. In this study, 16% of low-grade B-cell nodal lymphomas showed trisomy 3, and trisomies of chromosomes 7, 12, and 18 showed a similar incidence. Whereas trisomy 12 was found in cases of lymphocytic and lymphoplasmacytic lymphoma and might be a reflection of the importance of this in the pathogenesis of these tumors, the incidence of trisomy 3, 7, and 18 is broadly in keeping with previous metaphase studies and probably reflects the background incidence in low-grade B-cell NHL.2,14-20 Because MALT lymphoma and splenic marginal zone lymphoma are both thought to be derived from marginal zone B cells, it could be expected that they would share a common genetic abnormality.7 In this series we found that of the 11 cases of splenic lymphoma three showed trisomy of chromosome 3, each of which was known to have circulating villous lymphocytes. Oscier et al27 found none of the 31 cases of splenic lymphoma with villous lymphocytes to have additional copies of chromosome in their study using conventional metaphase cytogenetic techniques. The reason for a higher incidence in our study is uncertain. The lower frequency of trisomy 3 in splenic lymphomas suggests that, although both may arise from marginal zone B cells, lymphomas arising from the splenic marginal zone possibly differ from those arising at other extranodal, "mucosal" sites. This difference is also reflected in the differing clinical behavior of splenic marginal zone lymphoma that frequently involves the bone marrow and peripheral blood early in the course of the disease.28 In view of the variation in cytologic appearances and the architectural pattern of the splenic lymphoma, the origin from marginal zone B cells remains to be confirmed.28

The underlying genetic mechanism by which trisomy of chromosome 3 could play a role in the genesis of MALT lymphoma remains obscure. A recently described candidate proto-oncogene, bcl-6, has been mapped to 3q27.25 and rearrangements of this band have been associated with diffuse, large B-cell lymphomas arising at extranodal sites and appears to confer a better prognosis.30-32 Rearrangements of this proto-oncogene are not found in significant numbers of follicular lymphomas,30,32 but data for other low-grade B-cell lymphomas and particularly MALT lymphomas is not yet available. Because many primary extranodal high-grade B-cell lymphomas show features of lymphomas arising in MALT and show trisomy of chromosome 3,33 bcl-6 might play a significant role in the development of MALT lymphoma.

Several tumor-suppressor genes are thought to be located on the short arm of chromosome 3 and are involved in the development of carcinomas of the head and neck, lung, and kidney.34-38 It is possible that in low-grade MALT lymphomas one of the copies of chromosome 3 might contain an abnormal copy of a tumor-suppressor gene that could be transcribed to form a dominant negative protein that interferes with the wild-type tumor-suppressor activity.

It has been shown that the proliferation of the neoplastic cells of low-grade gastric MALT lymphomas is dependent on the presence of activated antigen-driven T cells.39 A possible mechanism for the role of an additional chromosome 3 might be the overexpression of a molecule or receptor that is involved in B- and T-cell interaction. The interleukin-5 (IL-5) receptor α chain gene has been localized to 3p26.30-34 In the murine model, IL-5 acts on B lymphocytes that have received a sustained mitogenic stimulus to sustain proliferation of high-density B lymphocytes stimulated with anti-Ig for 2 to 3 days.42-43 IL-5 has no stimulatory activity on quiescent B cells. Murine IL-5 may function synergistically with other cytokines such as IL-2 and IL-4 to stimulate growth and differentiation of B cells. IL-5 has also been found to cause increased synthesis of Ig, especially IgA.44 In humans, IL-5 is produced by the T cell subset of CD4+ T cells.45 and the IL-5 receptor β chain is common to the receptors for IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CSF).46 The role of IL-5 in human B-cell development is less well established.

The gene for B7 maps to 3q13.3-3q21.47 B7 is an activation antigen expressed on activated B cells and is the natural ligand for CD28 on T cells.48 After engagement of T-cell receptor with antigen in association with major histocompatibility complex class II, a second signal mediated through B7-CD28 binding greatly upregulates the production of lymphokines, particularly IL-2.49-51 It has been shown that the neoplastic cells of low-grade MALT lymphoma proliferate in a T-cell–dependent fashion.52 Overexpression of B7 as a consequence of trisomy 3 may therefore be important in the genesis of these tumors.

In conclusion, we have used interphase cytogenetic techniques with chromosome-specific probes to show trisomy 3 as a putative characteristic genetic abnormality in MALT lymphoma that is distinct from genetic abnormalities seen in nodal-type low-grade B-cell lymphoma. The presence of trisomy 3 in MALT lymphomas arising at different sites provides genetic evidence in support of the MALT lymphoma as a single entity. The presence of a consistent chromosomal abnormality in low-grade MALT lymphomas supports the contention that these are malignant tumors from the outset rather than a clonal reactive population in response to antigenic stimulation.

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