Cytogenetics as an Aid in the Diagnosis of Lymphomas

Armand B. Glassman, Vicki Hopwood, and Kimberly J. Hayes
Section of Cytogenetics, Department of Pathology and Laboratory Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Abstract. Multiple classifications of lymphomas are available. Generally, distinctions are made to identify low, intermediate, and high-risk groups. Histopathologic differentiation is at times difficult. The revised European-American lymphoma classification (REAL) uses histology, clusters of differentiation markers, histochemistry, and cytogenetics for definitive identification. This work reviews the karyotypic and FISH (fluorescent in situ hybridization) findings in some common lymphomas. B-Cell lymphomas, which make up approximately 85-90% of lymphomas, are associated with cytogenetic changes of +12, 13q14, 14q32, 2p11, and 22q13. Translocations help to support the diagnosis of follicular cell lymphoma t(14;18)(q32;q21), mantle cell lymphoma t(11;14)(q13;q32), and Burkitt's lymphoma t(2;8),t(8;14) and t(8;22). T-Cell lymphomas may show changes in 14q11,7p or 7q. Many of the lymphomas are characterized by complex karyotypic changes. Specific FISH probes are useful in determining characteristic or identifying marker chromosomes. Cytogenetic and FISH studies aid in the diagnosis, correct classification, and evaluation of therapy for a variety of lymphomas.

Keywords: Cytogenetics, lymphomas, fluorescent in situ hybridization, chromosomal translocations

Introduction

Lymphomas are generally divided into two large groups. They are Hodgkin's Disease (HD) and non-Hodgkin's lymphoma (NHL). Within each of these categories, there are multiple subcategories and classifications. Histology of the types of lymphoma are variable and controversies in the nomenclature and categorization exist [1]. In 1994, a group proposed the revised European-American lymphoma classification using a combination of histologic, histochemical, immunologic, and cytogenetic characteristics to arrive at a more definitive diagnosis [3].

Utilization of a consistent classification of lymphomas is of significant importance in determination of the natural history of disease, for prognosis, as a guideline for choice of correct therapy, and for follow-up of therapy. Classification for both NHL and HD continue to evolve and no single classification is accepted universally. Cytogenetic studies are complementary to histopathology and molecular studies in the diagnosis of NHL.

The objective of this work is to identify and report on cytogenetic changes, which can aid in classification and understanding of NHL of B-lymphocyte cell origin.

Methods

Cytogenetic karyotypes and FISH data from the Cytogenetics Laboratory of the University of Texas M.D. Anderson Cancer Center have been reviewed to determine common changes related to various classifications of NHL. Treatment status of the patients is not known. Cases were chosen on the basis of karyotype from the cytogenetics database.

Cytogenetic studies were conducted on cultures of bone marrow aspirates submitted to the laboratory. Marrow cells were adjusted to a concentration of 2 to 4 x 10^6 cells/ml. These were then cultured without addition of cytokines in HAM F10 nutrient media, L-glutamine, 10% fetal calf serum, and penicillin/streptomycin at 37°C for 24 to 48 hr. After exposure to colcemid (0.01 µg/ml) for 20 min, the cells were
 centrifuged, the supernatant removed, and 10 ml of hypotonic KCL (0.075 M) was added for 20 min at 37°C. The fixative step (3 parts methanol; 1 part glacial acetic acids) was repeated 3 times for 10 min each. Cells were centrifuged, supernatant removed, and the cells dropped on glass slides for air drying. The cells were warmed in an oven at 60°C overnight, trypsinized for 90 sec with Enzar T pancreatic enzyme, stained with Gurr's giemsa, analyzed, and karyotyped. At least 20 metaphases were counted and recorded. A clonal population was defined as additional numerical or structural aberrations (identical abnormalities) being found in 2 or more metaphases. For monosomy or absence of chromosomes, identical deletion abnormalities had to be found in 3 or more metaphases. Having no clonal abnormality among 20 or more metaphases studied identifies diploid karyotypes.

FISH techniques were performed using commercially available probes and manufacturer's recommended methods. Briefly, the method includes the use of 10 µl of the hybridization mixture to cover a 22 x 22-mm cover slip and sealed with rubber cement. The hybridization mixture was made up of an appropriate DNA probe mixture prepared according to the manufacturer's instructions. For satellite DNA probes, the undenatured probe mixture was applied directly on the slides. For whole chromosome painting probes, predenaturation and prehybridization are necessary. The probe DNA and specimen DNA were denatured simultaneously on a slide warmer at 75°C for 3 min, then the slide was immediately transferred to a humidified chamber. Hybridization occurred under incubator conditions of 37°C overnight. Signal detection was obtained with an antidigoxigenin FITC reagent and was followed by washing in a phosphate detergent buffer.

The histologic diagnosis was used as guidance for matching karyotypes with a specific diagnosis.

Results

Cases from January 1993 to February 1999 were reviewed. Fifty-six cases with a diagnosis of mantle cell lymphoma and t(11;14) or a variant were noted. Eighteen cases of follicular cell lymphoma (FCL) with a t(14;18) or a variant were recorded. Five cases of Burkitt's lymphoma with a t(2;8) were identified. Other translocations noted for B-cell lymphomas included 6 with t(8;14) and 15 with t(8;22). The breakpoints noted for each of these are listed in Table 1. Many of these karyotypes showed additional abnormalities in addition to the ones listed in the table. These included hypo- and hyperdiploid clones. In some instances, FISH techniques were used to identify marker chromosomes as cryptic translocations.

Table 1. Chromosomal changes in some lymphomas

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>n</th>
<th>Characteristic karyotype</th>
<th>Additional karyotypic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mantle cell lymphoma</td>
<td>56</td>
<td>t(11;14)(q13;q32)</td>
<td>+13, +14, del(6q), mar(s), del(s)</td>
</tr>
<tr>
<td>Follicular cell lymphoma</td>
<td>18</td>
<td>t(14;18)(q32;q21)</td>
<td>+3, del(6q), del(13q), +12, random del(s)</td>
</tr>
<tr>
<td>Burkitt's Group</td>
<td>5</td>
<td>t(2;8)(q33;q41)</td>
<td>random changes, including structural changes of 1, 13q, +mar</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>t(8;14)(q24;q32)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>t(8;22)(q24;q11)</td>
<td></td>
</tr>
</tbody>
</table>

Key:

n = number in category

Burkitt's Group includes large cell anaplastic lymphoma

Additional changes, not seen in each patient: del = deletion(s), mar = marker(s)
Discussion

Chromosomal aberrations detected by cytogenetics can be of assistance in the diagnosis of mantle cell lymphoma, follicular cell lymphoma, and B-cell lymphomas [4]. Approximately 70% of the patients with confirmed follicular lymphomas have chromosomal gains or losses. These gains involve chromosome 1q, 7, 12, and 18. Losses also have been reported from chromosome 6q and 13 [5].

The FCL karyotype is characterized by t(14;18) [6,7]. Mantle cell lymphoma, although previously classified as “low-grade” category, has been found to have a poor prognosis, probably due to overexpression of cyclin D1, which is the protein product of the chimeric gene formed from the translocation of chromosome 11 with chromosome 14 [8]. Abnormal karyotypes have been identified in approximately 63% of patients with MCL. Of these, approximately 90% had the t(11,14) abnormality. The breakpoint on chromosome 11 is in the region of the bc11/PRAD1 oncogene locus and the one on 14 in the region of the immunoglobulin heavy chain gene. In the use of karyotypes in evaluating patients with lymphomas and acute lymphocytic leukemias, it is noted that patients that are hyperdiploid, (that is, having 49 or more chromosomes) have a better prognosis than those that are diploid or hypodiploid [9].

Previous reports indicate that Burkitt’s lymphoma has the t(8;14) in approximately 75 to 85% of cases. The remainder have either the t(2;8) or t(8;22) [10]. The observation that only 6 of 26 patients in this study had t(8;14) may be the result of selection of patients sent to a referral center or the inclusion of large cell anaplastic lymphomas in the group. There have been cases of Burkitt’s lymphoma seen in which none of the three common translocations are found.

The role of cytogenetics continues to expand in its usefulness for categorization, guidance of therapy, and prognosis of lymphomas.

Acknowledgments

The secretarial assistance of Ms. Andrea G. West is gratefully acknowledged. The cytogenetic technologists of the Cytogenetics Laboratory of The University of Texas M.D. Anderson Cancer Center are thanked for their professionalism and competence.

References