Chromosome Abnormalities in Leukemia and Lymphoma

JANET D. ROWLEY, M.D.

Department of Medicine, University of Chicago, Chicago, IL 60637

ABSTRACT

Nonrandom chromosome changes have been identified in a number of malignant human tumors. The leukemias are among the best studied malignant cells and they provide the largest body of relevant cytogenetic data. In chronic myeloid leukemia, a reasonably consistent translocation [t(9;22)(q34;q11)] is observed in 93 percent of all Ph1 positive patients. In the other patients, translocations are either two-way, involving No. 22 with some other chromosome or complex translocations involving Nos. 9 and 22 and another chromosome. In acute lymphoblastic leukemia, two translocations are each specifically associated with leukemic cells arrested at two different stages of maturation. One of these, t(8;21)(q22;q22), is found mainly in patients with acute myeloblastic leukemia with maturation (AML-M2). The other, t(15;17)(q22;q21?), is seen only in patients with acute promyelocytic leukemia (APL-M3). Various translocations have been observed in B-cell acute lymphoblastic leukemia or in Burkitt lymphoma. The most common is t(8;14)(q24;q32), but variants of this, namely t(2;8)(p13?q24) and t(8;22)(q24;q11), have also been observed; in all of these, the consistent change involves 8q24. The various immunoglobulin loci are located on chromosomes 2, 14, and 22 in the same chromosome band affected by the translocations in B-cell leukemia.

These translocations may occur randomly. If a specific translocation provides a particular cell type with a growth advantage, then selection could act to cause the proliferation of this aneuploid cell line vis-a-vis cells with a normal karyotype. In this view, the chromosome change could be the fundamental event leading to the leukemic transformation of an otherwise normal cell. The challenge for the future is to define the genes located at the sites of consistent translocations in myeloid leukemias and to determine the alterations in gene function that are associated with the translocation.

Introduction

The study of the chromosome pattern in the affected cells of a number of human tumors has been one of the most exciting areas in cancer research over the last 20 years. Major advances in our understanding of the specificity of some of the abnormalities have occurred in the last ten years with the application of new
chromosome banding techniques. These techniques allow the identification of each human chromosome and of parts of chromosomes as well. Thus, the hypothesis put forward by Boveri, that an abnormal chromosome pattern was intimately associated with the malignant phenotype of the tumor cell, can now be tested with substantial hope of obtaining a valid answer.6

The study of chromosome pattern in human leukemias can be divided into two ten-year periods, 1960 to 1970 and 1970 to 1980. During the first period, the chromosome abnormalities seen in leukemic cells were identified without banding. The most significant observation was the identification of the Philadelphia (Ph1) chromosome in leukemic cells from patients with chronic myelogenous leukemia. This abnormality, discovered by Nowell and Hungerford,23 appeared to be a deletion of about one-half of the long arm of one G group chromosome either No. 21 or No. 22. The search for similar abnormalities associated with other malignant hematologic diseases was disappointing. Leukemic cells in about one-half of the patients with acute leukemia appeared to have a normal karyotype.27,33 Thus the accepted notion was that the Ph1 was a unique example of a consistent karyotypic abnormality, and the general rule was one of marked variability in karyotype.

The evidence obtained during the second period showed that this notion was incorrect. With the use of banding techniques, other specific abnormalities were found to be associated with certain leukemias and lymphomas.27 Moreover, banding techniques revealed that the gains and losses of chromosomes were distinctly nonrandom. It should be emphasized that the data presented here have been gathered primarily during the period 1974 to 1982. Most of the studies during this period used chromosomes that were relatively contracted, and the banding pattern often was fuzzy and poorly defined. Thus, subtle abnormalities such as a deletion or a duplication of one-third of a chromosome band, involving about $3 \times 10^6$ nucleotide pairs, would be undetectable.

A third period of analysis is now being embarked upon that will be characterized by substantial improvements in the quality of the chromosome preparations that are available for analysis. Yunis et al38 have recently reported that with the use of elongated (prophase) chromosomes from bone marrow cells of patients with acute nonlymphocytic leukemia (ANLL) everyone of 24 patients had an abnormal karyotype. Thus, the future emphasis will be to identify the abnormalities that have been overlooked in the past.

**Methods and Nomenclature**

An analysis of chromosomal patterns, to be relevant to a malignant disease, must be based on a study of the karyotype of the tumor cells themselves. In the case of leukemia, the specimen is usually a bone marrow aspirate that is processed immediately or cultured for a short time.35 In patients with a white blood cell count higher than 15,500, with about 10 percent immature myeloid cells, a sample of peripheral blood can be cultured for 24 or 48 hours without adding phytohemagglutinin (PHA). The karyotype of the dividing cells will be similar to that obtained from the bone marrow. It is more difficult to obtain chromosomes from lymph nodes or spleen; however, careful attention to the details of tissue culture can provide mitotic cells in about 85 percent of the samples in our laboratory. Lymph node cells are usually processed directly or are cultured for only 24 to 48 hours before chromosome preparations are made.

When an abnormal karyotype is found in a tumor, it is important to analyze cells from normal tissues, such as skin fibroblasts or peripheral blood lymphocytes stimulated to divide with the addition of PHA. In most instances, cells from these
unaffected tissues will have a normal karyotype. The chromosome abnormalities observed in the tumor cells thus represent somatic mutations in an otherwise normal individual.

The observation of at least two “pseudodiploid” or hyperdiploid cells or three hypodiploid cells, each showing the same abnormality, is considered evidence for the presence of an abnormal clone; patients with such clones are classified as abnormal. Patients whose cells show no alterations, or in whom the alterations involve different chromosomes in different cells, are considered to be normal. Isolated changes may be due to technical artifacts or to random mitotic errors. In malignancies with a very low mitotic index, however, a single abnormal cell may be the only malignant cell undergoing mitosis.

In the following discussion, the chromosomes are identified according to the international system for human cytogenetic nomenclature (1978), and the karyotypes are expressed as recommended under this system. The total chromosome number is indicated first, followed by the sex chromosome, and then by the gains, losses, or rearrangements of the autosomes. A + sign or − sign before a number indicates a gain or loss, respectively, of a whole chromosome; a + or − after a number indicates a gain or loss of part of a chromosome. The letters “p” and “q” refer to the short and long arms of the chromosome, respectively. Translocations are identified by “t” followed by the chromosomes involved in the first set of brackets; the chromosome bands in which the breaks occurred are indicated in the second brackets. Uncertainty about the chromosome or band involved is signified by “?”.

**Chronic Myelogenous Leukemia**

**Chronic Phase**

Nowell and Hungerford reported the first consistent chromosome abnormality in human cancer. They observed an unusually small G-group chromosome, which appeared to have lost about one-half of its long arm, in leukemic cells from patients with chronic myelogenous leukemia (CML). Chromosome banding techniques were first used in the cytogenetic study of leukemia for identification of the Ph chromosome as a deletion of No. 22 (22q−). Since quinacrine fluorescence revealed that the chromosome present in triplicate in Down’s syndrome was No. 21, the abnormalities in Down’s syndrome and CML were shown to affect different pairs of chromosomes.

The question of the origin of the Ph (22q−) was answered when Rowley reported that the Ph chromosome results from an apparently balanced reciprocal translocation (9;22)(q34;q11) rather than a deletion as many investigators had previously assumed.

Karyotypes of 1129 Ph+ patients with CML have been examined with banding techniques by a number of investigators, and the 9;22 translocation has been identified in 1036 (92 percent). It is now recognized that, in addition to the typical t(9;22), variant translocations may occur. These appear to be of two kinds: one is a simple translocation involving No. 22 and some chromosome other than No. 9, which has been seen in 42 patients. The other is a complex translocation involving three or more different chromosomes; except in two cases, two of the chromosomes involved were found to be No. 9 and No. 22. This type of translocation has been observed in 46 patients. Five patients have been reported who are said not to have had a translocation. The great specificity of the translocation involving Nos. 9 and 22 remains an enigma. The survival curves for patients with variant translocations appeared to be the same as those for patients with the standard t(9;22).

**Acute Phase of Chronic Myelogenous Leukemia.** When patients with CML enter the terminal acute phase, about 20
percent appear to retain the 46, Ph1+ cell line unchanged, whereas other chromosome abnormalities are superimposed on the Ph1+ cell line in 80 percent of patients.26 In a number of cases, the change in the karyotype preceded the clinical signs of blast crisis by two to four months. In general, if patients have a clone of Ph1+ cells with a unique marker during the chronic phase, this clone will be the one involved in the transformation.

Bone marrow chromosomes from 379 patients with Ph1 + CML, who were in acute phase, have been analyzed with banding techniques.26 Seventy-six showed no change in their karyotype, whereas 303 patients had additional chromosome abnormalities. The most common changes frequently occur in combination to produce modal numbers of 47 to 52. The following gains or structural rearrangements of particular chromosomes were observed in 303 patients who had relatively complete analyses: gain of No. 8, 119 patients; gain of an isochromosome No. 17q, 79 patients; gain of No. 19, 45 patients; and gain of Ph1, 113 patients.

**Specific Translocations in Acute Nonlymphocytic Leukemia**

The recognition of nonrandom gains and losses of chromosomes (notably +8 and −7) was one of the important observations in the study of acute nonlymphocytic leukemia (ANLL) with the use of banding techniques. The identification of specific chromosome translocations associated with maturation arrest of granulocytes at a particular stage in differentiation remains a fascinating phenomenon, the significance of which is currently unknown.

**The 8;21 Translocation in Acute Myeloblastic Leukemia**

In 1968, Kamada et al12 recognized that a subgroup of ANLL patients may be characterized by an abnormality most likely representing a translocation between G- and a G-group chromosome. The exact nature of this abnormality was resolved by Rowley25 who determined that it was a balanced translocation between chromosomes 8 and 21 (t(8;21) (q22;q22)). The frequency with which this translocation occurs seems to vary but it is about ten percent of the abnormal cases of ANLL. The abnormality appears to be largely restricted to patients with a diagnosis of M2 (acute myeloblastic leukemia [AML] with maturation) according to the French-American-British (FAB) classification.26 At the Fourth International Workshop on Chromosomes in Leukemia,10 of cases with a t(8;21) and adequate bone marrow material available for cytological review, all except four had a diagnosis of M2; the exceptions appeared to be M4. The 8;21 translocation is also of interest for two other reasons. First, chromosomes 8 and 21 can participate in threeway rearrangements similar to those involving chromosomes 9 and 22 in CML. Lindgren and Rowley15 reported on two patients with three-way translocations in whom the third chromosome was either a No. 11 or a No. 17. Second, the t(8;21) is often accompanied by the loss of a sex chromosome; of the cases reviewed at the Fourth International Workshop on Chromosomes in Leukemia,10 85 percent of the males with the t(8;21) were −Y and 50 percent of the females were missing one X. This association is particularly noteworthy because sex chromosome abnormalities are otherwise rarely observed in ANLL.

**The 15;17 Translocation and Acute Promyelocytic Leukemia**

A structural rearrangement involving chromosomes 15 and 17 in acute promyelocytic leukemia (APL) was first recognized by Rowley et al.30 The breakpoint in No. 15 appears to be distal to band q21, and in No. 17 it appears to be in q21
[t(15;17)(q2200;q2100)(14). Of the 82 patients with APL who were reviewed at the Fourth Workshop,10 50 had a t(15;17) alone (37 cases) or with other abnormalities; four had other types of chromosome changes; and 28 had a normal karyotype. The rearrangement was not found in patients with any other type of leukemia. Two cases with complex translocations involving Nos. 15 and 17 and either No. 2 or No. 3 were recently reported.5 Thus, the same pattern of variation of a specific translocation can involve the t(15;17) as well as the t(9;22) and the t(8;21).

In some cases, the granules typically seen in the leukemic promyelocytes may be too small to be seen by light microscopy, although they are present when the cells are examined ultrastructurally.34 The FAB Co-operative Group recently recognized that not all APL patients have coarse granules and has thus added a category called the M3 variant.2 The variant category was identified largely on the basis of the clinical features and the presence of the t(15;17).

These translocations are unusual in that the incidence of both is much higher in children and in young adults than is true for other karyotypic abnormalities in ANLL. Thus, the median age for the t(8;21) is 45 years and for the t(15;17) is 25 years.14 Neither translocation has been reported in other types of human cancer. It is also of interest that the t(8;21) has never been reported as a constitutional abnormality and the t(15;17) has been reported only once.9 Whether or not the breakpoints in the constitutional translocation are similar to those seen in APL has not yet been established.

**Consistent Translocations in Burkitt Lymphoma and in B-Cell Acute Lymphocytic Leukemia.**

**Burkitt Lymphoma.**

Burkitt lymphoma is an excellent example of a disease in which continued study of samples has revealed a complexity in the karyotype which was not suspected on the basis of the initial reports. Fresh tumor tissue obtained from six patients with African Burkitt lymphoma was studied with quinacrine banding by Manolov and Manolova.17 They reported on the presence of an extra band at the end of the long arm of one chromosome No. 14 (14q+) in five of the six tumors, and in five of six cell lines established from tumors from other patients. Zech et al59 reported that the material at the end of No. 14 represented a translocation from the end of No. 8 [(t(8;14)(q24;q32)] in eight of the 10 African Burkitt tumors in which it could be scored; two other tumors had the 14q+ chromosome, but the fluorescence was inadequate and involvement of No. 8 could not be determined. These observations have since been confirmed by others. The t(8;14) is found in non-endemic as well as in tumors from endemic areas. Moreover, it is present in those that are Epstein-Barr virus (EBV) positive and those that are EBV negative.

Recently, Manolova et al18 examined more elongated chromosomes from five Burkitt lymphoma cell lines. They determined that the break in No. 8 is in the proximal pale portion of band 8q24; in No. 14, it is the distal part of 14q32 in every case.

The heterogeneity of the translocation in Burkitt lymphoma has only recently been recognized. There are now several patients with non-African Burkitt lymphoma whose cells show a translocation involving the short arm of No. 2 and the long arm of No. 8 [t(2;8)(p12;q24)].52,57 Patients have been reported who have a second variant translocation, namely t(8;22)(q24;q11).5 Thus, the consistent change in all three translocations is the involvement of band 8q24. The data are too preliminary to determine whether or not there are important differences in the biological behavior of the Burkitt cells with translocations other than t(8;14).
Although the variants were originally reported in non-African Burkitt lymphomas, subsequent study has detected the same variants in tumors of African origin.4

**B-Cell Acute Lymphocytic Leukemia**

An apparently identical 8;14 translocation has been observed in acute lymphocytic leukemia (ALL) patients with B-cell markers and in patients with L3-type leukemic cells,20 indicating that Burkitt lymphoma and most B-cell ALL of the L3 type are probably different manifestations of the same disease. Sixteen patients with this rearrangement were reported at the Third International Workshop on Chromosomes in Leukemia (1981).26 In one patient with this translocation, the leukemic cells had a pre-B-cell phenotype and were of the L1 type;13 the morphology of the leukemic cells, however, changed to L3-type at relapse. Recently, one of the same variant translocations that were described in Burkitt lymphoma has been identified in B-cell ALL. This was a t(2;8) found in ALL patient with the L3 type.32

**Karyotype in other Non-Hodgkin Lymphoma**

**Poorly Differentiated Lymphocytic Lymphoma**

Partial or complete data on the chromosome analysis are available for 38 patients with poorly differentiated lymphocytic lymphoma (PDL).29 Among the 38 patients, cytogenetic analysis was performed on a clearly involved specimen (lymph nodes or effusions) in 27; peripheral blood was studied in the remainder. With one exception, a chromosomally abnormal clone was obtained from specimens that contained malignant cells, i.e., lymph nodes or effusions. In more than one-half of the lymph nodes, 12 to 50 percent of the cells had a normal karyotype.

Complete karyotypes were available for only some of the 35 chromosomally abnormal patients, and the changes appeared to be somewhat variable; some changes, however, occurred more often than others, the predominant one being the 14q+ chromosome. A 14q+ chromosome was identified in 24 of the 35 patients in whom chromosomally abnormal cells were found. A translocation between No. 14 and No. 18 was the most common one noted, and recent data suggest that it may occur in more than one-half of all PDL patients.*

**Diffuse Histiocytic Lymphoma**

Chromosome analyses with the use of banding have been performed on 32 patients with diffuse histiocytic lymphoma (DHL).28 In most cases, the analysis was done on involved lymph nodes or tissue from extra-nodal sites, although cells from pleural or ascitic fluid or circulating cells in the leukemic phase were also used. Every patient had a chromosomally abnormal clone of cells; in a few patients, two different, but related clones, were observed.

The chromosome pattern in some patients was extremely complex, with 15 or more structurally rearranged chromosomes per cell. Under these circumstances, correct identification of all the breaks and rearrangements becomes very difficult. Some valid conclusions can nevertheless be reached at this time, based on data from 28 patients in whom the analysis was sufficiently complete to give fairly adequate information. The single most common abnormality was a translocation to the end of the long arm of No. 14, usually to band 14q32; this occurred in 15 patients. The donor chromosome involved in the translocation tended to be variable.

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* Rowley, unpublished data.
Implications of Nonrandom Changes for Malignant Transformation

The evidence presented demonstrates that nonrandom chromosome changes are closely associated with a variety of human hematologic disorders. Similar associations have been identified in other human tumors and in animal tumors as well.28 The changes consist of gains or losses of part or all of certain specific chromosomes and of structural abnormalities, most frequently relatively consistent translocations, that are presumed to be reciprocal. The nonrandom translocation observed by us in malignant cells would represent those that provide a particular cell type with a selective advantage vis-a-vis in the cells with a normal karyotype. There is very strong evidence that many malignancies, CML and Burkitt lymphoma, for example, are of clonal origin. This means that a particular translocation in a single cell gives rise to the tumor or to the leukemia that ultimately overwhelms the host. Other rearrangements may be neutral, and the cells, therefore, will survive but will not proliferate differentially; still others may be lethal and thus would be eliminated. In such a model, the chromosome change is fundamental to malignant transformation.

The new data regarding the location of certain genes provides clues as to the nature of the genes that are affected by translocations. This evidence is most clear for the various translocations seen in Burkitt lymphoma and B-cell ALL. The most common translocation is t(8;14), but two variants, t(2;8) and t(8;22) are also observed. It is certainly significant that the gene for the immunoglobulin heavy chain is located on chromosome 14,7 while the gene for the lambda light chain is on No. 22,8 and for the kappa light chain is on 2p.16,19 Thus, each one of the various different chromosomes that is involved in a translocation with No. 8 carries a gene that would be of great importance in the normal function of a B-cell. What the role of 8q is in modifying this function so that the cell becomes a malignant B-cell is presently unknown. It seems almost certain, however, that the involvement in these translocations of the chromosomes carrying various immunoglobulin genes is not fortuitous.

The main stumbling block to making the same kinds of correlations for the consistent translocations seen in myeloid cells is our lack of understanding of which of the biologic markers currently under investigation has an analogous functional role in myeloid cells. It may be possible to work backward in myeloid cells by first defining the genes that are present at the sites of specific translocations and then determining the changes in gene function observed in myeloid cells with and without the translocation.

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References


