

Chromosome Abnormalities in Leukemia

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A. Introduction

The renewed interest in the study of chromosome abnormalities in hematologic malignancies, particularly in the leukemias, is the result of technical improvements which permit the precise identification of each human chromosome, and of parts of chromosomes as well. The information obtained raises a number of questions regarding the validity of older notions, such as the variability of the chromosome pattern (karyotype) in acute leukemia, or the rarity of associations of specific chromosome abnormalities with particular types of leukemia. One of the surprising observations of the last few years has been the frequent occurrence of consistent translocations in a variety of hematologic malignancies. The challenging questions at present are how and why nonrandom changes, particularly consistent translocations, occur.

B. Methods

An analysis of chromosome patterns in malignancy must be based on a study of the karyotype of the tumor tissue itself. In the case of leukemia, the specimen is usually a bone marrow aspirate that is processed immediately or is cultured for a short time [29]. Cells in metaphase from a 24-hour culture of peripheral blood will have a karyotype similar to that of cells obtained from the bone marrow. The chromosome analysis may be performed by means of one of several pretreatments prior to staining with Giemsa [34], or the slides can be stained with quinacrine mustard for fluorescence, as previously described [3,29]. The chromosomes are identified according to the Paris Nomenclature [22], and the karyotypes are expressed as recommended under this system.

C. Chronic Myelogenous Leukemia

I. Chronic Phase

Nowell and Hungerford [20] reported the first consistent chromosome abnormality in a human cancer; they observed an unusually small G-group chromosome, called the Philadelphia (Ph¹) chromosome, in leukemic cells from patients with chronic myelogenous leukemia (CML). Bone marrow cells from approximately 85% of patients who have clinically typical CML

contain the Ph¹ chromosome (Ph¹+) [38]. Chromosomes obtained from PHA-stimulated lymphocytes of patients with Ph¹+ CML usually are normal.

Chromosome banding techniques were first used in the cytogenetic study of leukemia for identification of the Ph¹ chromosome. Caspersson et al. [2] and O'Riordan et al. [21] reported independently that the Ph¹ chromosome was a No. 22q-. The question of the nature of this chromosome was answered in 1973, when Rowley [24] reported that it represents a translocation, rather than a deletion as many investigators had previously assumed. The first report in 1973 presented data on nine Ph¹ patients, in all of whom there was additional dully fluorescing chromosome material at the end of the long arm of one No. 9 (9q+). The amount and staining characteristics of this material were similar to those of the distal portion of the long arm of No. 22. The abnormality in CML is, therefore, an apparently balanced reciprocal translocation, t(9;22)(q34;q11). Measurements of the DNA content of the affected pairs (9 and 22) have shown that the amount of DNA added to No. 9 is equal to that missing from the Ph¹ [14]; thus there is no detectable loss of DNA in this chromosome rearrangement.

The karyotypes of 569 Ph¹+ patients with CML have been examined with banding techniques by a number of investigators, and the 9; 22 translocation has been identified in 529 cases (94%) (reviewed in Rowley [27]). Unusual or complex translocations were identified in 40 patients, in 17 of whom the translocation involved No. 22 and one of several other chromosomes. In two patients, the translocated material could not be detected and was presumed to be missing. Twenty-one cases have also been reported in which the rearrangement involved three or more chromosomes; in all of these cases, with one exception [13], two of the chromosomes were Nos. 9 and 22 with breaks in the usual bands.

The great specificity of the translocation involving Nos. 9 and 22 remains an enigma. At present, patients with a variant translocation appear not to differ clinically from those with the usual Ph¹ [32].

II. Acute Phase

When patients with CML enter the terminal acute phase, about 20% appear to retain the 46, Ph¹+ cell line unchanged, whereas other chromosome abnormalities are superimposed on the Ph¹+ cell line in 80% of patients [27,28]. In a number of cases, the change in the karyotype preceded the clinical signs of blast crisis by 2-4 months.

Bone marrow chromosomes from 178 patients with Ph¹+ CML, who were in the acute phase, have been analyzed with banding techniques [27,28].

Thirty-five showed no change in their karyotype, whereas 143 patients had additional chromosome abnormalities. The most frequent gains or structural rearrangements of particular chromosomes observed in 136 patients who underwent relatively complete analyses are summarized in Table 1. These changes frequently occur in combination to produce modal numbers of 47 to 52.

Table 1. The most frequent chromosome changes determined with banding in 136 Ph¹-positive patients in the acute phase of CML

Type of chromosome change	Number of chromosome						
	8	9	10	17	19	21	Ph ¹
	Number of patients						
Gain	53	8	11	8	24	11	48
Loss				5	1		2
Rearrangement ^a	3	4	3	36 ^b	2	1	4 ^c
Total	56	12	14	49	27	12	54

^a Includes translocations, deletions, inversions, and ring chromosomes.

^b Thirty of these were said to be an isochromosome for the long arm, i(17q)

^c All of these were said to be a dicentric Ph¹.

The single most common change in the acute phase of CML is the addition of a second Ph¹ chromosome. Prior to the use of banding, the most commonly observed abnormality was an additional C-group chromosome; of 64 patients whose cells contained additional C's, 53 had an additional 8.

The i(17)q, which was observed in 30 patients, appears to be the second most common structural rearrangement, after the 9;22 translocation. It was the only abnormality in addition to (9;22) in 16 cases, whereas in 14 it was associated with an extra C, identified as No. 8 in every patient. Fifty-one other structural rearrangements, such as balanced reciprocal translocations, deletions, and unidentified additions to chromosomes, were identified in combination with i(17)q and the dicentric Ph¹. In 13 cases, a second balanced reciprocal translocation (separate from the 9;22 translocation) was the only change noted in the acute phase as compared with the karyotype in the chronic phase. With one exception, the additional F noted in 25 cases was a No. 19; it was never seen as the only new abnormality in the acute phase of CML.

III. Identity of Ph¹-positive Cells

The identity of the cells that contain the Ph¹ chromosome has recently become a topic of considerable interest. This problem has at least two facets; one concerns the nature of the blast cells in the acute phase of CML and the other, the proper classification of patients with Ph¹+ acute leukemia.

In regard to the first aspect, Boggs [1] noted that the blast cells in some patients in the acute phase of CML appeared to be lymphoid rather than myeloid, and that some patients in the acute phase achieved a remission with vincristine and prednisone, which were usually effective primarily in lymphoid leukemias. Several laboratories are currently examining the surface markers of cells from patients in the acute phase of CML; unfortunately, the cytogenetic analyses are frequently not done with banding techniques, and often the karyotype is obtained only from the initial sample. Since Whang-Peng et al. [39] have identified the Ph¹ chromosome in two of four Ph¹+ ALL patients as a 21q-, banding is essential.

In regard to the second question, of 13 patients [26] with Ph¹+ ALL who had a 22q – chromosome identified with banding, six had a translocation of 22q to 9q34; two others had variant translocations, one to 14q and one to 21q. The presence of a translocation was not determined for the other five. Ten of the 13 patients were studied a second time; two of these had no remission and continued to have an abnormal karyotype. The remaining eight patients achieved a remission and had a normal karyotype in cells from the bone marrow or from unstimulated peripheral blood. It remains to be determined whether it is logical, or correct, to classify all Ph¹+ leukemias as CML, or whether we are dealing with two different diseases.

D. Acute Nonlymphocytic Leukemia (ANLL)

I. Nonrandom Patterns

Little information is available regarding the chromosome pattern determined with banding in acute lymphocytic leukemia; therefore this section includes the data available for ANLL only. Cells from approximately 216 patients with ANLL have been analyzed with banding; 113 patients (51%) had a chromosome abnormality, which was identified precisely in 100 [26,36]. The chromosome gains, losses, and rearrangements are summarized in Fig. 1. There is evidence that some portion of the apparent chromosome variability is related to evolution of the karyotype in ANLL. In an attempt to distinguish primary from secondary events, we have indicated the aberrations noted in 90 patients who had minimal changes, i.e., modal chromosome numbers of 45–47, in the shaded area of the figure. Although a gain of No. 8 and a loss of No. 7 are the most frequent changes in either case, other aberrations, such as a gain of Nos. 1, 6, or 7, are seen only in patients with higher modal numbers.

In some patients, it is possible to follow the development of other chromosome changes in the course of serial analyses of bone marrow samples. In a series of 90 patients with ANLL [35], 17 showed a change in their karyotype as the disease progressed. In 11 patients, this involved the gain of a chromosome, which was a No. 8 in nine cases. Thus, an additional No. 8 is a common occurrence both in the evolution of ANLL and in CML in the acute phase.

Two structural rearrangements are sufficiently important to merit special mention. The first occurs in acute myeloblastic leukemia (AML) and is seen in about 10% of all patients with aneuploidy. Prior to banding, it was described as – C, + D, + E, – G (37); Rowley [25] showed that this is a translocation, presumably reciprocal, involving Nos. 8 and 21, t(8;21) (q22;q22). This translocation is unique in that its presence is frequently associated with the loss of a sex chromosome, an X in females (33%) and the Y in males (59%); such loss is otherwise a rare occurrence.

The other consistent rearrangement has been identified only recently (30) as a 15;17 translocation, t(15;17) (q25;q22), in acute promyelocytic

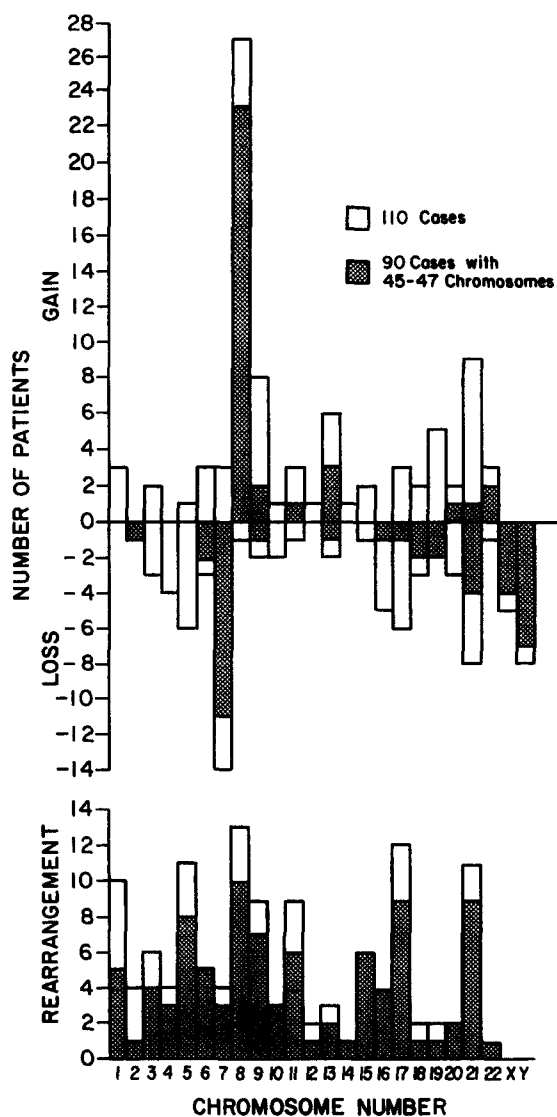


Fig. 1. Diagram of chromosome changes seen in 110 patients with ANLL; 45 patients were studied in my laboratory. The changes in 90 patients with modal chromosome numbers of 45-47 are indicated in the shaded portion.

leukemia (APL). Our first two patients with APL were found to have a deleted 17q [10]. Metaphase chromosomes from a third patient had clearer bands, and a structural rearrangement involving No. 15 as well as No. 17 was noted. Nine of 17 patients with APL included in data from the Workshop on Chromosomes in Leukemia had a 15; 17 translocation [7].

II. Clinical Significance of Chromosome Abnormalities

About 50% of patients with ANLL are found to have a normal karyotype even with the use of banding techniques. Sakurai and Sandberg [31] were the first to note (prior to banding) that the presence, in the initial bone sample, of even one cell with a normal karyotype was associated with a substantially better prognosis. In our first series of 50 patients studied with banding [11], particularly among those with acute myeloblastic leukemia (AML) who had a normal karyotype, 85% achieved a complete remission (median survival 18 months), compared with 25% of those with only abnormal cells (median survival 2.5 months). The difference in survival for patients with acute myelomonocytic leukemia (AMMoL) was not signif-

icantly related to the karyotype. In an enlarged series of 90 patients, we noted the same relationships [12]. These observations have also been confirmed by Nilsson et al. [19] and by data correlated by the Workshop on Chromosomes in Leukemia [7].

The significance of these findings is not clear. It may be that leukemic cells with a normal karyotype have not yet evolved to the same state of malignancy, and that patients with normal cells therefore have a better prognosis. Alternatively, it may be that the mechanism associated with leukemogenesis in cells with a normal karyotype is different and does not require chromosome changes for the malignant transformation. These patients may, therefore, represent a different etiologic category in which the cells could be more readily reversible or more sensitive to chemotherapy. In any event, hematologists might consider whether chemotherapeutic protocols should be specifically tailored to patients with normal karyotypes, whereas a different protocol may be appropriate for patients who have only chromosomally abnormal cells.

E. The Production of Consistent Translocations

The mechanism for the production of specific, consistent reciprocal translocations is unknown. Possibly, specific translocations are the result of cell selection. In such a model, chromosome breaks and rearrangements occur continuously at a low frequency. Many of these rearrangements do not lead to changes in cell metabolism, and the cells therefore do not proliferate preferentially; other rearrangements may be lethal to the cells. Still others provide the cell with a proliferative advantage, and cells with these changes not only persist, but eventually become the predominant cell type. In such a model, the chromosome change is the fundamental, initial event that leads to the neoplastic nature of the cell.

Other possible explanations depend on either [1] chromosome proximity, since translocations may occur more frequently when two chromosomes are close together, or [2] regions of homologous DNA that might pair preferentially and then be involved in rearrangements. The fact that many of the affected chromosomes, e.g., Nos. 1, 9, 13, 14, 15, 21, and 22, are involved in nucleolar organization supports these proposals.

On the other hand, proximity of homologous DNA sequences should lead to an increased frequency of these rearrangements in patients with constitutional abnormalities, but this has not been observed. It is possible that either or both of these mechanisms are subject to selection; a translocation might occur because the chromosomes are close together, but only certain specific rearrangements may have a proliferative advantage which results in leukemia and thus leads to their detection.

Another genetic mechanism that may account for consistent chromosome changes is related to transposable elements, called controlling elements in maize [6, 15] and insertion sequences in bacteria [4]. Transposable elements have been detected in every organism in which the genetic structure is known

with reasonable precision. In maize, for example, there are at least three distinct controlling elements, each with its own characteristics and with different chromosome locations that influence the production of anthocyanin pigment in each kernel of an ear of corn [6, 15]. Similar genetic systems that modify the action of host genes may be present in mammalian cells. If so, these transposable elements may play a role in malignant transformation. The following features of transposable elements are relevant to the "how" and "why" of consistent translocations: 1. Change in location within the DNA, 2. the transferring of adjacent DNA in this change, and 3. the alteration of the normal mechanism for genetic regulation, depending on the site and orientation of the inserted sequences. These properties, plus a selective system for removal of changes that do not have a proliferative advantage in hematologic cells, are just those required to explain consistent translocations occurring as somatic mutations.

F. The Role of Nonrandom Changes

There is good cytological [8] and biochemical [5] evidence that, in an individual patient with chronic myelogenous leukemia or Burkitt lymphoma, the tumor cells have a clonal origin. In CML, initially only a single cell has the 9;22 translocation, and when the patient comes to the physician, frequently all cells in division contain the Ph¹ chromosome. It is necessary to examine the kinds of genetic mechanisms that can provide the cell containing the 9;22 translocation with this proliferative advantage.

Two points that should be emphasized are the genetic heterogeneity of the human population and the variety of cells involved in malignancy. There is convincing evidence from animal experiments that the genetic constitution of an inbred strain of rats or mice plays a critical role in the frequency and type of malignancies that develop [23,33]. We are much more aware now than formerly of certain genes in man that predispose to cancer, such as the genes for Bloom syndrome, Fanconi anemia, and ataxia-telangiectasia [9]. We are completely ignorant of the number of gene loci in man which, in some way, control resistance or susceptibility to a particular malignancy.

The second factor affecting the karyotypic pattern relates to the different cells that are at risk of becoming malignant, and the varying states of maturation of these cells. There is good evidence that the same chromosomes may be affected in a variety of tumors; No. 8 is a good example [18]. On the other hand, some chromosomes seem to be involved in neoplasia involving a particular tissue; the involvement of No. 14 in lymphoid malignancies is an example.

When one considers the number of nonrandom changes that are seen in a single malignancy such as ANLL, it is clear that not just one gene, but rather a class of genes is involved. Our knowledge of the human gene map [17] has developed concurrently with our understanding of chromosome changes in leukemia. It is now possible to try to correlate the affected chro-

mosomes with the genes that they carry. Clearly, these efforts must be very tentative, since relatively few genes have been mapped, and since some of the chromosomes that are most frequently abnormal have few genetic markers.

Preliminary data suggest that chromosomes which carry genes related to nucleic acid biosynthesis may frequently be abnormal in hematologic malignancies. Moreover, specific chromosome regions associated with these genes may also be involved. Thus, the most frequent abnormalities of No. 17 result either in an isochromosome for the long arm or in a translocation with No. 15 in which the break in No. 17 is in band 17q22. This region of No. 17 contains genes for thymidine kinase, galactokinase, and a site that is particularly vulnerable to AD-12-induced breakage [16]. Furthermore, induction of host cell thymidine kinase and a high frequency of breaks in 17q22 are early functions of this virus, as is the synthesis of a tumor antigen which may play a role in the control of DNA synthesis.

Thus it is possible that nonrandom chromosome aberrations, when they occur, change the level of some enzymes related to nucleic acid metabolism, either through a change in location or through duplication of gene loci. Nonrandom chromosome changes, particularly consistent, specific translocations, now seem clearly to be an important component in the proliferative advantage gained by the mutant cell in neoplasia. The challenge is to decipher the meaning of these changes.

G. Summary

The consistent occurrence of nonrandom chromosome changes in human malignancies suggests that they are not trivial epiphenomena. Whereas we do not understand their significance at present, one possible role which they may fulfill is to provide the chromosomally aberrant cells with a proliferative advantage as the result of alteration in the number or location of genes related to nucleic acid biosynthesis. The proliferative advantage provided by various chromosome aberrations is likely to differ in patients with different genetic constitutions.

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