Quantification by DNA-based Cytophotometry of the 9q+/22q− Chromosomal Translocation Associated with Chronic Myelogenous Leukemia

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SUMMARY

DNA-based cytophotometry was used to analyze metaphase chromosomes in four patients with chronic myelogenous leukemia. In three of these patients, both Philadelphia chromosome (Ph')-positive and Ph'-negative cells were measured. On the basis of these three patients, the characteristic 9q+/22q− translocation of chronic myelogenous leukemia involves the net transfer of 0.325% of the autosomal genome; there is no evidence of net gain or loss of DNA (apart from duplication of the Ph' chromosome in one patient), and no significant difference is found in the amount of DNA transferred in different patients. Significant differences are found among patients in the derived Chromosome 9 and the Ph' chromosomes and are ascribed to preexisting variations in the Ph'-negative cells of these patients. There is no evidence in these patients of any further cytogenetic lesion associated with chronic myelogenous leukemia.

INTRODUCTION

CML shows the most consistent lesion of all the cytogenetic changes associated with malignant diseases. In 1961, Nowell and Hungerford (11) analyzed the karyotypes of leukemic cells from patients with CML and found that one of the G-group chromosomes had been replaced by a very small and characteristic acrocentric chromosome, the Ph' chromosome. They were not certain whether this chromosome resulted from deletion or translocation of the distal part of the long arm of one of the G-group chromosomes. Subsequently, the Ph' chromosome has been found in the karyotype of about 85% of all reported cases of CML (13). Cytophotometric analysis with UV absorption indicated that the Ph' chromosome had only 60% of the DNA content of the other chromosomes (16).

In 1973, Rowley (12) used the Q-banding method (1) to demonstrate that the Ph' chromosome of CML was associated with an apparent translocation, 9q+/22q−, in which there was a transfer of chromatin from the distal part of the long arm of a Chromosome 22 to the distal part of the long arm of a Chromosome 9. This translocation, 9q+/22q−, is found in about 90% of all Ph'-positive CML cases; other cases show variant translocations or more complex rearrangements (14).

The 9q+/22q− translocation of CML has been analyzed by DNA-based cytophotometry. In 1974, we reported measurements on a single case (5). These indicated that the translocation resulted in no net loss of DNA from the genome. The sensitivity of the measurements was such that any net loss involving more than 6% of Chromosome 22 (i.e., 6 × 10^-18 g of DNA or about 6 × 10^4 nucleotide pairs) would have been detected. The measurements on this initial case have now been analyzed more extensively by using the normal cells of the patient as an internal control, and the series has been extended to include 3 more cases. In 2 of these additional cases, we also were able to compare measurements on leukemic cells with normal cells from the same individual. The present data confirm and extend our initial findings.

MATERIALS AND METHODS

Patient Summary. Patient A is a 24-year-old white female studied previously (5). The diagnosis of CML was made on April 21, 1973. Tissue biopsies were taken on April 26, 1973. The patient was not on therapy during this period. Ph'-positive cells were prepared from a 24-hr bone marrow culture, and Ph'-negative cells were prepared from a 72-hr phytohemagglutinin-stimulated peripheral blood culture. A total of 11 Ph'-positive cells and 12 Ph'-negative cells were analyzed for chromosomal DNA content. The patient died on April 19, 1974.

Patient B is a 59-year-old black male. The diagnosis of CML was made on September 18, 1971. Peripheral blood for this study was taken on April 25, 1974. Therapy during this interval included treatment with busulfan. Chromosome preparations were made from nonstimulated 24-hr peripheral blood cultures. A total of 5 Ph'-positive cells and 5 Ph'-negative cells were analyzed for chromosomal DNA content. The patient died on July 25, 1974.

Patient C is an 18-year-old black male. The diagnosis of CML was made during July 1974. A bone marrow biopsy was taken in December 1974. The patient had been on
busulfan therapy, which was discontinued 1 month before the biopsy. Chromosome preparations were made from a 24-hr bone marrow culture. A total of 7 Ph'-positive and 6 Ph'-negative cells were analyzed for chromosomal DNA content. The patient is still under observation.

Patient D is a 31-year-old white male. The diagnosis of CML was made during December 1973. A bone marrow biopsy and peripheral blood were obtained on January 28, 1974. The patient was not on therapy during this period. Chromosome preparations for cytophotometric analysis were made from a 24-hr bone marrow culture. A total of 7 Ph'-positive cells were analyzed for chromosomal DNA content. No Ph'-negative cells were found that were suitable for analysis. The present status of the patient is unknown.

Culture and Slide Preparation. Peripheral blood was cultured according to the method of Moorhead et al. (10) in either McCoy's Medium 5A or minimal essential medium. Bone marrow biopsies were cultured as described (15). After the appropriate culture intervals the cells were harvested, swollen with 0.075 M hypotonic potassium chloride, fixed in absolute methanol:glacial acetic acid (3:1), dropped onto slides, and air-dried.

DNA-based Cytophotometry. Chromosomes from well-spread metaphase cells were preidentified with quinacrine banding, and each cell was karyotyped by 3 investigators to ensure accurate chromosome classification. Subsequent analysis followed our usual protocol (6). The quinacrine-stained preparations were destained, treated with RNase (150 μg/ml) for 3 hr at 37°C, and stained for 3 days with galbocyanin chrome alum, pH 1.64 [conditions that are specific and stoichiometric for DNA (3)], and mounted in oil (refractive index = 1.556).

The galbocyanin chrome alum-stained preparations were scanned and digitized with CYDAC, a flying-spot-scanning cytophotometer (7). This system operates at the limit of optical resolution; sample and line separation are 0.25 μm, absorbance is digitized in increments of 0.002 on a 256-level scale, and a focus-assist device ensures optimum focus (2). Each scan is 192 x 192 elements, and 2 or 3 overlapping scans are required for each cell.

Extensive computer processing is used in the analysis of the digitized images. A global threshold is chosen to generate a binary map of potential chromosomes. This map is displayed on a color television monitor and is edited interactively. Overlapping chromosomes are ignored, touching chromosomes are separated, fragmented chromosomes are joined, and karyotype information is entered. Subsequent processing sums all the absorbance values associated with each chromosome and corrects these by subtracting the local background value. The chromosome values are combined among overlapping scans for each cell and then are normalized on a cell-by-cell basis. Normalization involves a maximum likelihood procedure in which a normalization factor is obtained from measurements of normal chromosomes but is applied to all chromosomes (9). Normalization expresses chromosome values on a relative scale where 100 units is the sum of the values of all 44 autosomal chromosomes. Thus 1 unit corresponds to about 120 x 10^-15 g of DNA or 1.2 x 10^6 nucleotide pairs.

The sensitivity of this approach depends on the number of cells measured and the variation of the measurements among cells. The former is a function of chromosomal size; typically, the standard deviation of the measurements averages 4.2% of the chromosomal value. When 10 cells are measured, effects involving as little as 0.05 unit can be detected.

RESULTS

The normalized measurements of both normal and abnormal Chromosomes 9 and 22 are given in Table 1 for Patients A, B, and C. In the Ph'-positive cells, normal and abnormal Chromosomes 9 and 22 were readily identified and so could be measured independently. In the Ph'-negative cells, the homologous chromosomes could not be separately identified, and the tabulated values represent the mean measures for the homolog pairs. These means show substantial differences among cases. In order to minimize the effects of such DNA heteromorphisms, the values for the Ph'-negative cells are used as the basis for evaluation of the Ph'-positive cells. The expected value is the best estimate of the relative DNA content of the affected chromosomes before the translocation. It is calculated by subtracting the value for the normal homolog in the Ph'-positive cells from twice the mean in

| Table 1 |
| Normalized DNA-based measurements of Chromosomes 9 and 22 in Ph'-positive and Ph'-negative cells of 3 individuals |

| Sum of autosomal chromosomes is 100 units. |

<table>
<thead>
<tr>
<th>Ph'-negative cells</th>
<th>Ph'-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 9</td>
<td>Chromosome 22</td>
</tr>
<tr>
<td>Patient</td>
<td>9</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>A</td>
<td>2.48</td>
</tr>
<tr>
<td>(24)c</td>
<td>(25)</td>
</tr>
<tr>
<td>B</td>
<td>2.41</td>
</tr>
<tr>
<td>(9)</td>
<td>(9)</td>
</tr>
<tr>
<td>C</td>
<td>2.25</td>
</tr>
<tr>
<td>(9)</td>
<td>(12)</td>
</tr>
</tbody>
</table>

a Values expected for the affected homologs if they were not involved in the translocation.

b Differences between the means of the affected chromosomes and their expected normal means.

c Numbers in parentheses, number of measurements on which the means are based.

d Entries rounded to 2 decimals may lead to apparent inconsistencies.
Ph'-negative cells. The net change due to the translocation is equal to the difference between the mean of the affected chromosome and the expected value.

In Patient B, the Ph'-positive cells all contain 47 chromosomes, 2 of which are Ph' chromosomes. These pairs of Ph' chromosomes were compared, with the use of the homolog test of Moore (8). There is no evidence that the 2 Ph' chromosomes came from different distributions (\(p > 0.50\)), so their measurements were pooled for the subsequent analyses.

Consider first the measurement of the Ph' chromosomes. The mean values are 0.49, 0.54, and 0.57 unit for Patients A, B, and C, respectively. The variation among individuals is statistically significant (\(p = 0.006\)), suggesting that not all Ph' chromosomes are the same size. The estimated net losses are 0.35, 0.34, and 0.31 unit for Patients A, B, and C, respectively. For these 3 cases, the estimated mean loss is 0.34 unit with a standard error of the estimated mean of 0.03 unit. The variation among the net losses is not statistically significant. Thus the net losses show much less variation among individuals than do the Ph' chromosomes.

The mean values of the derived Chromosomes 9 are 2.77, 2.84, and 2.52 units for Patients A, B, and C, respectively. The variation among individuals is statistically significant (\(p < 0.005\)), suggesting that not all derived Chromosomes 9 are the same size. The estimated net gains due to the translocation are 0.30, 0.35, and 0.29 unit for Patients A, B, and C, respectively. The estimated mean gain is 0.31 unit, with a standard error of the estimated mean of 0.06 unit. The variation among the net gains is not statistically significant. Thus the net gains also show much less variation among individuals than do the derived Chromosomes 9.

When the net losses from Chromosomes 22 are compared with the net gains for Chromosomes 9, the differences are not significant on an individual basis. When the data from all cases are pooled, the estimated mean loss from Chromosome 22 is 0.335 unit, and the estimated mean gain for Chromosome 9 is 0.315 unit; these pooled values also do not differ significantly from one another. Thus the estimated overall effect of the translocation is a transfer of 0.325 unit.

For each of these 3 cases, measurements of all chromosomes in Ph'-positive and Ph'-negative cells were compared in order to test for possible further cytogenetic lesions associated with CML. Apart from Chromosomes 9 and 22, significant differences were found in Chromosomes 14 and 15 of Patient A (\(p = 0.005\) and \(p = 0.031\)), in Chromosome 4 of Patient B (\(p = 0.014\)), and in Chromosome 17 and 19 of Patient C (\(p = 0.005\) and \(p = 0.046\)). These comparisons required a total of 71 t tests; thus the 5 significant differences found at \(p \leq 0.05\) are not much more than the 3.6 differences expected by chance. Of the 2 differences with \(p = 0.005\), the measurements of Chromosome 14 in Patient A do not differ significantly from our standard normal data set (4) which is based on measurements from 10 phenotypically normal and healthy persons. The difference between measurements of Chromosome 17 in Patient C results from an abnormally low mean (1.41 units compared to a standard of 1.47 units) in the Ph'-negative cells.

Measurements also were made on chromosomes from a 4th case (Patient D). We were unable to obtain any Ph'-negative cells from this patient. The measurements for the Ph'-positive cells are given in Table 2. In this table, values for Ph'-positive cells are compared to our standard normal data set (4). Because of possible DNA heteromorphisms, there is much more uncertainty in these estimated differences than in the estimated differences given in Table 1; thus detailed analysis is unwarranted. However, the measured 9q+/ and 22q− values are almost identical with the measurements for Patient B given in Table 1.

As we have already reported (5), both Ph'-positive and Ph'-negative cells for Patient A show a presumptive 9q+/− translocation. In this case, there is evidence of a difference between measurements of the homologous Chromosomes 20. Comparison with the standard value indicates that the difference is due to a deficiency in 1 Chromosome 20 of 0.12 unit relative to the standard. There also is evidence of a difference between measurements of the homologous Chromosomes 9 in the Ph'-negative cells. Comparison with the standard value indicates the homolog difference is due to an excess in 1 Chromosome 9 of 0.11 unit relative to the standard. The estimated value for this large homolog is similar to the value of the unaffected Chromosome 9 in the Ph'-positive cells.

In Patients B, C, and D, the measurements also were tested for homolog differences and compared with the standard values. No evidence is seen for the 9q+/20q− translocation in these other cases, nor is there evidence that suggests any other translocation.

Table 2

<table>
<thead>
<tr>
<th>Sum of autosomal chromosomes is 100 units</th>
<th>Ph'-positive cells</th>
<th>Ph'-negative cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard values</td>
<td>Chromosome 9</td>
<td>Chromosome 22</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>2.37</td>
<td>0.89</td>
<td>2.28 (6)c</td>
</tr>
</tbody>
</table>

* Values expected for the affected homologs if they were not involved in the translocation and if the means of the unaffected homologs were the same as the standard karyotype.

* Differences between the means of the affected chromosomes and their expected normal means.

* Numbers in parentheses, number of measurements on which the means are based.
DISCUSSION

We have applied DNA-based cytophotometry to chromosomes from patients with CML and have demonstrated its utility as an adjunct to conventional techniques in the quantification and analysis of the characteristic cytogenetic lesion of this disease.

Measurements on 3 patients demonstrate that: (a) the lesion of CML in these patients involves the 9q+/22q− translocation with a net transfer of 0.325 unit (39 × 10⁻¹⁸ g of DNA). The net transfer does not differ significantly among the patients studied to date. In no case is there evidence of a significant overall change in cellular DNA content, except for duplication of the Ph1 chromosome in Patient B. Our measurements indicate that the net loss, if any, due to the translocation is less than 0.08 unit (10 × 10⁻¹⁸ g of DNA). If the loss had exceeded this it would have been detected (p > 0.95, 1-sided t test) by this analysis. (b) There are significant differences among individuals in the values of both the derived Chromosomes 9 and the Ph1 chromosomes. These differences are attributed to preexisting DNA heteromorphisms proximal to the lesion and present in these chromosomes in the nonleukemic (Ph1-negative) cells rather than to variation in the net amount of material transferred in the 9q+/22q− translocation in the leukemic (Ph1-positive) cells. (c) Nonleukemic (Ph1-negative) cells from these leukemic patients show variable patterns of DNA heteromorphisms, but in general these are not significantly different from those found in normal persons. Thus there is no evidence of a prodromal or independent cytogenetic lesion in the normal cells of these CML patients.

The relationship between the 9q+/22q− translocation and the phenotype of CML is remarkable. The cytogenetic lesion may result from the highly specific action of some etiological agent that induces the unique translocation. Alternatively, selection pressures may enhance the expression of this translocation over all other possible translocations. Regardless of the mechanism, it appears that the cytogenetic rearrangement characteristic of CML modifies the phenotype of the affected cells in that they escape partially from the normal controls to granulocyte proliferation, thus leading to the clinical manifestations of the disease. The lesion must be subtle in that many of the characteristics of these cells are unimpaired. It is tempting to speculate that the translocation results in positional modification of gene loci that control cell proliferation and growth and that CML is a lesion of genetic expression rather than of genetic loss. Validation of this speculation is beyond the resolution of current techniques.

REFERENCES

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