Genome-Wide Single Nucleotide Polymorphism Analysis Reveals Frequent Partial Uniparental Disomy Due to Somatic Recombination in Acute Myeloid Leukemias

Manoj Raghavan, Debra M. Lillington, Spyros Skoulakis, Silvana Debernardi, Tracy Chaplin, Nicola J. Foot, T. Andrew Lister, and Bryan D. Young

Cancer Research UK Medical Oncology Laboratory, Barts and the Royal London School of Medicine and Dentistry, Queen Mary, University of London, London, United Kingdom.

Abstract

Genome-wide analysis of single nucleotide polymorphisms in 64 acute myeloid leukemias has revealed that ∼20% exhibited large regions of homozygosity that could not be accounted for by visible chromosomal abnormalities in the karyotype. Further analysis confirmed that these patterns were due to partial uniparental disomy (UPD). Remission bone marrow was available from five patients showing UPD in their leukemias, and in all cases the homozygosity was found to be restricted to the leukemic clone. Two examples of UPD11p were shown to be of different parental origin as indicated by the methylation pattern of the H19 gene. Furthermore, a previously identified homozygous mutation in the CEBPA gene coincided with a large-scale UPD on chromosome 19. These cryptic chromosomal abnormalities, which seem to be nonrandom, have the characteristics of somatic recombination events and may define an important new subclass of leukemia. (Cancer Res 2005; 65(2): 375-8)

Introduction

A large proportion of acute myeloid leukemia (AML) has either a normal karyotype or nonrecurrent chromosomal abnormalities and the underlying pathogenesis remains obscure. The introduction of array-based analysis of single nucleotide polymorphism (SNP) allows the rapid determination of genome-wide allelic information at high density for a DNA sample (1,2). This approach has been used to search for loss of heterozygosity in cancers (3–6) and in the karyotype. The SNP signal values were calculated within the homozygous region. A DNA probe for the region of homozygosity, indicating a normal copy number for chromosome 11q results in a decrease in the ratio of heterozygous-homozygous calls but there was no significant reduction in signal ratio values for the region of homozygosity, indicating a normal copy number for chromosome 11. By contrast, the deletion of 7q, dic(7)(q22) in the same AML, resulted in leukemia-remission signal ratio values of ∼0.5 in the homozygous region. A DNA probe for the MLL gene (11q23), confirmed the presence of two copies of 11q23 in leukemic metaphase and interphase cells from the same patient (Fig. 1B). It was therefore concluded that these regions of homozygosity represented somatically acquired loss of heterozygosity, due to the presence of partial uniparental disomy (UPD).

Results and Discussion

A high-resolution genotype analysis was done on DNA from 64 presentation AML samples. Using the 10K SNP array (Affymetrix; ref. 7), a mean call rate of 93.3% resulted in >10,000 SNP genotype calls per sample. Large unexpected regions of homozygosity were observed in 12 (18.75%) AMLs (Table 1). These regions ranged from 16 million to 113 million bp and were not apparent as aberrations in the karyotype. The SNP signal values were calculated within the regions of homozygosity as described (5,10). In every example, the SNP signal values were indicative of two copies (Table 1). The presence of two copies was confirmed by fluorescence in situ hybridization in the cases analyzed. DNA from five remission bone marrow samples (available for patients 1,3,6,7, and 10 in Table 1) was subjected to SNP genotype analysis. The SNP call data (not shown) showed clearly that the homozygosity seen in the leukemic DNA was not present in the respective remission bone marrow DNA. All of the SNP calls in the homozygous regions were concordant with the equivalent calls in the remission bone marrow DNA. A comparison between leukemic DNA and remission DNA for patient 3 is presented in Fig. 1A for relevant chromosomes. The large region of homozygosity on chromosome 11q results in a decrease in the ratio of heterozygous-homozygous calls but there was no significant reduction in signal ratio values for the region of homozygosity, indicating a normal copy number for chromosome 11. By contrast, the deletion of 7q, dic(7)(q22) in the same AML, resulted in leukemia-remission signal ratio values of ∼0.5 in the homozygous region. A DNA probe for the MLL gene (11q23), confirmed the presence of two copies of 11q23 in leukemic metaphase and interphase cells from the same patient (Fig. 1B). It was therefore concluded that these regions of homozygosity represented somatically acquired loss of heterozygosity, due to the presence of partial uniparental disomy (UPD).
Table 1. The 12 leukemias exhibiting UPD are listed below with their respective karyotypes

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>FAB classification</th>
<th>Karyotype</th>
<th>Chromosomal Region of UPD</th>
<th>Signal value ratio</th>
<th>Centromeric heterozygous SNP Base range</th>
<th>FISH copy no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (M1)</td>
<td>46 XY[20]</td>
<td>11p13-11pter</td>
<td></td>
<td>0.92</td>
<td>SNP_A-1509863 1-34938966</td>
<td>ND</td>
</tr>
<tr>
<td>2 (M2)</td>
<td>46 XX[20]</td>
<td>11p11-11pter</td>
<td></td>
<td>0.88</td>
<td>SNP_A-1511180 1-52461565</td>
<td>ND</td>
</tr>
<tr>
<td>3 (M1)</td>
<td>46,XX,dic(7;22)</td>
<td>11q12-11pter</td>
<td></td>
<td>0.85</td>
<td>SNP_A-1512785 65274905-137400000</td>
<td>11q23(MLL×2)[100]</td>
</tr>
<tr>
<td>4 (M1)</td>
<td>46 XX[20]</td>
<td>11q13-11pter</td>
<td></td>
<td>0.80</td>
<td>SNP_A-1512811 66987057-137400000</td>
<td>Failed</td>
</tr>
<tr>
<td>5 (M4)</td>
<td>46,XX[20]</td>
<td>11p11-11pter</td>
<td></td>
<td>0.85</td>
<td>SNP_A-1508814-2581306-50853201</td>
<td>ND</td>
</tr>
<tr>
<td>6 (M4)</td>
<td>48,XY,−3,−10[10]</td>
<td>6p21-6pter</td>
<td></td>
<td>0.90</td>
<td>SNP_A-1518080 1-422272</td>
<td>ND</td>
</tr>
<tr>
<td>7 (M1)</td>
<td>46,XX[20]</td>
<td>6p11-6pter</td>
<td></td>
<td>1.09</td>
<td>SNP_A-1507946 1-57365515</td>
<td>ND</td>
</tr>
<tr>
<td>8 (M3)</td>
<td>46,XY(15q17)</td>
<td>9q33-9pter</td>
<td></td>
<td>1.05</td>
<td>SNP_A-1516948 116551991-132400000</td>
<td>9q34(ABL×2)[100]</td>
</tr>
<tr>
<td>9 (M5)</td>
<td>46,XY,der(12)(q11)</td>
<td>9pcen-9pter</td>
<td></td>
<td>0.88</td>
<td>SNP_A-1517078 1-43264784</td>
<td>9p21(p16×2)[100]</td>
</tr>
<tr>
<td>10 (M1)</td>
<td>46,XY[25]</td>
<td>19q12-19pter</td>
<td></td>
<td>0.94</td>
<td>SNP_A-1515117 28798570-60000000</td>
<td>19q13(CEBPAX2)[100]</td>
</tr>
<tr>
<td>11 (M1)</td>
<td>46,XX[20]</td>
<td>13</td>
<td></td>
<td>0.80</td>
<td>Whole Chr 13 1-113400000</td>
<td>Failed</td>
</tr>
<tr>
<td>12 (M2)</td>
<td>46,XX[20]</td>
<td>21q21.1</td>
<td></td>
<td>1.07</td>
<td>SNP_A-1509329 20420648-44464366</td>
<td>ND</td>
</tr>
</tbody>
</table>

NOTE: In patient 8, the presence of the PML-RARα gene fusion was confirmed by FISH in the majority of interphase cells. In total, 64 diagnostic acute myeloid leukemia bone marrow or peripherally taken blood samples from the frozen tissue bank at St. Bartholomew’s Hospital were analysed using the 10K SNP array that encodes approximately 11,500 SNP loci (Affymetrix; ref. 7). For each SNP on the chip, there are 20 probe pairs, 10 for allele A, and 10 for allele B. A probe pair contains a perfect match and a mismatch. The signal value is calculated as the average intensity difference between a perfect match and a mismatch for all probe pairs and is given by 1/20, where PMi is the intensity of perfect match of pair i and MMi is the intensity of mismatch for the same pair. The signal value ratio is the ratio of mean signal within UPD region divided by mean signal outside, for the same chromosome. For patient 11, we have calculated the ratio of mean signal of chromosome 13 to the mean of the mean signal of all other chromosomes. All the statistical analysis is done with R.

Abbreviation: FISH, fluorescence in situ hybridization.

Figure 1. Analysis of UPD in AML 3. A. SNP array analysis was performed on remission bone marrow of patient 3 and compared with the genotype of the leukemia of the same patient. The ratio of the number of heterozygous to homozygous calls was calculated in a running window of 20 Mbp. The loss of heterozygosity score (black) is calculated by dividing the above ratio in the diagnosis sample by the same ratio in the remission sample from the same patient. The signal score (red) is calculated as the ratio of the mean signal (Table 1), in a running window of 20 Mbp, between diagnosis and remission samples. Results for analysis of chromosome 11 (top) and chromosome 7 (bottom) in patient 3. B, fluorescence in situ hybridization of a probe for the MLL gene on 11q23 shows two copies in leukemic interphase cells.
There seems to be a nonrandom distribution of these events with three examples of UPD11p and two examples each of UPD11q and UPD6p (Fig. 2). In 10 of the 12 examples, the region of loss of heterozygosity continued to the telomere implying that this phenomenon is due to somatic recombination. It was also apparent that the points of recombination are clearly different in all examples. In one example, the entire chromosome 13 was homozygous (patient 11) suggesting either a nondisjunction event followed by chromosomal duplication or a recombination close to the centromere.

The expression of imprinted genes in the homozygous regions would be profoundly affected. Congenital disorders associated with UPD have been linked to a single parental origin (e.g., Beckwith-Weidemann syndrome and paternal UPD of 11p15; ref. 11), suggesting the parental pattern of methylation is important to the pathogenesis of such disorders. Any parental bias in the UPD in AML would indicate a role for imprinted genes. The H19 gene, located at 11p15, is normally methylated only on the paternal allele (9). Two leukemias exhibited UPD including 11p15 and the methylation status of the H19 gene was therefore determined by bisulfite sequencing. One leukemia with UPD11p (patient 1) exhibited a homozygous methylated paternal H19 genes, whereas the other example of UPD11p (patient 2) showed homozygous nonmethylated maternal pattern (Fig. 3). A control leukemia with heterozygous SNP calls for 11p showed a mixed methylated and nonmethylated pattern (Fig. 3). Hence, the homozygosity on 11p is not restricted to a single parental origin. In a previous analysis (12), one of the leukemias studied here (leukemia 10 in Table 1) was homozygous for a CEBPA mutation and fluorescence in situ hybridization showed two copies of the CEBPA gene. This gene is located at 19q13.1, within the area of UPD and we conclude that the mutation occurred before the UPD. An important consequence of UPD could be to unmask preexisting mutations.

Microsatellite markers have shown UPD in other clonal hematologic disorders such as polycythaemia vera (13) and...
childhood acute leukemia (14). A study of childhood acute lymphoblastic leukemia has shown the consistent loss of maternal alleles on 9p without evidence of cytogenetic deletions in the same region (15). In solid tumors, UPD has been observed in breast cancer (16), uveal melanoma (17), Wilms’ tumors (18), retinoblastoma (19), and in tumors associated with Beckwith-Weidemann syndrome (11). A recent study of solid tumor cell lines using SNP genotyping has suggested that somatic recombination may be playing a role because regions of homozygosity were observed which corresponded to normal copy number values (10). In the general population, UPD is a rare occurrence (20).

The discovery of somatically acquired UPD in leukemias has potentially important clinical implications. In this study, 20% of the normal karyotype AMLs were found to have UPD, and this could offer a valuable new approach to the classification of this important subgroup of AML. The prognostic consequences of UPD for the patient are uncertain, and larger studies will be required to assess the clinical significance of this phenomenon.

Acknowledgments

Received 10/12/2004; revised 11/15/2004; accepted 11/18/2004.

Grant support: Kay Kendall Leukaemia Fund, Barts and Royal London Charitable Foundation, and Barts Foundation for Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank John Amess for phenotype analysis of leukemias.

References


Genome-Wide Single Nucleotide Polymorphism Analysis Reveals Frequent Partial Uniparental Disomy Due to Somatic Recombination in Acute Myeloid Leukemias

Manoj Raghavan, Debra M. Lillington, Spyros Skoulakis, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/65/2/375

Cited articles  This article cites 19 articles, 9 of which you can access for free at: http://cancerres.aacrjournals.org/content/65/2/375.full.html#ref-list-1

Citing articles  This article has been cited by 58 HighWire-hosted articles. Access the articles at: /content/65/2/375.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.