Association between Acquired Uniparental Disomy and Homozygous Gene Mutation in Acute Myeloid Leukemias

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Abstract

Genome-wide single nucleotide polymorphism analysis has revealed large-scale cryptic regions of acquired homozygosity in the form of segmental uniparental disomy in ~20% of acute myeloid leukemias. We have investigated whether such regions, which are the consequence of mitotic recombination, contain homozygous mutations in genes known to be mutational targets in leukemia. In 7 of 13 cases with uniparental disomy, we identified concurrent homozygous mutations at four distinct loci (WT1, FLT3, CEBPA, and RUNX1). This implies that mutation precedes mitotic recombination which acts as a "second hit" responsible for removal of the remaining wild-type allele, as has recently been shown for the JAK2 gene in myeloproliferative disorders. (Cancer Res 2005; 65(20): 9152-4)

Introduction

Recently, a novel form of abnormality in acute myeloid leukemia (AML), uniparental disomy, has been revealed through the application of single nucleotide polymorphism (SNP) genotype array technology (1). Uniparental disomy, which cannot be detected by conventional cytogenetic methods, was observed in ~20% of the AMLs studied. The affected regions are evident as large stretches of somatically acquired homozygosity, usually continuing from a certain point on the chromosome to the telomere and have the hallmarks of mitotic recombination, because there is no net change in copy number in the affected region. A consequence of a mitotic recombination event is the generation of two daughter cells, each with the reciprocal chromosomal products. The homozygosity observed in AML is due to the outgrowth of one of these daughter cells. Because this phenomenon has only recently been described, the nature of the selective advantage to the cell, gained by uniparental disomy, has been uncertain. However, it was noted, first, that the distribution of affected chromosomal regions seemed nonrandom, and second, that some of the regions of uniparental disomy included genes known to be mutated in a proportion of AMLs studied. The potential relationship between acquired uniparental disomy and gene mutation was therefore investigated.

Materials and Methods

10K GeneChip assay. DNA was extracted using standard phenol-chloroform techniques or from the organic phase of TRIzol (Invitrogen, Carlsbad, CA). DNA probes were prepared using the GeneChip mapping assay protocol (Affymetrix, Inc., Santa Clara, CA) with the modification that PCR products were purified using the Ultrafree-MC filtration column (Millipore Corp., Billerica, MA). Signal intensity data was analyzed by the GeneChip DNA analysis software, which uses a model algorithm to generate SNP calls.

Mutation analysis. Bone marrow samples were collected and analyzed in accordance with the requirements of the Local Research Ethics Committee. Cases were classified according to the French-American-British system and subsequently according to WHO. DNA was extracted using standard phenol-chloroform methodologies. Primers and precise amplification conditions were derived either from previously published studies or cDNA/genomic comparison and are available on request. Mutation screening was done for the entire coding region (CCND3, CDKN2A, CDKN2B, PUD1, and CEBPA) or specific exons [WT1 (7-10), FLT3 (exons 14-15 and 20), RUNX1 (exons 3-5), and MLL (PTD)]. PCR products were sequenced directly or cloned by TOPO TA cloning (Invitrogen Ltd., Paisley, United Kingdom), plasmid DNA extracted and sequenced by use of an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, CA). For direct sequencing, unincorporated primer was removed by ultrafiltration using a Centricon YM-100,000 filter device (Millipore). Sequencing data were analyzed using DNASTAR, Inc. (Madison, WI).

Results and Discussion

SNP genotype arrays were used to analyze DNA samples from a series of AMLs. As described previously (1), such an analysis can detect the presence of acquired homozygosity and by evaluation of the signal values can determine whether it is due to mitotic recombination or deletion. To investigate this phenomenon further, we selected a total of 13 primary AMLs with uniparental disomy encompassing regions known to harbor genes potentially mutated in AML (Table 1). These included previously described and new cases of uniparental disomy which were confirmed by analysis of the SNP signal values and the karyotype.

Sequence analysis was done on nine genes in the appropriate leukemias (Table 1). Mutations were detected at four distinct loci (WT1, FLT3, CEBPA, and RUNX1) in 7 of the 13 leukemias examined. In all cases, sequence analysis indicated a homozygous mutation with no evidence of the wild-type sequence. In Fig. 1, the sequence traces showing a homozygous insertional mutation of the WT1 gene are illustrated along with the homozygosity map for chromosome 11. Similarly, a homozygous single-base insertion of the WT1 gene is shown with the concurrent homozygosity of 21q (Fig. 1). It was notable that uniparental disomy of 13q was associated with homozygous internal tandem duplication mutation of FLT3 (FLT3-ITD) in four separate cases.

The presence of a mutation and the absence of the wild-type sequence has previously been reported in AML: WT1 (3, CEBPA (4), and RUNX1 (5, 6). Such a situation could arise by several mechanisms, including the deletion of wild-type allele to yield a hemizygous state, amplification of the mutant allele, or mitotic...
recombination. Without accurate copy number information, it has been difficult to distinguish between these possibilities. The high degree of association between uniparental disomy and homozygous mutation presented here suggests that uniparental disomy may account for a substantial proportion of cases exhibiting a degree of association between uniparental disomy and homozygous mutation. However, there may be other targets in UPD6p, UPD9p, and UPD13q, respectively.

FLT3-ITD mutation in the heterozygous state is a recognized poor prognostic risk factor for AML. Additionally, the loss of the wild-type FLT3 allele in leukemias with a FLT3 mutation in ~10% of patients with normal karyotype AML is associated with a worse outcome (7). Hence, the association between loss of the wild-type FLT3 allele and UPD13q could suggest that the presence of uniparental disomy may be linked to clinical outcome. The close proximity of FLT3 to the centromere and the small number of SNP markers proximal to FLT3 on chromosome 13 means that in some cases, we cannot exclude the possibility of mechanisms other than mitotic recombination leading to uniparental disomy of chromosome 13. This could include nondisjunction followed by chromosome duplication. Mitotic recombination is clearly the case for WT1, RUNX1, and CEBPA mutation (Fig. 1) and suggests that mutation precedes mitotic recombination that acts to remove a normal copy of a gene and replace it with a mutated copy.

Before the introduction of SNP genotyping arrays, only a limited number of microsatellite markers could be used to detect the occurrence of mitotic recombination and the resultant uniparental disomy in tumors. In this manner, uniparental disomy has previously been shown on chromosome 9p in polycythemia vera (8). Recently, several groups have shown that a frequent mutational target in myeloproliferative disorders such as polycythemia vera is the JAK2 gene, located within 9p (9–12). Loss of heterozygosity at 9p is frequently observed in association with mutations to the JAK2 gene and has been shown to be due to mitotic recombination (9). Thus, mitotic recombination is playing a role in both myeloproliferative disorders and in AMLs, although clearly different mutational targets are involved.

In solid tumors, uniparental disomy has been observed in breast cancer (13), uveal melanoma (14), Wilms’ tumors (15), and retinoblastoma (16). In addition, the background rate of mitotic recombination has been measured (17) in normal human and mouse cells to be ~10^-4 to 10^-5. Thus, given the widespread nature of mitotic recombination, it is therefore very probable that the acquisition of uniparental disomy has a similar role in other tumors. In this context, it is interesting that a recent SNP array study has shown that acquired uniparental disomy of chromosome 9q is common in basal cell carcinomas (18).

Our study indicates that mitotic recombination and the resultant uniparental disomy has a role in rendering a leukemic cell homozgyous for a preexisting mutation. However, there may be other potentially important consequences. Uniparental disomy will, in principle, lead to alterations in expression levels of imprinted genes. In addition, it has recently been shown that there are allelic differences in gene expression levels (19) and therefore, if these are maintained after mitotic recombination, uniparental disomy could be associated with specific gene expression patterns.

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### Table 1. Thirteen examples of uniparental disomy were investigated for mutation

<table>
<thead>
<tr>
<th>Patient</th>
<th>FAB</th>
<th>Karyotype</th>
<th>Uniparental disomy</th>
<th>Gene</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M1</td>
<td>46,XY</td>
<td>11p13-11pter</td>
<td>WT1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>M1</td>
<td>46,XY</td>
<td>19q12-19qter</td>
<td>CEBPA</td>
<td>970insA (RUNX1)</td>
</tr>
<tr>
<td>3</td>
<td>M2</td>
<td>46,XY</td>
<td>21q21-21qter</td>
<td>RUNX1</td>
<td>1038ins57bp (CEBPA)</td>
</tr>
<tr>
<td>4</td>
<td>M1</td>
<td>46,XX</td>
<td>13q</td>
<td>FLT3</td>
<td>ITD 21bp (FLT3)</td>
</tr>
<tr>
<td>5</td>
<td>M2</td>
<td>46,XY (t(6;9)</td>
<td>13q</td>
<td>FLT3</td>
<td>ITD 63bp (FLT3)</td>
</tr>
<tr>
<td>6</td>
<td>M4</td>
<td>46,XX</td>
<td>13q</td>
<td>FLT3</td>
<td>ITD 36bp/39bp* (FLT3)</td>
</tr>
<tr>
<td>7</td>
<td>M1</td>
<td>46,XX</td>
<td>13q</td>
<td>FLT3</td>
<td>ITD 108bp (FLT3)</td>
</tr>
<tr>
<td>8</td>
<td>M2</td>
<td>46 XX</td>
<td>11p11-11pter</td>
<td>WT1</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>M4</td>
<td>46 XX</td>
<td>11p11-11p14</td>
<td>WT1</td>
<td>None</td>
</tr>
<tr>
<td>10</td>
<td>M1</td>
<td>46,XX, dic(7;22)(q11.2q10), +8[10]</td>
<td>11q12-11qter</td>
<td>MLL</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>M4</td>
<td>48,XY;+3,+10[10]</td>
<td>6p21-6pter</td>
<td>CCND3</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>M1</td>
<td>46 XX</td>
<td>6p11-6pter</td>
<td>CCND3</td>
<td>None</td>
</tr>
</tbody>
</table>

**NOTE:** Mutational analysis was done on the following genes/exons: CCND3, CDKN2A, CDKN2B, PU.1, and CEBPA (entire coding region; ref. 2); WT1 (exons 7–10); FLT3 (exons 14–15); RUNX1 (exons 3–5); and MLL (PTD). Numbering of mutations is according to the following Genbank accession nos.: NM_024426 (WT1), Y11525 (CEBPA), and NM_001754 (RUNX1).

*Clonal evolution of one FLT3-ITD mutation was observed in patient 6 following uniparental disomy resulting in the addition of 3 bp (ITD = 39bp).
Figure 1. Display of uniparental disomy on 11p and 21q in two AML samples. Heterozygous calls (red) and homozygous calls (blue). The corresponding sequencing traces showing 1584ins16bp WT1 and 970insA RUNX1 homozygous mutations. WT1 and RUNX1 duplicated segments (underlined in orange).

References
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