Frequent Deletion of hSNF5/INI1, a Component of the SWI/SNF Complex, in Chronic Myeloid Leukemia

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Abstract

During routine two-fusion fluorescence in situ hybridization analysis of patients with blast crisis of chronic myeloid leukemia (CML), we observed that yeast artificial chromosome 29GD7, which is distal to BCR at 22q11, failed to hybridize to the 9q+ derivative chromosome in 3 of 11 (27%) cases. This deleted region is close to hSNF5/INI1 (SMARC1), a gene that encodes a widely expressed component of the SWI/SNF chromatin remodeling complex and that suffers biallelic mutations in malignant rhabdoid tumors. To determine whether hSNF5/INI1 was also deleted in patients with CML, we performed fluorescence in situ hybridization analysis with a specific cosmids probe. Deletion of hSNF5/INI1 on the 9q+ chromosome was found in 9 of 25 (36%) cases in blast crisis (lymphoid, n = 3; myeloid, n = 6). For the three of these nine patients for whom material was available prior to transformation, deletions were also seen in chronic phase, indicating that they are early events. Analysis of an additional 21 patients in chronic phase revealed heterozygous loss of hSNF5/INI1 in 5 (24%) cases. Of the 14 patients who had hSNF5/INI1 deletions, 7 showed a mosaic pattern of hybridization in which only a proportion of CML cells that harbored both the t(9;22) derivative chromosomes had a deletion, indicating that loss of hSNF5/INI1 was acquired during the course of the disease. Single-strand conformation polymorphism analysis of all nine hSNF5/INI1 exons and splice junctions failed to reveal any mutations for 31 patients in transformation, including 8 who had deletions, although two polymorphisms were identified. We conclude that deletions of hSNF5/INI1 are frequent in patients with CML. Such deletions may be associated with reduced levels of hSNF5/INI1 expression, which could contribute to leukemogenesis by altering chromatin-mediated transcriptional control. Alternatively, the deletions could target another unidentified gene at 22q11 that plays a role in the pathogenesis of CML.

Introduction

CML is characterized in ~95% of cases by the presence of the chimeric BCR-ABL fusion gene, usually visualized cytogenetically as t(9;22)(q34;q11). The clinical course of CML is generally triphasic, with presentation in chronic phase followed, in the absence of effective treatment, by progression though an ill-defined accelerated phase to blast crisis. Although the BCR-ABL fusion gene plays a central role in the pathogenesis of CML, it is unclear whether this chimeric product alone is sufficient to establish chronic phase or whether other cooperating genetic events are also required (1, 2). Furthermore, the additional genetic changes that are presumed to be required for progression of CML to blast crisis are incompletely understood.

Elucidation of these changes is important because transformation is associated with a very poor prognosis.

Currently, the only consistent changes known to take place during progression of CML are mutations of the p53 gene in 20–30% of cases in myeloid transformation (3, 4), the absence of detectable Rb protein in most or all cases in megakaryoblastic transformation (5), and homozygous deletions of the p16INK4a gene in up to 50% of cases in lymphoid transformation (6, 7). A proportion of cases in myeloid blast crisis show overexpression of the EVII gene (8), occasionally in the form of an AML1-EVII fusion (9). Furthermore, up to 50% of patients in either lymphoid or myeloid blast crisis show loss of heterozygosity at 1p36 suggesting the presence of a tumor suppressor gene that may be inactivated during disease progression (10). Loss of imprinting at the Igf2 locus (11) and methylation of the ABL 1a promoter (12) are seen in the majority of patients in advanced phases, although the significance of these findings is unclear.

Here, we demonstrate that the region of 22q11 downstream of BCR is frequently and extensively deleted on the 9q+ derivative chromosome in a subset of patients with CML. The recently described tumor suppressor hSNF5/INI1 (13) localizes to this region, but we were unable to identify any point mutations in this gene. Although it is possible that haploinsufficiency of hSNF5/INI1 may play a role in the pathogenesis of CML, it is perhaps more likely that these deletions target another gene at 22q11.

Materials and Methods

Patient Material. A total of 72 patients with CML were analyzed (chronic phase, n = 24; accelerated phase or blast crisis, n = 48). Of these 72 patients, 41 (chronic phase, n = 24; accelerated phase or blast crisis, n = 17) were tested by FISH analysis alone, 23 (all in accelerated phase or blast crisis) were tested by SSCP analysis alone, and 8 (all in accelerated phase or blast crisis) were tested by both techniques. Of the 49 patients studied by FISH, 42 had 100% or nearly 100% metaphases showing both the Philadelphia (Ph) and 9q+ derivative chromosomes. The remaining 7 patients had complex rearrangements with all derivative chromosomes clearly visible. As controls, DNA samples were obtained from 25 individuals who had no evidence of malignancy.

FISH. Two-fusion FISH for BCR-ABL was performed as described previously (14). An hSNF5/INI1 cosmide clone (118d7) was isolated from a chromosome 22-specific library (LL22NC03; obtained from the MRC Human Genome Resource Center, Hinxton, United Kingdom) by screening with a specific cDNA probe amplified by reverse transcriptase-PCR from peripheral blood leukocyte cDNA. PCR with exon-specific primers showed that this clone contained the whole of the hSNF5/INI1 gene. In some cases, FISH results were confirmed with 77A2, an hSNF5/INI1 cosmide clone kindly provided by Dr. O. Delattre (Institut Curie, Paris, France). Cosmid DNA was isolated from bulk cultures using standard procedures. The relative positions of these clones are shown in Fig. 1. Probes were labeled with biotin by nick translation, tested on metaphases from phytohemagglutinin-stimulated peripheral blood lymphocytes from a normal individual, and subsequently hybridized to patient metaphases as described previously (15). Hybridization signals were detected using FITC-labeled avidin (Vector, Peterborough, United Kingdom). To confirm the presence of the 9q+ and/or Ph derivative chromosomes, we also cohybridized some metaphases with a chromosome 22-painting probe (Cam-

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The abbreviations used are: CML, chronic myeloid leukemia; FISH, fluorescence in situ hybridization; SSCP, single-strand conformation polymorphism; YAC, yeast artificial chromosome.
bridge Biotech, Cambridge, United Kingdom), two-color BCR-ABL probes, or the AS3 probe (Vysis, Richmond, United Kingdom). The AS3 probe is upstream of ABL and, therefore, normally hybridizes to the normal chromosome 9 and the 9q+ derivative in CML cells (16). Chromosomes were counterstained with 4',6-diamidino-2-phenylindole/antifade (Biovation, Aberdeen, United Kingdom) and examined using an Olympus Vanox microscope. Images were captured using a charged coupled device camera and SmartCapture Software (Vysis). Whenever possible, at least 20 metaphases were analyzed.

SSCP Analysis. Primers were designed to amplify individual hSNF5/INI1 exons from genomic DNA, including donor and acceptor splice sites. PCR was performed for 30 cycles at 97°C for 20 s, 60°C for 50 s, and 73°C for 60 s. Amplified fragments were labeled by reamplification and sequencing.

Results

Deletions 3' of BCR Detected by Two-Fusion FISH. We have previously described a two-color BCR-ABL FISH technique that uses four YAC clones to detect both the Ph and 9q+ derivative chromosomes in patients with CML (14). During routine screening of CML patients in transformation, we noted that YAC 29GD7 failed to hybridize to the 9q+ chromosome in 11 of 25 (44%) cases, indicating that most or all of this region had been deleted (Fig. 2, A and B). YAC 29GD7 is telomeric of BCR and contains the markers D22S131 and D22S301 (18). This region is known to be close to hSNF5/INI1, a component of the SWI/SNF complex that has recently shown to suffer biallelic mutations in malignant rhabdoid tumors (13).

Frequent Deletion of hSNF5/INI1 in CML. To determine whether hSNF5/INI1 was also deleted, we performed FISH analysis on a larger series of patients with a cosmids (118d7) that contained the entire hSNF5/INI1 gene. As expected, on normal metaphases, this clone hybridized to 22q11 only. However, for patients with CML in blast crisis, this cosmids hybridized to the normal chromosome 22 but failed to hybridize to the 9q+ chromosome in 9 of 25 (36%) of cases (Fig. 2, C and D). Of the 11 patients tested with both YAC 29GD7 and cosmids 118d7, 3 patients were deleted for both probes and 8 patients retained both probes. In some cases, the deletions were confirmed by use of a second hSNF5/INI1 cosmids clone. In deleted cases, the 9q+ chromosome was clearly visible by microscopic analysis after enhancement of the 4',6-diamidino-2-phenylindole banding pattern, but its presence was confirmed in some cases by cohybridization with either a chromosome 22-painting probe or the AS3 probe, which is upstream of ABL and, therefore, hybridizes to the normal chromosome 9 and the 9q+ derivative (18). Deletions were seen in both cases with lymphoid (n = 3) or myeloid transformation (n = 6).

For three of the nine blast crisis patients with deletions, material was available for analysis prior to transformation. In each of these 3 cases, deletions were also seen in chronic phase 14–19 months prior to the diagnosis of blast crisis, indicating that loss of hSNF5/INI1 occurs relatively early in the course of the disease. FISH analysis was also performed on an additional 21 cases of CML in chronic phase. Of these, deletion of hSNF5/INI1 on the 9q+ chromosome was observed in five (24%) cases. FISH results are summarized in Table 2.

Of the 14 patients who had hSNF5/INI1 deletions, 7 (chronic phase, n = 4; blast crisis, n = 3) showed a mosaic pattern of hybridization. In these patients, a proportion (6–97%; median, 36%) of CML cells that harbored both the 9q+ and 22q− derivative chromosomes had a deletion of hSNF5/INI1, whereas the remaining metaphases retained both alleles (Fig. 2, E and F). This indicates that the deletions, at least in some cases, are acquired during the course of the disease.

Mutational Analysis of hSNF5/INI1. To determine whether the hSNF5/INI1 gene was the target of the deletions, we performed SSCP analysis for all nine exons of this gene, including the donor and acceptor splice junctions. Of 31 blast crisis patients analyzed, 8 of whom were found to have a heterozygous hSNF5/INI1 deletion by FISH analysis, apparently identical bandshifts were noted for in 4 patients for exon 7 and in 10 patients for exon 9 (Fig. 3). Sequencing of the aberrantly migrating bands for exon 7 revealed an identical silent change at Ser-299 (position 966, G→A; GenBank accession no. U04837) in all four cases. This change creates a novel DdeI recognition site. The second change resulted from a G→A change in exon 8, 41 bp upstream from exon 9. This change did not alter a restriction enzyme recognition site but was seen in all four aberrantly migrating bands that were sequenced. The finding of identical changes in different patients suggested that they were probably polymorphisms of no pathogenic significance. To test this, we analyzed DNA from 25 control individuals by PCR amplification and digestion with DdeI.

Table 1 Primers used for amplification of individual hSNF5/INI1 exons

<table>
<thead>
<tr>
<th>Primer sequence</th>
<th>Primer number</th>
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<tbody>
<tr>
<td>5'-TTTCGCATTCTCGGCTTCGCTGTT-3'</td>
<td>Exon 1 (forward, outer)</td>
</tr>
<tr>
<td>5'-AAAAATTTCGCTCGGCTTCGCTGTT-3'</td>
<td>Exon 1 (reverse, inner)</td>
</tr>
<tr>
<td>5'-GGCGGGCATTCTCGGCTTCGCTGTT-3'</td>
<td>Exon 2 (inner, reverse)</td>
</tr>
<tr>
<td>5'-TCATTTGCTCTCGGCTTCGCTGTT-3'</td>
<td>Exon 3 (forward, inner)</td>
</tr>
<tr>
<td>5'-TCAGTATGGCCCTCGGCTTCGCTGTT-3'</td>
<td>Exon 4 (forward, inner)</td>
</tr>
<tr>
<td>5'-TTACGTGAGGGGTCGCTTCGCTGTT-3'</td>
<td>Exon 5 (forward, inner)</td>
</tr>
<tr>
<td>5'-TTTGACGATGGGTCGCTTCGCTGTT-3'</td>
<td>Exon 6 (forward, inner)</td>
</tr>
<tr>
<td>5'-TTAGGACGATGGGTCGCTTCGCTGTT-3'</td>
<td>Exon 7 (forward, inner)</td>
</tr>
<tr>
<td>5'-TTACGTGAGGGGTCGCTTCGCTGTT-3'</td>
<td>Exon 8 (forward, inner)</td>
</tr>
<tr>
<td>5'-TTACGTGAGGGGTCGCTTCGCTGTT-3'</td>
<td>Exon 9 (forward, inner)</td>
</tr>
</tbody>
</table>

Fig. 1. Map of chromosome 22q11 showing the positions of the YAC and cosmids probes used. Positions are approximate, but the radiation hybrid distance between BCR and hSNF5/INI1 is reported to be ~ 70 cR _2000_ (20). Other maps, however, have reported a different order of markers in this region, suggesting a distance between BCR and hSNF5/INI1 of 25–30 cR _2000_ (31).

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or by SSCP analysis, followed by sequencing. The changes in exons 7 and 9 were seen in four (16%) and five (20%) of these individuals, respectively (data not shown), confirming that they are polymorphic in the normal population.

Unfortunately, no patient material that was suitable for protein or RNA analysis was available from individuals who were known to have heterozygous hSNF5/INI1 deletions.

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<table>
<thead>
<tr>
<th>YAC 29GD7</th>
<th>Cosmid 118d7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic phase</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blast crisis</td>
<td>5/24 (24%)</td>
</tr>
</tbody>
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<sup>a</sup> ND, not determined.

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**Fig. 2.** FISH analysis. YAC 29GD7 (<em>green</em>) cohybridized with a chromosome 22 paint (<em>red</em>) to a nondeletected CML metaphase (A) and a CML metaphase with a 22q11 deletion (B); cosmid 118d7 hybridized to a CML metaphase and interphase cell without a deletion (C); cosmid 118h7 (<em>green</em>) cohybridized with a chromosome 22 paint (<em>red</em>) to a CML metaphase with a deletion (D); and cosmid 118d7 (<em>green</em>) cohybridized with the Vyssis 2 color BCR-ABL probe (<em>BCR, green; ABL, red</em>) on CML metaphases without (E) and with (F) deletions in the same patient sample. The normal chromosomes 9, 22 and the t(9;22) derivatives 9q+ and Ph are indicated. The <em>green</em> signal on the Ph chromosome is from the Vyssis BCR probe, the <em>green</em> signal on the 9q+ (if present) is from the hSNF5/INI1 cosmid, and the <em>green</em> signal on the normal chromosome 22 is from both probes.

**Fig. 3.** SSCP analysis of hSNF5/INI1 exons 7 and 9 demonstrating polymorphic changes in patients with CML in transformation.
Here, we found frequent deletions of 22q11 on the 9q+ chromosome in patients with CML. Deletions of the 9q+ chromosome that remove the 3′ end of the BCR gene have been reported previously, on the basis of Southern blot analysis (19), and were believed to be a by-product of t(9;22). However, the deletions we found here are more telomeric, and the mosaic pattern of hybridization seen by FISH analysis indicated that they are acquired during the course of the disease, at least in some cases. It is likely, therefore, that these deletions are pathogenetically significant and may indicate the presence of a tumor suppressor gene, abrogation of which is required for establishment or progression of CML. However, because we found deletions in both chronic phase and blast crisis patients, loss of this putative tumor suppressor gene alone cannot be sufficient for transformation. It is currently unclear why deletions are only seen on the 9q+ and not the normal chromosome 22, but conceivably, this could relate to genomic imprinting in this region and/or alterations in local chromatin structure as a result of the t(9;22). The size of the deleted region remains to be defined but must, in many cases, be at least 1 Mb because both YAC 29GD7 and hSNF5/INI1 were missing. Recent maps show that this region of 22q11 is relatively gene-rich and that at least 40 genes lie between BCR and hSNF5/INI1 (20).

We focused our analysis on hSNF5/INI1 because biallelic truncating mutations of this locus have been described recently in malignant rhabdoid tumors (13, 21), suggesting that it may act as a tumor suppressor gene. Furthermore, there are potential links between the known function of hSNF5/INI1 and leukemia: hSNF5/INI1 is a widely expressed component of the SWI/SNF complex, which is believed to facilitate the inducible expression of certain genes via the remodeling of local chromatin structure (22, 23). Remodeling of chromatin has been implicated as the mechanism of leukemogenesis brought about by several distinct fusion genes. (a) Chromatin changes mediated by histone acetylation are believed to underlie leukemias with the MOZ-CBP, MLL-CBP, and MOZ-TIF2 fusions, associated with the (8;16), t(11;16), and inv(8), respectively (24–26). CBP is known to have histone acetyltransferase activity, whereas MOZ and TIF2 contain domains that are highly homologous to other histone acetyltransferases. (b) Both ETO and PLZF, involved in t(8;21) and t(11;17), respectively, bind to transcriptional corepressors and histone deacetylases. Consequently, the AML1-ETO and PLZF-RArRα fusions that result from these translocations constitutively repress transcription from AML1- or RARα-responsive promoters by chromatin-mediated mechanisms (27–29). (c) MLL, the gene involved in a wide range of leukemias with translocations of 11q23, belongs to the trithorax family of genes that are believed to play a primary role in the establishment of open chromatin states. Recently, MLL was shown to directly interact with hSNF5/INI1 via its COOH-terminal SET domain and may serve to recruit the SWI/SNF complex to target genes (30). MLL fusion genes lack the SET domain and may, therefore, interfere with this process.

Although we found a high frequency of heterozygous hSNF5/INI1 deletions in patients with CML, we did not find any mutations of the remaining hSNF5/INI1 allele. This is conceivable, however, that reduced levels of hSNF5/INI1 might contribute to leukemogenesis by relaxing constraints on chromatin-mediated control of transcription. We are currently defining the minimum area of deletion at 22q11 to help resolve these possibilities.

References


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