Loss of heterozygosity in acute leukemia: evidence of frequent submicroscopic deletions

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Although chromosomal abnormalities are detected by conventional cytogenetic analysis (CCA) in 40-60% of patients with acute myeloid leukemia (AML), cryptic chromosomal deletions may only be detected by molecular analysis. To determine their frequency, we studied 74 cases of AML by microsatellite allelotype assay using 35 microsatellites spanning eight chromosomal regions known to be frequently involved in AML. In 42 (57%) we found DNA imbalance at the screened loci. This was detected by CCA only in 4 cases. Our data show that cryptic deletions are a common event in AML.

Key words: LOH, acute leukemia, recurrent chromosomal aberrations.

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ABSTRACT

Clonal chromosomal abnormalities are detected by conventional cytogenetic analysis (CCA) in 40-60% of patients with de novo acute myeloid leukemia (AML). Thus, CCA is a fundamental component of modern leukemia cytogenetics. Nevertheless, cryptic chromosomal aberrations may escape CCA, only being detected by molecular genetic analysis. Loss of heterozygosity (LOH), resulting from deletion of one allele at a given locus, may be used as a marker of genetic imbalance in several neoplasms. Its ability to detect smaller DNA losses, in the range of a few kilobases, results in a higher resolution power than CCA and FISH. To identify patients who have submicroscopic deletions at various chromosomal regions, we performed a microsatellite allelotype assay based on fluorescence-PCR in 74 patients with AML.

Design and Methods

Sample selection

A total of 74 consecutive patients diagnosed with AML at our institution between 2002 and 2004 were eligible for this study. The diagnosis of AML was established according to the criteria of the French-American-British (FAB) classification by standard morphological, cytochemical and immunophenotypic methods. Their FAB distribution was as follows: M0, n=6; M1, n=13; M2, n=18; M3, n=10; M4, n=14; M5, n=6; sAML, n=5, and MDS, n=2. These were 33 males and 41 females with a median age of 52 years (range 10-77) (Table 1, online version only).

Karyotype was available in 63 of the 74 cases (85%). Twenty-five patients had a normal karyotype. Favorable cytogenetic alterations were found in 19 patients, such as t(8;21), t(15;17), and inv(16). Twelve patients had unfavorable alterations, such as −7/del(7q), 11q23, 3q abnormalities, t(6;9), hyperdiploidy and complex karyotype. Trisomy 8 as a single aberration was found in two patients, and the remaining 5 cases carried miscellaneous clonal abnormalities. Chromosomal abnormalities were classified according to the International System for Cytogenetic Nomenclature. The cytogenetic risk classification of AML patients was performed according to current criteria.
Microsatellites analysis

Diagnostic samples from bone marrow and/or peripheral blood containing a high proportion of leukemic cells (>80%) were used for molecular studies. We used epithelial cells from buccal mucosa at diagnosis (8 pts), or T lymphocytes, from peripheral blood at clinical remission (66 patients) as a source of constitutional DNA for each patient. These were immunoselected using magnetic beads (MiniMacs; Miltenyi Biotec Bergish Gladbach, Germany). LOH was assessed by using oligonucleotide primers for 35 highly polymorphic microsatellite repeat markers chosen among those spanning chromosomal 5q31-32, 6q16-23, 7q22.1-35, 9q21.3-22, 12p12.2-13.3, 13q13, 17p12-13.3 and 20q11 (Figure 1). Commercial primers with fluorescent dye phosphoramidites FAM, TET or NED were used (Applied Biosystem, CA, USA). PCR-amplification of microsatellite sequences was performed and analyzed by capillary electrophoresis as previously described.6

Only heterozygous loci were considered useful. LOH was scored as positive when the degree of reduction in allelic signal intensity was greater than 70% in one of the alleles of the blast population compared with control DNA. All samples showing LOH were subjected to repeat amplification and analysis for data confirmation. The presence of novel alleles in leukemic cells combined with their absence in normal cells was referred to as microsatellite instability (MSI), whereas at least a double intensity signal of one of the alleles in tumor cells was defined as genomic amplification. Examples of LOH and amplification are shown in Figure 2.

Results and Discussion

Matched pairs of constitutional and clonal DNA from 74 patients were analyzed for LOH by using 35

Figure 1. Diagrammatic representation of microsatellite markers chosen for LOH study.

Figure 2. Examples of allelotyping results. A. Loss of heterozygosity (LOH). B. Heterozygosity. C. Genomic amplification.
microsatellites covering eight chromosomal regions known to be frequently involved in AML. A total of 2,028 loci were successfully screened, and informative allelotypes were found in 1,560 examined loci (77%). All samples were informative for at least 75% of the 35 microsatellite markers. Balanced allelotypes were observed in 32 cases (43.2%), while microsatellite DNA imbalance at the screened loci was observed in the remaining 42 cases (57%). LOH was detected in 37 cases, microsatellite instability in 2 cases, and amplification in 6 cases (Table 1, online version only). LOH events were randomly present in all analyzed chromosomal bands, except for clustering at 7q22-32, observed in 14 patients. CCA had failed to detect any ch7 abnormality in ten of these.

Among the 42 cases with genetic imbalance, 9 had unfavorable cytogenetic (cases #1, 7, 15, 19, 31, 34, 36, 38 and 40), 9 patients (21%) had favorable cytogenetic (cases #10, 16, 21-26, 32), 10 patients (23%) showed a diploid karyotype (cases #3, 4, 6, 9, 12, 18, 27, 30, 33 and 39) and the remaining 6 patients showed miscellaneous chromosomal abnormalities (cases #8, 11, 14, 17, 20 and 41). Remarkably, all chromosomal monosomies or deletions (cases #1, 14, 17, and 40) have been confirmed by the microsatellites data, whereas 38 cases with genetic imbalance had no cytogenetically detectable abnormalities at the screened bands. Our data suggest that LOH is a common event in adults with acute myeloid leukemia, involving at least one half of patients. This percentage may be an underestimation since we used a restricted panel of microsatellites. The percentage of patients with LOH may significantly increase if a broader panel is used.

Possible advances in the treatment of acute leukemia also depend on understanding the pathogenic mechanisms. Identification of genetic events which facilitate or start the leukemic process represents a major objective of current research in this field. If one assumes that LOH may impact on the mechanisms of leukemogenesis, definition of its real frequency is of interest. Nevertheless the incidence of LOH in de novo acute leukemia remains controversial, since only limited data are available. In the only reported genome-wide screening study of childhood AML, microsatellite LOH was found in 17 out of 53 samples (32%).

It has recently been suggested that LOH arises not only from chromosomal deletion, but also from somatic recombination with acquired uniparental dysomy (UPD). This results in large homozygous regions spanning from a specific point to the telomere. This novel form of abnormality was documented by genome-wide SNP analysis in about 20% of AML by Raghavan et al. UPD mechanism could explain LOH only in a minority of our patients (cases #5, 11, 12, 39 and 42) while the remaining had interstitial LOH, compatible only with loss of genetic material. This agrees with the findings of Gorletta et al.

In our series, LOH was not apparently restricted to, or associated with, any specific AML FAB subtype or chromosomal region, with the only exception of the 7q band, which was involved in 10 cases with normal chromosome 7 at CCA. Conflicting results have been reported on the 7q region involvement in patients with cytogenetically normal chromosome 7. In a previous study, we found 7q LOH in 16 of 50 (32%) cases of acute leukemia, including 13 cases which were LOH+ and despite cytogenetically normal chromosome 7. In this series, submicroscopic deletions at D7S486 were confirmed by FISH.

What has been learned from the comparative study of conventional cytogenetic and molecular data? Submicroscopic deletions, which may be detected by FISH, have been reported proximal to the translocation breakpoints of some non-random leukemia-associated translocation. This suggests that the underlying mechanism is likely to be associated with the translocation process itself and may be dependent on the flanking sequences. No deletions were found in adult AML with normal karyotype by Cuneo et al. In a 24-color FISH study, Schoch et al. showed that loss of genetic material is a frequent event in AML with a complex aberrant karyotype. Furthermore, a large spectrum of genomic imbalances were identified in a recent study using comparative genomic hybridization array (array-CGH).

The frequency of LOH may depend heavily on the methods used for its screening. Novel techniques such as array-based SNP and CGH allow a high resolution screening and confirm that cryptic chromosomal abnormalities are a common event in leukemia.

In conclusion, on the basis of our findings, LOH appears to be a very common event in adult AML, not associated with recurrent chromosomal alterations, and may also be found in patients with diploid karyotype. LOH may occur not only adjacent to usual breakpoint regions, but also some distance from them, suggesting that loss of chromosomal material occurring throughout the genome may be a pivotal mechanism for leukemogenesis. Cryptic deletions may cause homozygosity of tumor-sup-
ressor genes and may influence gene expression pattern by loss of microRNA or as a consequence of gene dosage.\(^{17,18}\) Moreover, LOH due to acquired uniparental dysomy has been associated with homozygous gene mutation in AML.\(^{19}\) All of these mechanisms may have important implications in leukogenesis. Whether LOH is a primary event or a secondary molecular lesion remains to be clarified.

### Authors’ Contributions

CA and RB contributed equally to this work. They performed the LOH study and contributed to the discussion of results; FF is involved in clinical management of acute leukemia. He addressed the leukemic samples to the Laboratory of Hematology and contributed to the discussion of results; VR and LC performed standard cytogenetic and FISH analysis and contributed to the discussion of results; GC and MLR were involved in molecular investigation and contributed to the discussion of results; AM performed the diagnostic Immunophenotype analysis of leukemic blasts by flow-cytometry; SM, the Head of the Division, was in charge of clinical assistance; he contributed to the study design and partially to funding the study; AS the principal investigator, was responsible for the main study design. She overviewed the research plan from its design to development, was responsible for the analysis of the results, and drafted the manuscript.

### Conflict of Interest

The authors reported no potential conflicts of interest.

### References