De Novo Erythroleukemia Chromosome Features Include Multiple Rearrangements, With Special Involvement of Chromosomes 11 and 19

Juan C. Cigudosa, Maria D. Odero, M. José Calasanz, Francesc Solé, Marta Salido, Eva Arranz, Angel Martínez-Ramirez, Miguel Urioste, Sara Alvarez, Jose V. Cervera, Donald MacGrogan, Miguel A. Sanz, Stephen D. Nimer, and Javier Benitez

1Cytogenetics Unit, Department of Human Genetics, Spanish National Cancer Center, Madrid, Spain
2Department of Genetics, University of Navarra, Pamplona, Spain
3Laboratory of Cytology, Department of Pathology, Hospital del Mar, IMAS, IMIM, Barcelona, Spain
4Service of Hematology, Hospital La Princesa, Madrid, Spain
5Service of Hematology, Hospital La Fe, Valencia, Spain
6Laboratory of Molecular Aspects of Hematopoiesis, Memorial Sloan-Kettering Cancer Center, New York, NY

Erythroid leukemia (ERL or AML-M6) is an uncommon subtype of acute myeloid leukemia, the clinical, morphological, and genetic behavior of which needs further characterization. We analyzed a homogeneous group of 23 de novo AML-M6 patients whose bone marrow cells showed complex karyotypes. We also analyzed eight leukemia cell lines with erythroid phenotype, performing detailed molecular cytogenetic analyses, including spectral karyotyping (SKY) in all samples. The main features are: (1) A majority of patients (56%) had hypodiploidy. Loss of genetic material was the most common genetic change, especially monosomies of chromosome 7 or 18, and deletions of chromosome arm 5q. Taken together, 87% of the cases displayed aberrations involving chromosome 5 or 8. (2) We describe a novel, cryptic, and recurrent translocation, t(11;19)(p11.2;q13.1). Another translocation, t(12;21)(p11.2;q11.2), was found to be recurrent in a patient with ERL and in the K562 cell line. (3) MLL gene rearrangements were detected in 20% of cases (three translocations and three amplifications) and, overall, we defined 52 rearrangements (excluding deletions) with a mean of 2.3 translocations per patient. (4) Of the structural aberrations, 21% involved chromosomes 11 and 19. Most of the rearrangements were unbalanced; only 13 reciprocal translocations were observed. The general picture of chromosomal aberrations in cell lines did not reflect what occurred in patient samples. However, both primary samples and cell lines shared three common breakpoints at 19q13.1, 20q11.2, and 21q11.2. This is the first molecular cytogenetic description of the karyotype abnormalities present in patients with ERL. It should assist in the identification of genes involved in erythroleukemogenesis.
or trisomy 8, which are commonly found in myelodysplasias and several subgroups of AML, specific genetic abnormalities have not been recurrently found in ERL. The most commonly identified karyotypes in ERL reflect a great complexity (frequently with more than three rearrangements), and a lack of recurrent breakpoints (other than those affecting chromosome 5 or 7). More powerful molecular cytogenetic tools are now available, such as fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH), and spectral karyotyping (SKY), which can help in identifying the genetic changes that take place in ERL. We used molecular cytogenetics to study a series of homogeneously defined de novo ERLs, with complex karyotypes, as well as eight erythroid leukemia cell lines. We found several recurring distinctive and novel genetic features of this subtype of leukemia.

We studied 23 patients, 10 male and 13 female, whose ages ranged from 1 to 85 years, with a median of 69 years. All samples were collected at diagnosis. The clinical history of previous hematologic disorders and/or chemotherapy or exposure to other known genotoxic agents was ruled out in every case. Morphological classification criteria were applied according to the WHO proposal (Jaffe et al., 2001). Erythroleukemia (erythroid/myeloid) was defined by the presence of ≥50% erythroid precursors in the entire nucleated cell population and ≥20% myeloblasts in the non-erythroid population; pure erythroid leukemia: >80% of marrow cells of the erythroid lineage with no evidence of a significant myeloblastic component. All slides were reviewed centrally by some of the authors. We also analyzed eight leukemia cell lines with erythroid phenotype, irrespective of the type of leukemia from which they were derived. Five cell lines were derived from chronic myeloid leukemia (CML) in blast crisis, SAM-1 (provided by J. Cossman), AP-217 (provided by R. Berthier), K-562 (in-house), HEL-R (provided by T. Papayannopoulou), and LAMA 84 and KU812 (provided by J. Goldman). Two cell lines were derived from AML-M6: KMOE-2 (from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany) and TF-1 (in-house). The cells lines were cultured, according to their respective requirements, and they were harvested after 2 hr of exposure to Colcemid, for standard cytogenetic procedures.

All samples had a complex aberrant karyotype at diagnosis. We considered a karyotype complex when three or more clonal chromosome abnormalities were identified. Conventional cytogenetics was performed on bone marrow samples after direct and/or short-term culture without mitogens. Metaphase cells were prepared from all samples. G-banding analysis was performed by standard procedures. Karyotypes were described according to the ISCN (Mitelman, 1995). After conventional cytogenetic analysis, slides were prepared from the fixative-stored material and hybridized using the SKY method according to the manufacturer’s protocol (Applied Spectral Imaging, Migdal Ha-Emek, Israel). Images were acquired with an SD300 Spectra cube (Applied Spectral Imaging) mounted on a Zeiss Axioplan microscope using a custom-designed optical filter, SKY-1 (Chroma Technology, Brattleboro, VT). For each case, 8–30 metaphase cells were captured and analyzed. Breakpoints corresponding to the chromosome abnormalities were assigned based on the corresponding DAPI banding and G-banded karyotype of the same cells. An abnormality or breakpoint was considered as recurrent if it was identified in two or more different cases by G-banding or SKY. A whole chromosome painting (WCP) probe for chromosome 19, telomeric 19p and 19q FISH probes (Appligene Oncor, France), and BCR/ABL and MLL FISH probes (Vysis, Downers Grove, IL) were used to define the involvement of these genes or chromosome regions. Hybridizations were performed according to the manufacturer’s protocols.

All 23 patients were selected based on their fulfillment of the WHO criteria for ERL and the presence of complex karyotypes at diagnosis as detected by G-banding. Bone marrow morphological analysis showed that two thirds of the patients had trilineage dysplasia. All but two cases (cases 18 and 22) were classified as erythroleukemia (erythroid/myeloid), according to the WHO classification criteria (Table 1). The clinical course of the patients was poor, with a mean survival of only 4.3 months (range, 1–25 months). To characterize the chromosome profile completely, we performed spectral karyotyping in all cases. The SKY karyotypes are given in Table 1. All data discussed below make reference to the SKY analysis.

Hypodiploidy seems to be a rather common genetic feature of ERL. Thirteen cases (56%) showed hypodiploid karyotypes. The proportion of hypodiploid cases (excluding cases with only monosomy 7) has been reported (Mitelman et al., 2002) to range between 2% for M5 and 11% for M1, with ERL showing a significantly higher ratio of 38%. In our series, which included only de novo cases, 70% of the cases showed chromosome 5 monosomy or 5q deletions; trisomy 8, either alone
or with other abnormalities, was seen in 52% of the cases; monosomy or 7q deletions were observed in 43% of the patients. Taken together, 87% of the cases displayed aberrations involving chromosome 5 or 8, and 91% of the cases demonstrated an abnormality of chromosome 5, 7, or 8.

Two recent papers have demonstrated that hypodiploidy and loss of genetic material are the most frequent finding in AML with complex karyotypes (Mrozek et al., 2002; Schoch et al., 2002). Those series both included de novo and therapy-related cases; the most frequent whole-chromosome losses were monosomies 7 and 18, and the most frequent interstitial deletions involved 5q, 17p, 12p, and 20q. In our series of de novo ERL cases, we observed a nearly identical profile, suggesting a common genetic pattern of chromosomal aberrations in myeloblast proliferation.

Fifty-two rearrangements (excluding deletions) were defined in our series, with a median of 2.3 per case (range, 0–10), of which 13 were balanced translocations. SKY analysis refined the vast majority of structural aberrations. None has been reported in de novo ERL cases, we observed a nearly identical profile, suggesting a common genetic pattern of chromosomal aberrations in myeloblast proliferation.

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the karyotypes of ERL have generally been characterized by the presence of numerous chromosomal rearrangements, without specific and recurrent breakpoints. Only the t(3;5) translocation has been seen in several cases of M6 (10 of 378 cases), but the exact breakpoints involved are not identical (Kwong, 1998; Mitelman et al., 2002). We did not find this rearrangement in our series. As in other acute myeloid leukemias with complex karyotypes, unbalanced rearrangements led to the net loss of genetic material in several segments, mainly 5q, 7q, 17p, and 12p. Although it has been reported that some 5q or 7q deletions can be redefined as unbalanced translocations, we did not find such deletions.

A novel and cryptic recurrent balanced translocation was found in cases 6 and 10 of our study. The t(11;19)(p11.2;q13.1) has not been reported previously for any kind of malignancy. The translocation was detected only by SKY, whereas it had been described as a deletion on 11p by conventional cytogenetics. We characterized the translocation by WCP and FISH hybridizations and used telomeric probes for chromosome 19 (Fig. 1A–D). The lack of sufficient biological material from the patients precludes specific molecular genetic studies; however, both chromosomal regions, 11p11.2 and 19q13.1, harbor genes that regulate the cell cycle or cell proliferation (e.g., KAI1, HPTP eta, MLL2, and others) (Honda et al., 1994; Huntsman et al., 1999; Silver et al., 1999; Verma et al., 1999).

We found three translocations with breakpoints at 11q23, the chromosomal site of the MLL gene: t(11;19)(q23;p13.3), t(11;16)(q23;q22), and t(1;11)(?;q23) in cases 1, 6, and 17, respectively. By FISH analysis, we detected that MLL was rearranged in these three cases. The first two translocations have been reported in small series of AML-M6 that occurred after exposure to DNA-damaging agents (Groupe Franc¸ais de Cytoge´n e´tique He´matologique, 1984; Moorman et al., 1998). Our cases had no antecedents of such exposure, although, because the MLL gene is recurrently involved in acute leukemia (Rowley, 1998), we analyzed its status by FISH in all our samples. We found that MLL was rearranged in these three cases. The first two translocations have been reported in small series of AML-M6 that occurred after exposure to DNA-damaging agents (Groupe Franc¸ais de Cytoge´n e´tique He´matologique, 1984; Moorman et al., 1998). Our cases had no antecedents of such exposure, although, because the MLL gene is recurrently involved in acute leukemia (Rowley, 1998), we analyzed its status by FISH in all our samples. We found that MLL was amplified in the same case 1 and in two additional cases (5 and 13) (Table 1). In all three cases, five to seven copies of MLL were located on a ring chromosome that was identified as being derived from chromosome 11 (Fig. 1E). Five of the 23 cases showed MLL rearrangements, a relatively high frequency that is similar to the fre-
Figure 2.
The cytogenetic rearrangements showed 51 chromosome breakpoints that were non-randomly distributed (Fig. 2). Chromosomes 11 and 19 were most frequently involved in chromosomal aberrations, clustering 11 and 10%, respectively, of all of the rearrangements. The most recurrent breakpoints were bands 11p15, 19q13.1, involved in four rearrangements each; and bands 9q13, 11p11.2, 11q23, 12p13, 15q24, 16q22, 18q21, 19p13.1, and 21q11.2, involved in three rearrangements each. A recent report of the cytogenetic profile of M7 AML (Dastigue et al., 2002), by conventional G-banding, shows that, despite the high complexity of the karyotypes, the distribution of the breakpoints between the ERL and M7 subtypes of AML is rather different both in their location and in their frequency.

Molecular cytogenetic analysis of the erythroid cell lines showed a different pattern (Table 2). Five cell lines were established from before the blast crisis phase of CML. Their cytogenetic profile fits this condition: the presence of an extra Philadelphia chromosome and of isochromosome 17q, and trisomy 8. At the ploidy level, all cell lines but TF-1 were tri- or tetraploid, whereas patient samples were mostly diploid and hypodiploid. Probably these differences were attributable to the late passage status of the cell lines.

A comparison of the chromosome breakpoint pattern between patients and cell lines (Fig. 2) shows only a few common sites of recurrent breakpoints: these were 19q13.1, 20q12.1, and 8q24.1. As for the translocations, only one was recurrent in case 21 and in the K562 cell line. The t(12;21)(p11.2;q11.2) was unbalanced translocation and only the derivative chromosomal breakpoints involved in structural rearrangements in erythroleukemia. Arrowheads to the left of each chromosome indicate breakpoints observed in patients. Numbers above each chromosome are the number of breakpoints that could not be assigned to a specific band. The left number corresponds to cell lines and the right number corresponds to patient samples.

### TABLE 2. SKY Karyotype of the Structural Rearrangements of Erythroleukemic Cell Lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>SKY karyotype</th>
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<tbody>
<tr>
<td>TF-1</td>
<td>2n:der(Y)(Y;3;11:Ypter→Yq11.2:3</td>
</tr>
<tr>
<td>HEL-R</td>
<td>3n:der(Y)(Y;12);der(Y)(Y;22);der(Y)(Y;14)</td>
</tr>
<tr>
<td>AP217</td>
<td>5n:der(Y;22)</td>
</tr>
<tr>
<td>Ku812</td>
<td>4n:der(Y;9);der(Y;22)</td>
</tr>
<tr>
<td>LAMA84</td>
<td>3n:der(Y;3)</td>
</tr>
<tr>
<td>KmoE-02</td>
<td>3n:der(Y;3)</td>
</tr>
<tr>
<td>K562</td>
<td>3n:der(Y;3)</td>
</tr>
</tbody>
</table>

*Figure 2. Ideogram showing the breakpoints involved in structural rearrangements in erythroleukemia. Arrowheads to the left of each chromosome indicate breakpoints observed in patients. Numbers above each chromosome are the number of breakpoints that could not be assigned to a specific band. The left number corresponds to cell lines and the right number corresponds to patient samples.*

This is the first report of molecular cytogenetics and karyotype features of a large number of cases of de novo erythroid leukemia with a complex karyotype. The use of new molecular cytogenetic techniques demonstrated several interesting features of ERL: (1) Hypodiploidy and multiple unbalanced translocations are very common, with net losses of chromosome material; (2) we have found a novel, cryptic translocation t(11;19) in two patients; (3) MLL rearrangements, both as translocated or as amplified, are frequent in the absence of exposure to genotoxic agents; (4) the breakpoint analysis pointed to chromosomes 11 and 19 as the most frequently involved. This distribution of the breakpoints shows a different pattern for ERL than for other subtypes of AML. These features suggest a characteristic genetic picture of ERL and helps focus on a more intensive molecular study of this disease.

REFERENCES


