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BRIEF COMMUNICATION

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

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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.

De novo erythroleukemia (ERL) is a rare type of acute myeloid leukemia (AML), representing 2–7% of de novo AML cases. Because of its heterogeneous nature, ERL has posed difficulties in its precise classification. Most authors agree that two main subtypes exist, according to the WHO classification of acute erythroid leukemias: (1) erythroleukemia (erythroid/myeloid), M6 acute leukemia according to the FAB criteria, in which mixed granulocytic (>20% myeloblasts) and erythroblastic cellular components are present; and (2) pure erythroid leukemia with erythroblasts constituting more than 80% of the marrow cells, with no significant myeloblastic component (Bennett et al., 1985; Goldberg et al., 1998; Mazzella et al., 1998; Jaffe et al., 2001; Domingo-Claros et al., 2002; Park et al., 2002).

In contrast to other types of acute leukemia, no specific cytogenetic or molecular markers are available for ERL for classification or prognostic purposes (Cuneo et al., 1990; Olopade et al., 1992; Kwong, 1998). About 70% of de novo ERL cases show chromosome abnormalities (Cuneo et al., 1990; Olopade et al., 1992; Goldberg et al., 1998). In fact, 378 different abnormal karyotypes have been recorded in the Mitelman Database of Chromosome Aberrations in Human Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman) (Mitelman et al., 2002). Other than a high frequency of loss or partial deletions of chromosomes 5 and/or 7,

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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 $\geq$50% erythroid precursors in the entire nucleated cell population and $\geq$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. Papayannnopoulou), 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-Emck, 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

TABLE 1. Clinical and Molecular Cytogenetic Description of the Abnormal Clones Found in de Novo ERL Patients Using GTG Banding and SKY Techniques

Case no.	Sex/ age	Survival[a]	Dysplasia[b]	SKY karyotype
1	M/73	1	Tri	37~58,XX,der(3)t(3;21)(q11.2;q11.2),del(5)(q12q33),+8,der(8)t(8;11)(p21;p11.2)t(8;12)(q22;q13),t(11;19)(q23;p13.3),der(12)t(12;18)(q24;q11.2),der(16)t(3;16)(?;p11.2),der(18)t(3;18)(?;q21),der(19)ins(19;17)(p13.1;?)t(3;19)(q11.2;p13.3),r(11)×2amp(*MLL*)
2	M/77	1	Tri	44~46,XX,del(5)(q13q33),−7,−19
3	F/63	5	Tri	46~48,XX,del(5)(q13q33),+8,der(11)t(6;11)(p15;?),−13,−18,+19,+21
4	F/57	2	Tri	44,XY,t(1;7)(q32;q32),−7,+8,−9,−13,−17,−20,+21×2
5	M/69	3	Tri	45~55,XX,+X,+1,+2,del(5)(q13q33),+6,+8,+11,+r(11)×2amp(*MLL*),+14,+22
6	M/67	5	Tri	45~48,XX,inv(3)(q21q26),del(5)(q13q33),−7,+8,del(10)(q24),der(11)t(11;19)(p11.2;q13.1),del(12)(p11),+del(13)(q14),der(16)t(11;16)(q23;q22),der(17)ins(17;3)(q22;?),der(19)t(11;19)(p15;p13.1),+20,+r(?)
7	M/68	4	Ery	45~46,Y,der(X)t(X;13)(p22.1;q22),i(3)(q10),del(5)(q22),inv(6)(p23q12),der(13)t(2;9;13)(?;?;q22),der(16)t(1;16)(?;q21–22),+19,−21
8	M/65	3	Tri	44~45,XY,del(5)(q13),−7,+8,del(12)(p13),der(16)t(2;16)(?;q?),+20
9	M/70	4	Tri	61~65,XYY,+1×2,+2,+3,+4,+5,der(5)t(5;14;16)(q13;q13;?),+inv(6)(p23q12)×2,+7,+8×2,+16,−19,der(19)t(19;20)(q13.1;p11.2)×2,+21×2,+22,+der(22)del(22)(q11)×2
10	M/71	8	Tri	42~45,X,del(X)(q13),+1,del(5)(q13q31),+del(5)(p13),+6,−7,+8,t(11;19)(p11.2;q13.1),hsr(12)(p13),t(13;14)(p11;q11)c,der(15)t(13;15)(q24;?),−17,t(18;21)(p11.2;q11.2),r(19) 51~54,X,del(X)(q13),+1,del(5)(q13q31),+del(5)(p13),+6,i(7)(q10),+8,t(11;19)(p11.2;q13.1),t(12;15)(p13;q22),t(13;14)(p11;q11),+14,+15,+20×2,+21,r(19)
11	F/69	1	Tri	51~52,XX,+8,del(10)(q24),del(11)(p15),−3,der(16)t(7;16)(?;q24)
12	F/76	6	Ery	35~42,XX,r(3),−5,der(7)t(6;7)(?;q22),t(7;10)(p15;q22),−9,−10,der(11)t(9;11)(p12;p15),del(13)(q22),−17,−18
13	F/59	14	Tri	45,XX,der(1;19)(p32;p13.1),t(3;9)(q21;q13),del(5)(q13q31),t(14;21)(q10,q10),−17,−19,+r(11)amp(*MLL*)
14	F/72	5	Ery	45,XX,−5,der(7)t(7,9)(q32;q13),der(9)t(9,19)(q13;q13.1),−18,+21,dmin,amp(c-MYC)
15	M/82	1	Tri	46~48,XY,der(2)t(2;3)(q31;?),+4,−7,+8
16	F/69	3	Tri	44,XX,t(1;12)(q42;p13),t(2;15)(q31;q24),der(5)t(5;17)(q13;q22),t(6;18)(q27;q21),−7,−17
17	F/71	24	Tri	46,XX,del(1)(p13),−7,der(11)t(1;11)(?;q23),ins(12;1)(q24;?),der(14)t(1;14)(?;q13),+20
18	F/1	7	Ery[c]	49,XX,inv(X)(p22.1q13),+5,+8,+18 [15]
19	F/37	25	Ery	46,X,t(X;11)(q13;p15),del(14)(q23q32) [20]
20	F/85	7	E/M	46~47,XX,−5,+8,del(20)(q12)
21	M/50	25	Ery	44~47,XY,der(4)t(4;7)(p12;q21),del(5)(q13),der(8)t(3;8)(?;q24)×2,der(12)t(12;21)(p13;q11.2),der(14)t(7;14)(?;q32)
22	F/76	8	E/M	45~47,XX,del(5)(q13q31),−7,del(7)(q11.2),der(7)t(7;9)(q11.2;?),der(7)t(7;20)(q11.2;q11.2),der(16)t(1;16)(?;q22),der(18)t(7;18)(q22;q21)
23	F/71	6	Tri	46~47,XX,t(X;9;22)(?;q34;q11.2),+8,i(17)(q10)

[a]Survival is given in months.
[b]Lineage involvement: Tri, erythroid–myeloid–megakaryoblastic; Ery: erythroid; E/M: erythroid–myeloid.
[c]Pure erythroid leukemia. M6b type, according to the WHO classification.

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 the Mitelman Database (Mitelman et al., 2002) or in the most recently reported ERL cases (Mrozek et al., 2002; Schoch et al., 2002). In fact,

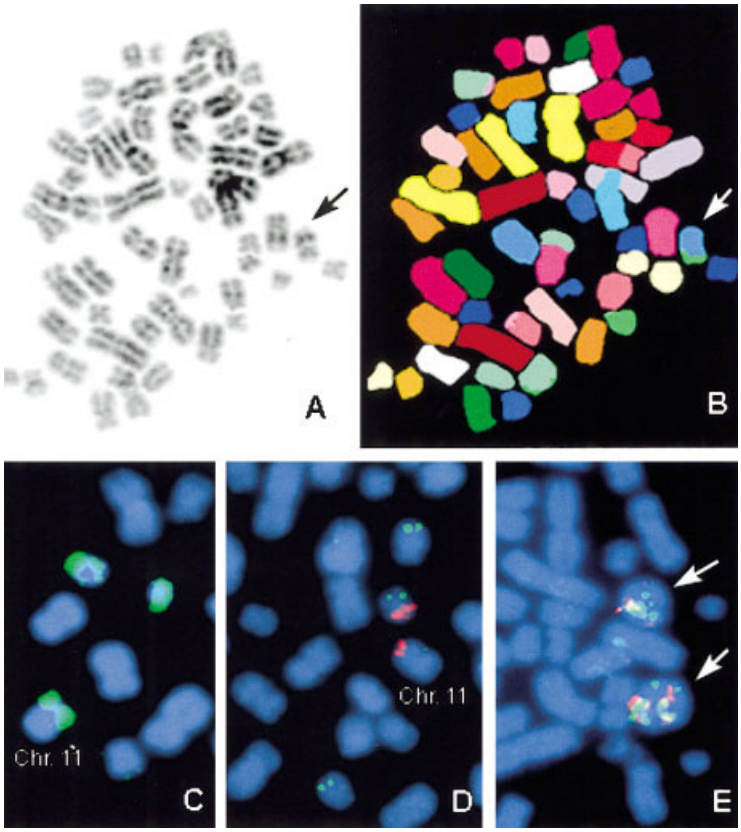

Figure 1. Molecular cytogenetic findings in acute erythroleukemia. **A:** An inverted DAPI-stained metaphase spread of case 6 with an arrow pointing to the cryptically altered chromosome 11. **B:** Spectral image of the same metaphase cell from case 6. The arrow indicates the derivative chromosome 11 from the translocation t(11;19)(p11.2;q13.1). Blue and green colors correspond to chromosomes 11 and 19, respectively. **C:** WCP of chromosome 19 (green signal) on a partial metaphase from case 10. Green color corresponds to the genetic material from chromosome 19, which has been translocated onto chromosome 11. Additional green signals correspond to normal and derivative chromosome 19. **D:** FISH assay on case 6 with telomeric probes from the short (green signal) and long (red signal) arms of chromosome 19. Red signals on the translocated chromosome 11 confirmed the involvement of the long arm of chromosome 19 in t(11;19). A normal chromosome 19 with both red and green signals is also shown. **E:** FISH assay with the *MLL* gene probe on a metaphase from case 1. The *MLL* gene probe gives a two-color (green and red) signal, usually seen as a yellow fusion signal. The partial metaphase shows two ring chromosomes (arrows) with multiple (4 to 6) copies of the *MLL* genes that are seen as multiple spots on the abnormal chromosomes.

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 Français de Cytogénétique Hématologique, 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-

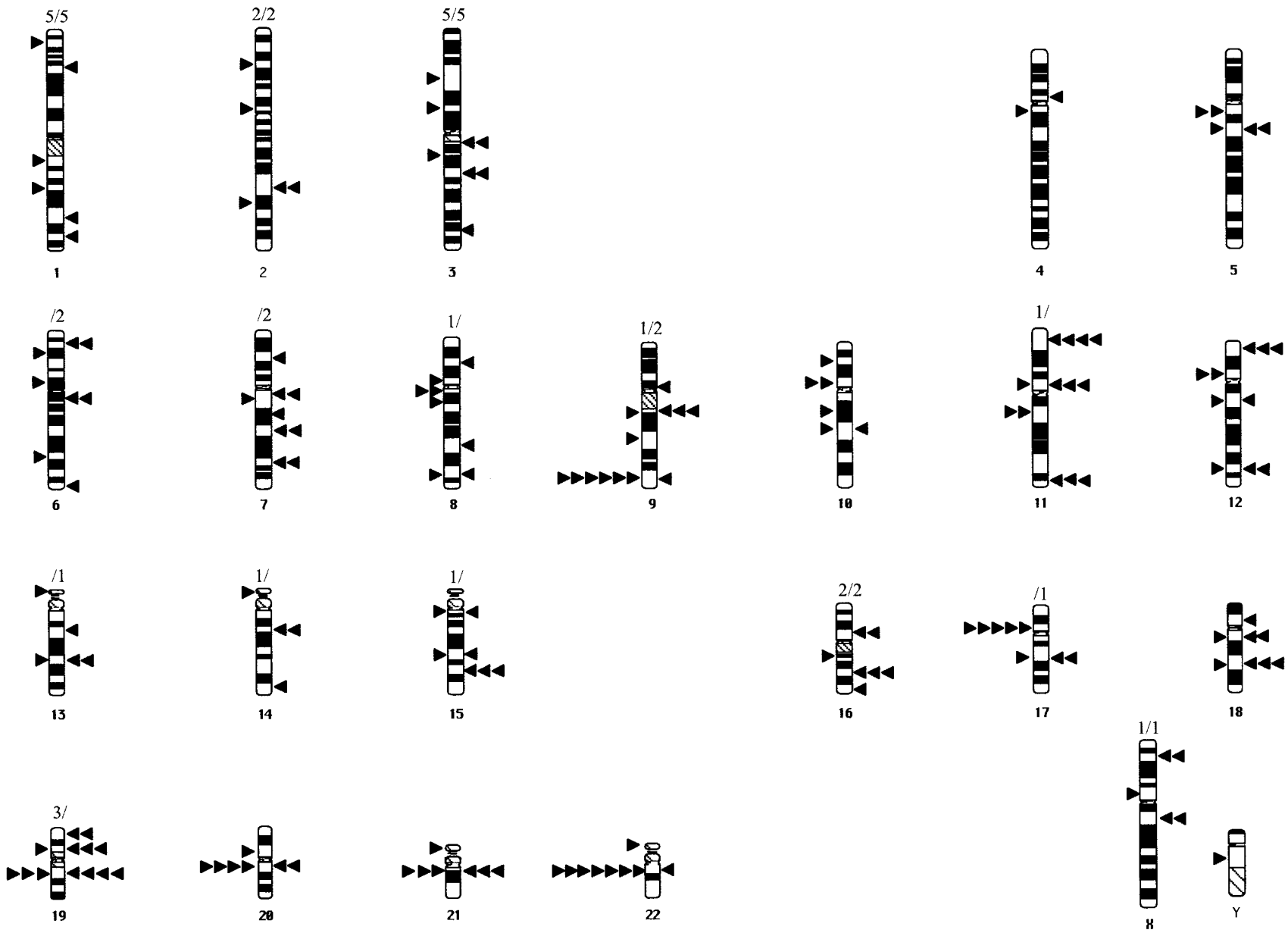
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Figure 2.

TABLE 2. SKY Karyotype of the Structural Rearrangements of Erythroblastoid Leukemic Cell Lines

Cell line	SKY karyotype
TF-1	2n±,der(Y)t(Y;3;11)(Ypter→Yq11.2::3 ?::11q13→11qter),der(1)t(1;8)(p36.3;q?),der(2)t(2;15)(q32;q22),del(3)(p13),del(3)(q12),der(3)(3?::14?), der(5)t(5;19)(q12;q13.1),del(8)(p11.2),der(8)t(8;19)(q11.2;q13.1),der(12)t(10;12)(q21;q13),der(12)(3?:: 12p11.2→12q24::1?),der(14)(2?::14p12→14qter),der(15)(15p12→15q11::1?::8q12→8qter),der(17)t(3;17) (?;p11.2),der(19)t(19;19)(q?;?),der(20)t(3;20)(?;q11.2),der(22)t(2;22)(?;p12)
HEL-R	3n±,der(1)t(1;5)(q21;q13),del(2)(q21),t(2;8)(p11.2;q11.2),t(6;11)(q23;q13),der(8)t(8;20)(p12; q11.2),der(8)t(1;8)(q?;q24.1),del(9)(q13),der(9)dup(9)(q13;q34)t(9;22)(q34;q11.2),der(9)dup(9)(q13; q34)t(9;11;19)(q34;q13;?),der(17)t(3;17)(q13;p11.2),der(19)t(10;19)(p11.2;q13.1),dup(21)(q11.2qter)
AP217	5n±,t(9;22)(q34;q11.2),der(10)t(6;10)(q?;p13),i(17)(q10),der(17)t(15;17)(q?;p11.2),der(19)t(11; 19)(q13;p13.1),hsr(20)(q?)
Ku812	4n±,del(9)(q21),t(9;22)(q34;q11.2),der(14)tas(14;14),del(17)(p11.2),i(17)(q10),der(17)t(17; 21)(p11.2;q11.2),der(20)t(4;20)(q12;q11.2)
LAMA84	3n±,der(3)del(3)(p?)del(3)(q?),t(9;22)(q34;q11.2),der(11)t(11;16)(p11.2;?),der(17)t(11;17)(?; p11.2),der(22)t(9;22)(q34;q11.2)
KmoE-02	3n±,der(X)t(X;16)(p11.2;q12),der(1)del(1)(p22)del(1)(q25),der(1)del(1)(p13)del(1)(q?),der(1)t(1; 13)(q23;q22),der(2)t(2;9)(p23;?),del(3)(q21),der(3)t(3;21)(p12;q11.2),del(7)(p22),der(7)t(X;7)(?; q11.2),del(12)(p12),der(16)t(1;16)(?;p?),i(17)(q10),hsr(19)(q?),der(20)t(9;20)(?; p11.2),der(20)t(20;22)(q11.2;q11.2),der(20)t(20;22)(q13;q11.2)
K562	3n±,der(5)t(5;6)(q11.2;?),dup(6)(pter→p12::p22qter),der(7)t(7;7)(p?;q?),del(9)(p12),der(9)t(9;9)(p1?3;q22), der(10)t(3;10)(p21;q22),der(10)t(3;10;17)(?;p11.2;q22),der(12)t(12;21)(p11.2;q11.2),der(13)t(9;13)(?;13p11), der(17)t(9;17)(?;p11.2),der(18)t(1;18)(?;q21–q22),dic(6;20)(?;p?),der(21)t(1;21)(?;p?),der(22)t(9; 22)(q34;q11.2), der(22)t(22pter→22q11.2::hsr(9q34;22q11.2))

quency found in AML M5a, M5b, or M4. About 40 cases of AML with amplifications of the *MLL* gene have been documented thus far (Avet-Loiseau et al., 1999; Cuthbert et al., 2000; Michaux et al., 2000; Park et al., 2000; Streubel et al., 2000; Mrozek et al., 2002; Schoch et al., 2002), suggesting its important role in leukemogenesis. Other genomic amplifications were detected: one case with double-minute chromosomes, which were shown to be C-MYC amplifications, and a homogeneously staining region (HSR) on chromosome arm 12p. Neither C-MYC nor *MLL* amplifications, nor HSRs have been reported previously in patients with ERL.

The cytogenetic rearrangements showed 51 chromosomal 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 *BCR/ABL*-positive cells during 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, 20q13.1, and 21q11.2. The remaining breakpoints were distributed according to a different pattern. 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). It was an unbalanced translocation and only the derivative chro-

Figure 2. Ideogram showing the breakpoints involved in structural rearrangements in erythroleukemia. Arrowheads to the left of each chromosome indicate breakpoints observed in the cell lines. Right of each chromosome indicates 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.

mosome 12 was retained in the cell, resulting in loss of 12p and gain of chromosome 21 material. In general, the available erythroid cell lines are not comparable to the de novo ERL cases, most likely because of their CML-derived origin.

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.

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