



## Establishment and characterization of a megakaryoblast cell line with amplification of *MLL*

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**A new cell line with megakaryoblastic features, designated UoC-M1, was established from the malignant cells of a 68-year-old patient with acute myeloid leukemia. The patient's leukemic cells reacted with  $\alpha$ -naphthyl acetate esterase and acid phosphatase and expressed CD7, CD24, CD34, CD38, CD45, HLA-DR and CD61. Cytogenetic analysis of the patient's malignant cells (and of the UoC-M1 cells) showed a human, male hypodiploid karyotype with many chromosome rearrangements and marker chromosomes. Spectral karyotyping (SKY) analysis complemented the G-banded karyotyping and clarified several chromosomal translocations and identified the marker chromosomes. Fluorescence *in situ* hybridization (FISH) and SKY analysis demonstrated that one marker chromosome contained three segments of chromosome 9 interspersed with three segments of chromosome 11, as well as a portion of chromosome 19. FISH analysis with a probe for *MLL* revealed that the UoC-M1 cells contained four copies of the *MLL* gene. Southern blot analysis determined that the *MLL* gene had a germline profile while Northern and Western analyses showed that the *MLL* mRNAs and protein were of the appropriate sizes. This is the first report of amplification of the *MLL* gene which may be an additional mechanism of leukemogenesis or disease progression.**

**Keywords:** cell line; megakaryoblast; *MLL* amplification

### Introduction

The establishment of several immortal human cell lines with megakaryoblastic features has been reported.<sup>1–9</sup> A few cell lines have been established from infants with acute megakaryoblastic leukemia (both with and without Down syndrome), as well as from adults with chronic myelogenous leukemia with a megakaryoblastic blast crisis.<sup>4–6</sup> Several cell lines have been established from adults with AML-M7 which share common features including: (1) the expression of platelet-associated (ie CD41, CD61) and early myeloid (ie CD34, CD33) antigens; (2) growth factor (commonly interleukin-3, stem cell factor, and GM-CSF) dependence and (3) erythroid marker expression with or without induction with phorbol esters.<sup>1,7–9</sup> Cell lines provide an unlimited supply of DNA, RNA and proteins which permits an extensive evaluation of chromosomal and other genetic changes.<sup>10</sup>

Translocations involving the *MLL* gene, which occur in both acute myeloid and lymphoid leukemia, have been demonstrated in approximately 70% of infants with acute leukemias, 5–10% of acute leukemias in all age groups and in 2–3% of cancer patients who develop therapy-related leukemias (reviewed in Refs 11 and 12). *MLL* is involved in translocations with at least 30 different partner genes, 12 of which have been cloned (reviewed in Refs 13–16). These translocations result in an in-frame fusion joining the amino-terminal

portion of *MLL* to the carboxy portion of the partner genes creating novel fusion proteins which are critical for leukemogenesis.<sup>17</sup>

Amplification of some genes has been reported in cancers but rarely in leukemias or in leukemia cell lines. Exceptions are the amplification of the *MYC* oncogene in the HL-60 cell line and of multidrug resistance genes in drug-resistant cell lines.<sup>18,19</sup> More recently, amplification of the *E2F1* transcription factor gene was observed in HEL erythroleukemia cells, and it was hypothesized that overexpression of *E2F1* in erythroid progenitors may stimulate abnormal cell proliferation by overriding negative regulatory signals mediated by tumor suppressor proteins.<sup>20</sup> However, to date there has been no description of an amplification of normal *MLL*. We report the establishment of a new cell line, UoC-M1, which has megakaryoblastic features and amplification of the *MLL* gene.

### Materials and methods

#### Case report

A previously healthy 68-year-old male presented in February 1992, with progressive dyspnea and pancytopenia of several weeks duration. A bone marrow aspirate and biopsy revealed a hypercellular marrow with >50% blast cells. Based upon a review of the bone marrow morphology, the immunophenotype and the cytochemical staining pattern; a diagnosis of undifferentiated acute myeloid leukemia (FAB M1) was made. The patient's leukemia was refractory to chemotherapy and the patient died 3 months after diagnosis.

#### Source of malignant cells

Peripheral blood and bone marrow cells were collected at diagnosis and separated into aliquots for cell culture experiments, cytochemical studies, immunophenotyping and karyotype analysis. The protocol procedures were approved by the Institutional Review Board and informed consent was obtained.

#### Establishment and maintenance of UoC-M1 cell lines

The techniques for culturing the leukemia cells were a modification of previously reported techniques.<sup>21</sup> Briefly, Ficoll-Hypaque gradient separated cells were plated ( $5 \times 10^5$  cells/ml; 0.3 ml) onto 24-well Petri dishes and cultured in an incubator gassed with 5% O<sub>2</sub>, 6% CO<sub>2</sub>, 89% N<sub>2</sub>. Each well contained a feeder layer consisting of media, agar (0.5%) and human serum (10%).

### Cytochemical stains

Peripheral blood, bone marrow and UoC-M1 cells were evaluated for reactivity with myeloperoxidase,  $\alpha$ -naphthyl butyrate esterase,  $\alpha$ -naphthyl acetate esterase (with and without sodium fluoride) and acid phosphatase (with and without tartrate) by established methods.

### Characterization of cellular antigens

Cell surface antigens were evaluated on the patient's peripheral blood and the UoC-M1 cells by indirect immunofluorescence using fluorescein isothiocyanate conjugated antibodies and analyzed by flow cytometry.

### Electron micrographs

Cells were fixed with 2.5% glutaraldehyde, centrifuged, washed with sodium cacodylate and post-fixed with 1% osmium tetroxide. The cell pellet was stained with 1% uranyl acetate, dehydrated and embedded with LX-112 medium. Ultrathin sections were cut, mounted on uncoated 200-mesh grids, stained with uranyl acetate and lead citrate and examined with a JEOL-CXII electron microscope.

### Cytogenetic analysis

Cytogenetic analyses using a trypsin-Giemsa banding technique were performed on the bone marrow cells as previously described.<sup>22</sup> Metaphase cells were prepared following a 24-h culture without mitogens. The UoC-M1 cell line was karyotyped after 2 months of growth *in vitro*. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature.<sup>23</sup>

### Fluorescence *in situ* hybridization (FISH) and spectral karyotyping (SKY)

Metaphase cells were hybridized with various probes as described previously,<sup>24</sup> including painting probes and centromere probes for chromosomes 9, 11 and 16 (Vysis, Downers Grove, IL, USA), probes for *MLL* at 11q23 (Oncor, Gaithersburg, MD, USA), C48 for *AF9* at 9p22,<sup>25</sup> D9S967 at 9p21 and D9S748 at 9q31<sup>26</sup> and *ABL* at 9q34.<sup>27</sup> SKY analysis was performed using 24 fluorescently labeled chromosome painting probes which allows a color display of all the human chromosomes.<sup>28</sup> Metaphase chromosomes were also analyzed using SKY (case 20955 in Ref. 29).

### Southern blot

High molecular weight DNA was extracted from the UoC-M1 cells, digested with *Bam*HI restriction endonuclease, electrophoresed on 0.8% agarose gel, transferred to a positively charged nylon membrane, (Hybond N+; Amersham, Life Sciences, Arlington Heights, IL, USA) and hybridized with the 0.74 kb *Bam*HI *MLL* cDNA probe (which spans exons 5–11, excluding exon 8) as previously described.<sup>14</sup> Hybridization was performed at 42°C and the final washing stringency was  $1 \times$  SSC, 0.1% SDS, at 65°C for 20 min.

### Northern hybridization

Leukemia cell lines with characteristic cell surface markers of the myeloid lineage (BV173, HL-60, KG-1, U937, YK-M2), T-lymphoid lineage (K-T1, SUP-T1, SUP-T3, SUP-T13) and B-lymphoid lineage (SUP-B2, UoC-B6, UoC-B10) were evaluated by Northern blot analysis and the results compared to the UoC-M1 cells. Poly(A)<sup>+</sup> RNA was isolated from cells in log phase growth using the Micro-Fast Track kit (Invitrogen, San Diego, CA, USA). Five  $\mu$ g of poly(A)<sup>+</sup> RNA was electrophoresed on a 0.8% agarose gel, transferred to a nitrocellulose membrane which was hybridized with the 0.74 kb *Bam*HI *MLL* cDNA probe. For determination of RNA integrity and loading, the membrane was hybridized with a  $\beta$ -actin probe.<sup>30</sup>

### Western blot

For whole cell extracts, cells ( $1 \times 10^7$  cells per ml) were washed once in PBS and re-suspended in PBS with protease inhibitors (PMSF, pepstatin, EDTA and aprotinin). Cells were sonicated and extracts were centrifuged at 12 000 g for 10 min, the supernatant aliquoted and snap frozen. Cytoplasmic and nuclear extracts were prepared using a modification of the Dignam method.<sup>31,32</sup> The extracts from  $1 \times 10^7$  cells were incubated on a nutator with either preimmune or immune serum for 1 h at 4°C, then protein A-agarose (Sigma, St Louis, MO, USA) was added and nutated for 1 h at 4°C. SDS sample buffer was added to the agarose-bound proteins which were heated to 95°C and electrophoresed on a 4.5% SDS-polyacrylamide gel. The proteins were electroblotted on an Immobilon membrane (Millipore Corporation, Bedford, MA, USA), blocked with milk, incubated with preimmune or immune antisera against the *MLL* repression domain<sup>33</sup> and processed for chemiluminescent detection according to the manufacturer's guidelines (Amersham).

## Results

### Establishment of the UoC-M1 cell line

In cultures of the bone marrow cells, cell viability gradually fell to less than 1% during the first 14 days of culture. However, during the third week, cell proliferation was observed which continued after the cells had been transferred to suspension culture. The UoC-M1 cell line has sustained growth for >150 passages and has proliferated for more than 4 years in suspension culture.

### Cytochemical stains

While the patient's leukemic blasts were non-reactive when stained with myeloperoxidase and  $\alpha$ -naphthyl butyrate esterase, the majority of the blast cells reacted with  $\alpha$ -naphthyl acetate esterase (which was inhibited by NaF) and acid phosphatase (which was inhibited by tartrate). The UoC-M1 cells had the same staining pattern except that the ANA staining was limited to the Golgi in 25% of the cells.

### Immunophenotype

The patient's leukemic cells expressed CD7, CD24, CD34, CD38, CD45, HLA-DR and CD61 (Table 1). The T cell antigen

**Table 1** Immunophenotype of the patient's peripheral blood (PB) leukemic cells and the UoC-M1 cell line

Lineage	Cluster of differentiation	PB cells (%)	UoC-M1 (%)
T-lymphoid	CD1a (T6)	1	1
	CD2 (T11)	11	1
	CD3 (T3)	14	2
	CD4 (T4)	7	2
	CD5 (T1)	14	1
	CD7 (Leu9)	80	100
	CD8 (T8)	6	2
	B-lymphoid	CD10 (J5)	5
CD19 (B4)		2	1
CD20 (B1)		1	1
CD24 (BA1)		90	100
kappa		1	1
lambda		1	1
Myeloid	CD13 (My7)	7	100
	CD14 (My4)	10	1
	CD14 (MO2)	5	1
	CD15 (LeuM1)	10	1
	CD33 (My9)	3	100
Platelet associated	CD61 (GPIIIa)	13	100
	CD41 (PLT-1)	ND	1
	GPIb	ND	1
	GPIIb/IIIa	ND	52
Others	CD24 (IL2R1)	2	88
	CD34 (HPCA)	68	100
	CD38 (Leu17)	78	100
	CD45 (KC56)	96	100
	CD56 (NKH-1)	5	1
	HLA-DR (12)	50	99
	Glycophorin	ND	1

ND, not determined.

**Table 2** Karyotype analysis of the patient's bone marrow cells and the UoC-M1 cell line

Cells analyzed	Karyotype <sup>a</sup>
Bone marrow (AML) <sup>b</sup>	43,X,-Y,add(5)(q13),dic(5;9)(p15;p13),-7,+der(9)t(9;19)(q11;q11),dic(9;?;16;?)(9qter->9p13::?:16p11->16q22::?)-11,+16,dic(16;21)(q11;p12),der(17)t(7;17)(p14;p12),-19,-19,+mar1,+mar2,+mar3[21]
UoC-M1 <sup>c</sup>	Related clonal abnormalities in nine cells 42,X,-Y,der(9)t(Y;9)(q1?2;p22)t(9;19)(q1?2;p12 or q12),del(5)(q1?2q3?4),der(5)t(5;9)(p1?5;q1?3),-7,-9,dic(11)t(9;11;19),der(14;21)(q10;q10)del(14)(q1?3),-16,der(17)t(7;17)(p14;p12),-19,der(19)(p1?1q1?2),+21,der(21)t(11;21)(q22;q22)dup(21)(q11q22),+der(21)t(16;21)(p11;p11),der(22)t(19;22)(p12 or q12;p1?1)[5]

<sup>a</sup>The difference in the modal chromosome number is related to a change in nomenclature; dicentric chromosomes are currently counted as one chromosome.

<sup>b</sup>Standard G-banding analysis.

<sup>c</sup>Using FISH and SKY analysis.

CD7 is expressed on about one third of patients with AML M1 and, whereas the B-lymphocyte antigen CD24 is usually not evaluated in myeloid leukemia, it has been reported on some myeloid cell lines.<sup>34,35</sup> The UoC-M1 cells expressed this same antigen profile as well as the myeloid lineage antigens CD13 and CD33. Some of the patient's leukemic cells (13%) expressed CD61 (Table 1) while all of the UoC-M1 cells expressed this antigen which suggests that the UoC-M1 cell were an outgrowth of this subpopulation of cells. The UoC-M1 cells expressed GPIIb/IIIa but lacked expression of CD41 and Gp1b. The expression of myeloid and platelet-associated antigens suggests a megakaryoblastic lineage.<sup>36</sup>

### Electron micrographs

The UoC-M1 cells displayed prominent nucleoli and a high nuclear-cytoplasmic volume ratio (Figure 1a). The cytoplasm contained abundant polyribosomes, a well-developed Golgi complex, numerous mitochondria, and some rough endoplasmic reticulum. Also both electron dense and 'bull's eye' granules (an electron-dense core with a peripheral electron-lucent zone) were identified (Figure 1b, c) features which were present in other megakaryoblast cell lines.<sup>2,5</sup> Although irregular cytoplasmic projections were observed frequently, no membrane demarcations were noted.

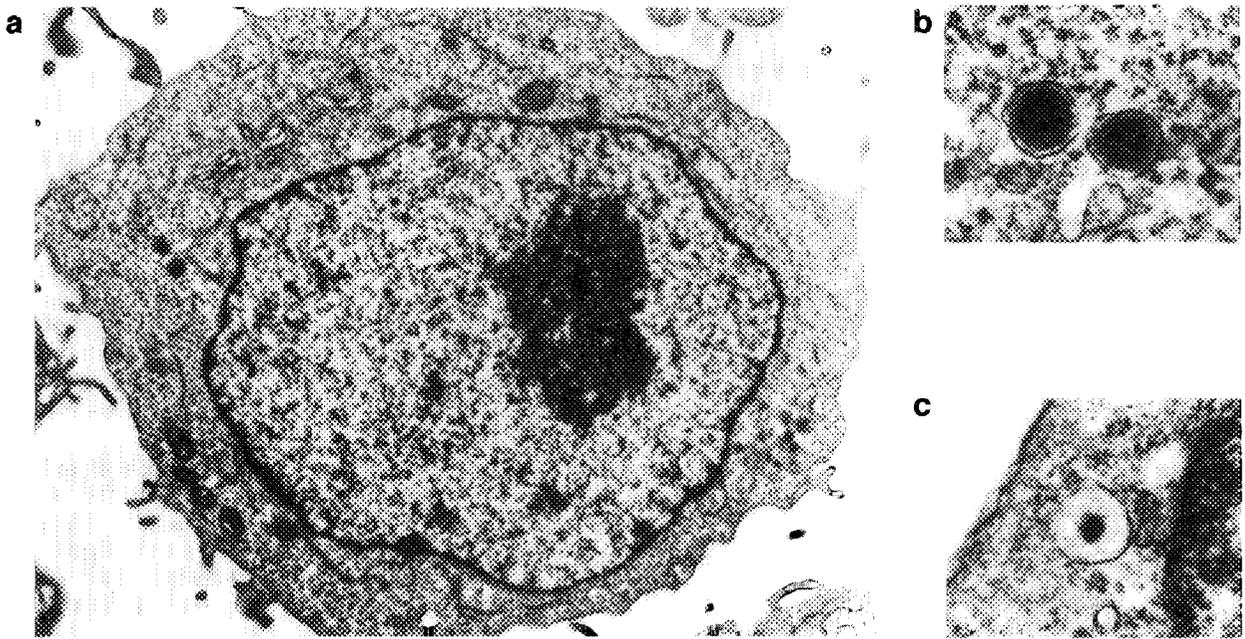
### Karyotype analysis

The G-banded karyotype of the patient's bone marrow cells demonstrated an abnormal mosaic karyotype with multiple complex changes. Specific chromosome gains and losses as well as the sites of many of the chromosome breaks were identical in the patient's karyotype and the UoC-M1 cell line (Table 2, Figure 2).

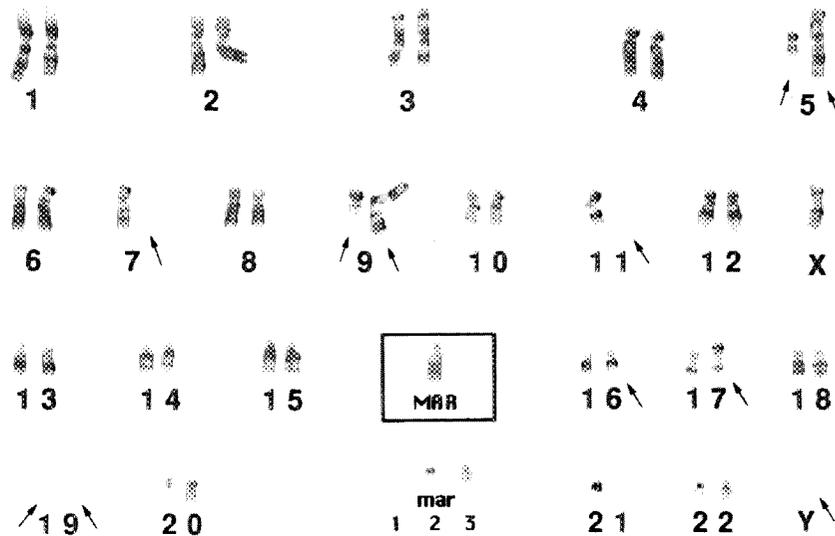
### FISH and SKY

Hybridization of the UoC-M1 cells with FISH probes and SKY analysis helped to clarify the composition of several of the rearranged chromosomes. The large marker chromosome, originally thought to be a 9;?;16;? dicentric chromosome, was shown to be a very complex rearrangement involving chromosomes 9, 11 and 19 (Figure 3a). Using the painting probes for 9, 11 and 16, this chromosome was shown to contain three segments of chromosome 9 interspersed with three segments of chromosome 11 (Figure 3a, b and summarized in e). Using the *MLL* probe, two copies of *MLL* were observed in the marker (Figure 3a) indicating that there was a duplication of 11q including 11q23. The other two segments of chromosome 11 in this marker each included the centromere so that this chromosome is a dicentric chromosome 11 (dic(11), Figure 3e). The three probes for 9q (*ABL*, D9S967 and D9S748) all hybridized only to the opposite end of this large marker chromosome; there was no hybridization with probe C48 at 9p22 or with the chromosome 9 specific probe (qh) so the origin of the other two segments of chromosome 9 is unknown (Figure 3b, c, d, e). With SKY, the marker was shown to contain part of chromosome 19 (Figure 4 and Ref. 29, case 20955) and the provisional description of this chromosome is 9qter->9q21::11?p11->11?q11::9::11?p11->11?q11::9::19::11q?12->11q2?4::11q2?2->11qter.

FISH analysis of another rearranged chromosome showed



**Figure 1** Transmission electron micrographs. (a) Micrograph of the UoC-M1 cells  $\times 16\,000$ . (b) Micrograph of two dense granules  $\times 52\,000$ . (c) Micrograph of a 'bull's eye' granule  $\times 58\,000$ .

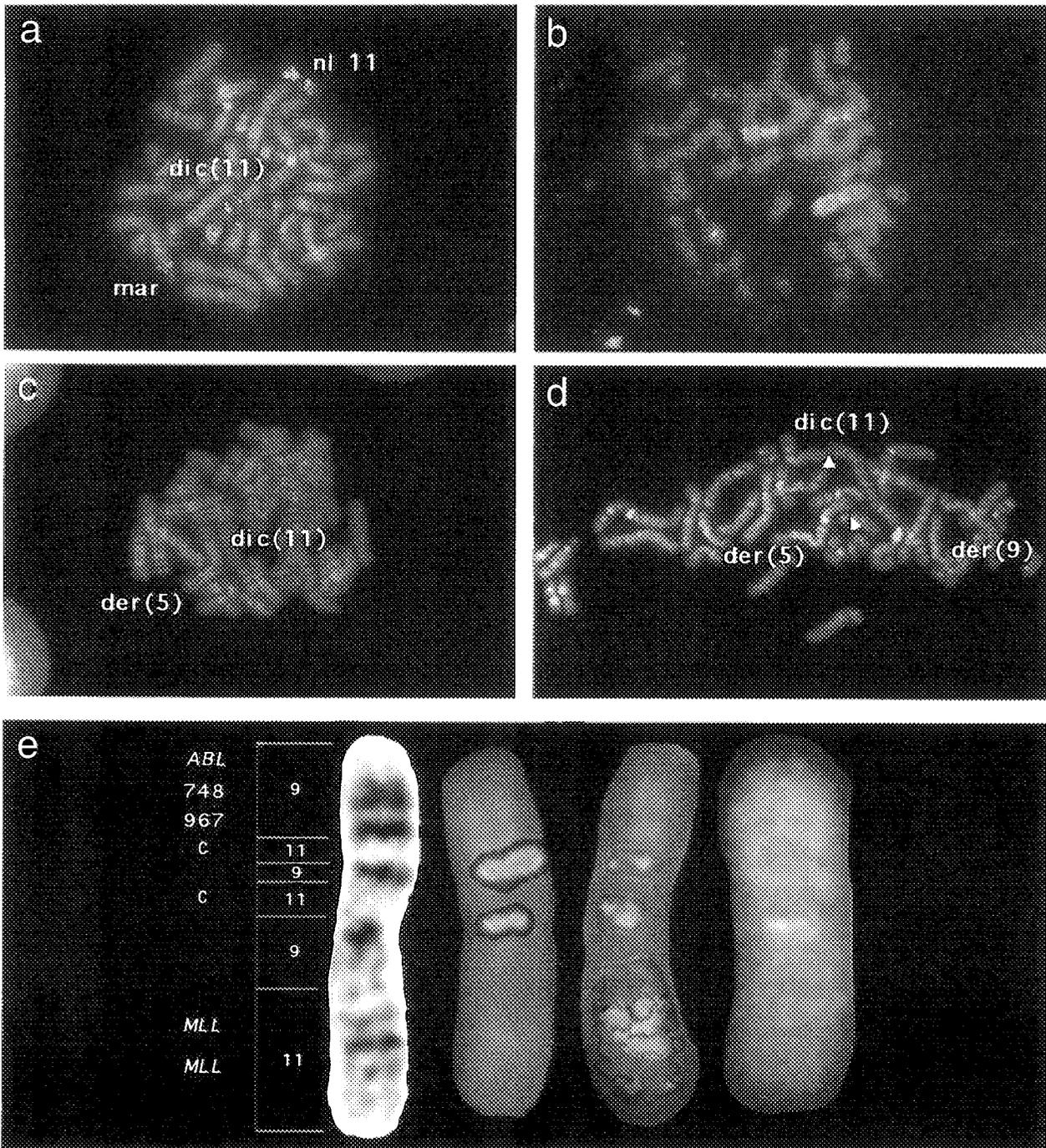


**Figure 2** Karyotype of metaphase cell from the cell line harvested in May 1992 which is virtually identical to that of the leukemic cells obtained at diagnosis in March 1992. Both chromosomes 5 are abnormal; the left hand 5 shows a deletion of 5q, whereas the right hand 5 shows the translocation of 9q to 5p. Both chromosomes 9 are abnormal; the left hand 9 shows the translocation interpreted as 9p and 19p or q; the right hand 9 is the complex rearrangement of chromosome 9 that was thought to involve chromosome 16 as well. The second 16 is involved in the translocation with 21q and the second 17 is involved with chromosome 7. There are no normal chromosomes 19 and there are a number of small markers and one larger marker found in the cell line. The larger marker is the  $\text{der}(21)\text{t}(11;21)$  and the small markers (mar 1, 2 and 3) involved chromosome 19 (see Table 2 for complete karyotype).

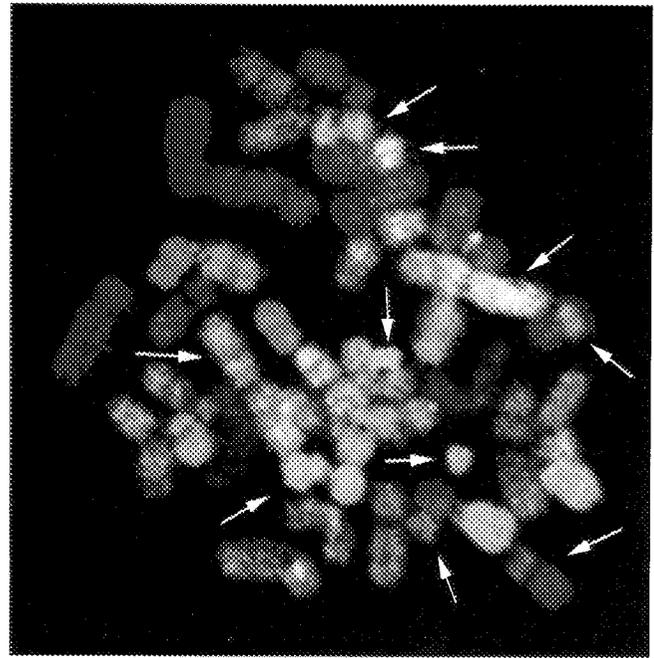
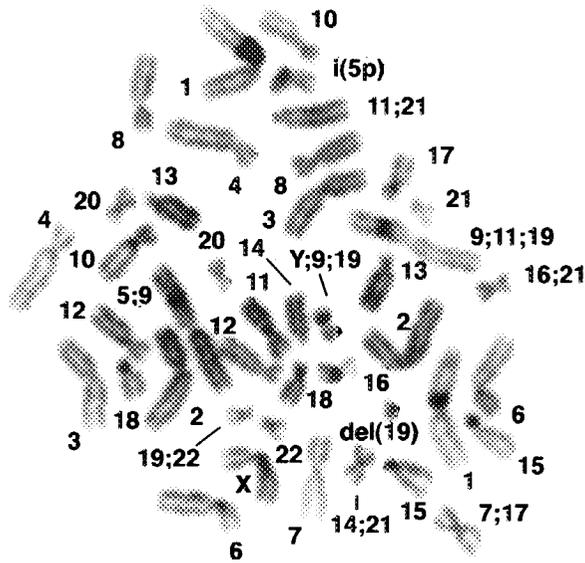
an acrocentric marker that contained 11q material including one copy of *MLL* at the end of the long arm (Figure 3a, b). SKY analysis showed that the centromere and proximal portion of the marker contained chromosome 21 which was present in at least duplicate (Figure 4). Thus, for chromosome 11, there was one normal 11 and two marker chromosomes with a total of four copies of *MLL*.

A second large marker chromosome, originally identified as a dicentric 5;9 was shown to lack any chromosome 9 centro-

mere material and therefore it should be described as a  $\text{der}(5)$  chromosome. A single copy of each of the three probes for 9q but not for 9p was present (Figure 3c, d). The analysis was confirmed by SKY, so that this chromosome presumably contains virtually all of chromosome 5 with 9q translocated to the short arm of chromosome 5. The  $\text{der}(9)$  chromosome (Figures 3b, 2d and 4), which was thought to involve only 9 and 19 on standard karyotyping, was shown by SKY also to contain material from the Y chromosome. The Y chromosome was



**Figure 3** FISH analysis. (a) Metaphase cell hybridization with chromosome 11 painting probe (pink) and the *MLL* probe (green). The normal chromosome 11, dic(11) and marker were labeled and contain one, two and one copies of *MLL*, respectively. (b) Metaphase cell hybridization with chromosome 9 (green) and 11 (orange) painting probes. The normal 11, dic(11) and markers were labeled with the chromosome 11 painting probe as in panel a (orange) and the chromosome 9 components of the dic(11) (right), der(5) (top) and der(9) (just to the right of top) were labeled in green. (c) Metaphase cell hybridized with probes D9S967 (red) at 9q21 and D9S748 at 9q31 (green). The dic(11) and the der(5) chromosomes each had a single copy of these probes with D9S748 more telomeric than D9S967. (d) Metaphase cell hybridized with probes C48 (red) at 9p22 and *ABL* (green) at 9q34.2. C48 labeled the der(9) chromosome and the *ABL* labeled the dic(11) and der(5) chromosomes as identified by the arrowheads. (e) Composite of the dic(11) with a diagram of the position of chromosome components and genes at the very left (c is centromere), followed by a G-banded chromosome and examples of hybridization (left to right) with the centromere 11 probe (red); the *MLL* (green) and chromosome 11 painting probes (red) (from panel a); and painting probes for chromosome 9 (green) and 11 (orange) (from panel b). Composite (e) does not include the location of chromosome 19 material because a probe for chromosome 19 was not used in these studies.



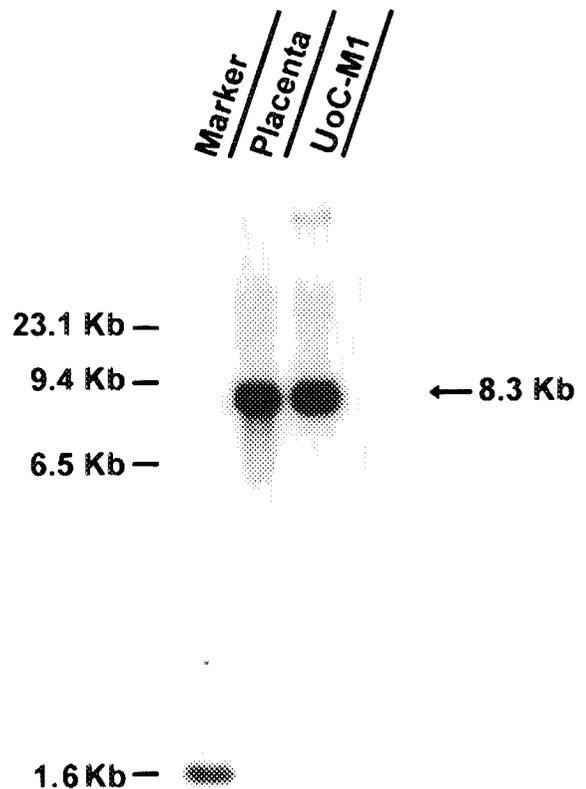
**Figure 4** SKY analysis of the UoC-M1 cell line. Left panel, DAPI (4'6-diamidino-2-phenylindole dihydrochloride) image with identification of each chromosome. Right panel, the spectral karyotyping image of the same cell (case 20955).<sup>29</sup>

attached to 9p whereas chromosome 19 was attached to the 9 centromere (Figure 4). Thus, for chromosome 9, there was no normal 9, one copy of 9p and 9 centromere, and two copies of 9q, as well as other unidentified chromosome 9 material.

Based on the SKY analysis, the add 5(q13) could also be del(5q), the der(21)t(16;21) and der(17)t(7;17) were confirmed and the origins of three small markers were clarified. Mar1 contained material from 14q and 21q, mar2 contained material from 19 and 22 and mar3 was a chromosome 19 which lacked most of both the long and short arms. As detected by FISH and SKY analysis, the multiple chromosome rearrangements resulted in loss of part of the Y chromosome, loss of 5q (5q13 to q34), loss of most of chromosome 7 (either all of 7q and part of 7p or all of 7p and most of 7q), loss of part of chromosome 9 (possible 9p), duplication of 11q resulting in four copies of *MLL*, loss of about 2/3 of 11q, loss of part of 16 (possible 16q), loss of 17p, loss of part of chromosome 19, duplication of 21q resulting in at least five copies of 21q and loss of part of 22 (probably 22p).

#### Southern blot

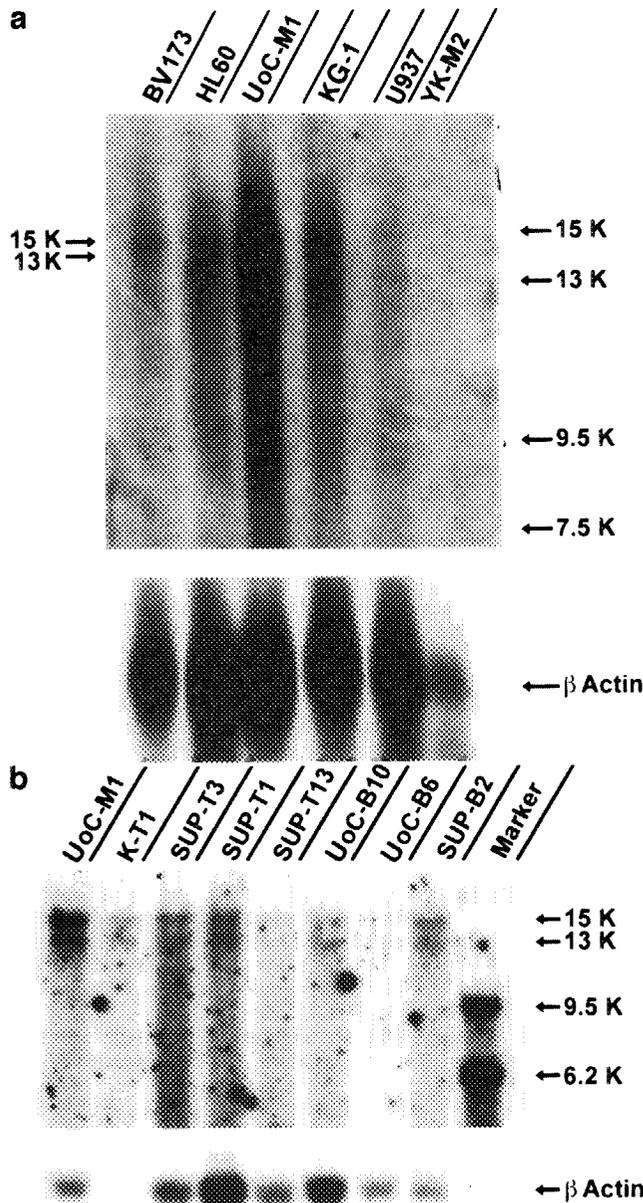
After finding that the UoC-M1 cells contained four copies of *MLL*, we wished to determine whether *MLL* was rearranged in this cell line. Using the *MLL* breakpoint cluster region probe (which detects virtually all *MLL* rearrangements),<sup>14</sup> a single 8.3 kb *Bam*HI fragment was detected which co-migrated with the unrearranged gene (Figure 5). Thus, even though two additional copies of *MLL* are present on one of the marker chromosomes, there is no rearrangement of the *MLL* breakpoint cluster region; on FISH analysis, the size of the *MLL* signal is similar to that seen on the normal chromosome 11.



**Figure 5** Southern blot analysis of placental DNA and UoC-M1 DNA. Comigration of the 8.3 kb bands indicated that there was no rearrangement of the *MLL* gene in the UoC-M1 cell.

Northern blot

*MLL* RNAs were normal in size (15 kb and 13 kb), the UoC-M1 cells expressed relatively more *MLL* RNA than the myeloid (Figure 6a) and lymphoid cell lines (Figure 6b) examined. Because both messages were equally overexpressed, *MLL* may be overexpressed as a result of duplication of the *MLL* gene plus the surrounding promoter and/or enhancer elements. There was no expression of additional aberrant-sized *MLL* RNAs, which is consistent with the germline configuration of the DNA.



**Figure 6** Northern blot analyses of the UoC-M1 cells and myeloid cell lines (a) and T- and B-lymphoid cell lines (b) using the 0.74 kb *MLL* probe. The arrowheads correspond to the normally expressed 15 k nt and 13 k nt messages. The UoC-M1 cells overexpressed the *MLL* mRNA relative to the other cell lines evaluated.

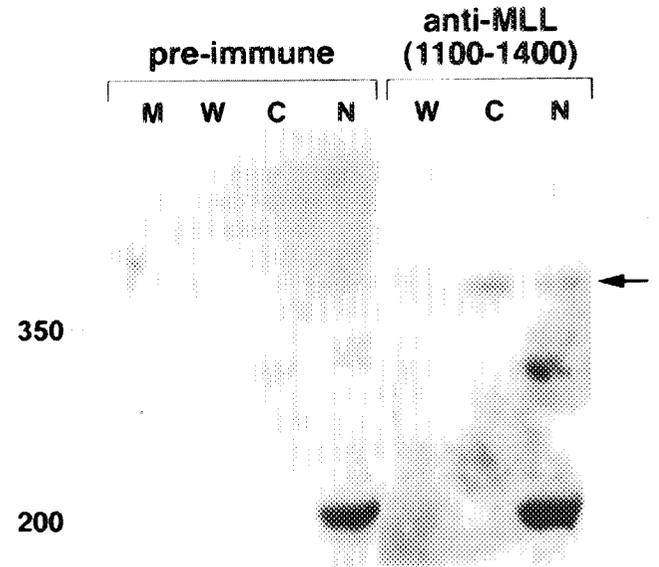
Western blot

A protein analysis was performed using a *MLL* domain-specific rabbit polyclonal antisera against bacterially expressed protein that corresponds to the *MLL* repression domain (amino acids 1100–1400).<sup>33</sup> This antibody was used to immunoblot *MLL* protein from the UoC-M1 cells. A specific *MLL* protein (>350 kDa) was detected in whole cell extracts, as well as in the nuclear and cytoplasmic fractions (Figure 7).

Discussion

We report the establishment and initial characterization of a new cell line, UoC-M1, which has megakaryoblastic features. The UoC-M1 cell line has good general concordance with the patient's primary leukemia as evaluated by cytochemical staining, immunophenotyping and karyotype analysis. UoC-M1 cells express antigens which are associated both with undifferentiated megakaryoblasts, as well as mature platelets. In comparison with other cell lines with megakaryoblastic features, UoC-M1 cells are similar to ELF-153 cells because both cell lines: (1) were established from adults with poorly differentiated myeloid leukemia; (2) have monosomy 7 and loss of 5q; (3) lack erythroid markers; and (4) were growth factor independent.<sup>2</sup> These cell lines are distinct, though, because the ELF-153 cells did not express CD38, had a t(12;14)(p11.2;q11.2) and some of the cells were polyploid (up to 32N). Also, the ELF-153 cells proliferate in response to IL-3 and GM-CSF while the UoC-M1 cells were not stimulated by these factors in serum-containing or serum-free media (data not shown). However, both cell lines grew in limiting dilution experiments and probably are dependent upon autocrine growth factors.<sup>37</sup>

Chromosome analysis of the UoC-M1 cells by standard G-banding identified a complex karyotype with many complicated chromosome rearrangements and markers. FISH analy-



**Figure 7** Western blot analyses, using either pre-immune or immune antisera, were performed on extracts of the UoC-M1 cells: whole cell (W), cytoplasmic (C) or nuclear (N). The arrowhead corresponds to the full length *MLL* protein of approximately 430 kDa. The *MLL* protein was detected in both the cytoplasmic and nuclear fractions of the UoC-M1 cells. M, protein markers.

sis using probes for chromosomes 9, 11 and 16, revealed that the large marker chromosome had three segments of chromosome 9 interspersed with three segments of chromosome 11 resulting in duplication of *MLL*. Using painting probes, the location of material from chromosome 9, 11 and 16 was determined and, with specific DNA probes, the location of 9p, 9q, 9 and 11 centromeres and *MLL* were identified. Using SKY analysis, the location of all chromosome segments could be defined and thus, in one hybridization, we determined that the large marker contained part of chromosome 19, as well as 9 and 11. Moreover, we found that the proximal part of the large acrocentric marker was chromosome 21 (present in at least two copies), and that a marker thought to contain chromosome 9 and 19, also carried much of the Y chromosome. However, although SKY can identify the chromosomal origin, it cannot define the region of the chromosome; this requires band-specific or gene-specific probes. Nonetheless, SKY analysis is a powerful new tool which will help elucidate complex karyotypic abnormalities occurring in normal and malignant cells.<sup>28,29</sup> Our unique analysis of the chromosomes of the UoC-M1 cells with G-banding techniques, SKY and specific FISH probes illustrates the complementarity of the three techniques.

This is the first report of a cell line that contains an amplification of the normal germline configuration of the *MLL* gene. Previously, Rovigatti et al<sup>8</sup> reported a patient with AMMoL who had a homogeneously staining region at 11q23 and a 30-fold amplification of *ETS1*. Because *MLL* is located at the same chromosome region, it is possible that *MLL* was amplified in this patient's leukemic cells. Translocations involving *MLL* are found in acute lymphoid leukemia, in *de novo* acute myeloid leukemia and in acute myeloid leukemia associated with chemotherapy with DNA topoisomerase II-targeting drugs.<sup>11-16</sup> The translocations involving *MLL* are varied and up to 30 different partner genes may be involved.<sup>11-16</sup> One of the rearrangements that has been cloned is a partial duplication of *MLL* which results in an aberrant in-frame self-fusion protein which contains an additional amino portion of *MLL*.<sup>15,39,40</sup> There has, however, been no observed amplification of the normal *MLL* gene. In the UoC-M1 cells, four copies of the *MLL* gene are present resulting in a higher level of *MLL* RNA compared with other cell lines. It is probable that the *MLL* promoter or enhancer sequences (which have yet to be identified and characterized) have been amplified along with the coding sequences, resulting in the higher level of *MLL* expression. We also demonstrate detection of the putative full-length *MLL* protein by immunoblotting. The difficulties of working with an extremely large protein (430 kDa), as well as the fact that very little of the *MLL* RNA and protein are produced, has made analysis of this protein very challenging. UoC-M1 cells are useful because they overexpress the normal *MLL* protein. We are attempting to identify targets for the normal *MLL* protein function to determine whether overexpression of a normal *MLL* protein had contributed to the leukemia phenotype in this patient.

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