Key words
TTV DNA; blood donors; ALT.

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Karyotype refinement in five patients with acute myeloid leukemia using spectral karyotyping

Five patients with acute myeloid leukemia showing several complex rearrangements were examined by spectral karyotype (SKY) analysis. Twenty rearrangements, misclassified or undetected by standard cytogenetic techniques, were identified. The present study supports the ability of SKY to detect the organization of chromosome rearrangements and to identify new prognostic markers.

Sir,

Highly rearranged karyotypes and indecipherable marker chromosomes often found in leukemia cells are usually difficult to define using standard cytogenetic techniques. Fluorescence *in situ* hybridization (FISH) has partially overcome these limitations by using specific chromosome probes.[1,2] More recently, spectral karyotype (SKY) analysis, based on the cohybridization of 24 fluorescently labeled chromosome painting probes provides precise identification of marker chromosomes and cryptic translocations.[3,4] Improvement in karyotype definition is important for investigating genes involved in hematologic diseases. We studied five patients with different acute myeloid leukemia (AML) showing complex chromosome rearrangements at standard cytogenetic investigation. SKY analysis identified six marker chromosomes, 11 translocations and three insertions which were undetected by G banding (Table 1). In the first patient with an AML-M0 subsequent to a RAEB, SKY analysis showed a number of anomalies including a t(11;12) and a trisomy 11q (Figure 1) which allowed us to uncover loss of the TEL gene and triplication of the MLL gene. TEL on 12p12 and MLL on 11q23 have been implicated in development of either myelodysplastic syndrome (MDS) or acute leukemia.[1,5-6] We suggest that loss of TEL had a role in the development of MDS while the other abnormalities, including amplification of MLL, could have been implicated in acute transformation as well as in the severe and rapid course of the disease; indeed this patient was unresponsive to therapy and died three months later. In the second patient SKY analysis, performed during RAEB to AML transition, showed a t(1;15) together with a 12q insertion onto 5q13. Rearrangements involving the 5q arm are well documented both in MDS and in AML patients.[1,7] We argue that this rearrangement was the primary cause of RAEB and in association with other chromosome changes had a role in the acute transformation into AML. The third patient with AML-M1 had a trisomy 8 and i(8q) resulting in a pentasomy 8q which SKY analysis revealed was associated with i(5p). Both pentasomy 8q and i(5p) have rarely been reported in AML.[1,8] Chromosome 8 numerical abnormalities are known to have a poor prognostic significance.[1] We suggest that pentasomy 8q, through gene amplification, was implicated in the origin and in the severe course of the disease (three months survival) while a role for i(5p) is unclear at present but requires further study. In the fourth patient with a diagnosis of AML-M2 without typical t(8;21) as shown by FISH, SKY revealed del(5)(q13) which could well be implicated in the origin of AML.

Table 1. Hematologic, cytogenetic and SKY data in the five AML patients.

Pts	Sex/ age(years)	Disease	Treat.	Survival in mos.	GTG banding	Karyotype by SKY	FISH
1	N.D. M/55	M0	DCA	5	46,XY,-2,-5,der(9)t(9;?),der(11)t(11;?)(p12;?),-12, add(13)(q21),add(17)(q25), +3mar [12]	46,XY,-2,-5,der(9)t(3;9)(?;p24)ins(3;14)(?;q?)t(9;13)(q34;q?),der(11)t(11;12)(p12;q13),+der(11)(p11),-12,der(13)t(2;13)(?; p10),der(13)t(13;20)(q12;?) der(16)t(16;18)(q24;?),der(17) t(14;17)(q?;q25),+20,+21	Confirmed.
2	M.A. F/75	M1	no	3	46,XX,t(5;12)(q26;q21) [8] 45,XX,-12,i(15)(q10) [12]	46,XX,ins(5;12)(q13;q14q24) 45,XX,-12,der(15)t(1;15)(p12;p10)	Painting for chromosomes 1, 12, and 15 confirmed t(1;15) and ins(5;12).
3	D.E. F/62	M1	DCA	3	48,XX,+8,+i(8)(q10) [12] 49,XX,+8,+i(8)(q10),+mar [8]	48,XX,+8,+i(8)(q10) 49,XX,+i(5)(p10),+8,+i(8)(q10)	Confirmed.
4	I.A. F/74	M2	CAT	2+	41,XX,add(1)(p32),add(1)(p32),5,del(5)(q13),-9,-13,-17, add(18)(q23),add(20)(?),-22 [18]	41,XX,-1,der(1)t(1;17)(p32;q?),del(5)(q13),-9,-13,-17,der(18)t(1;18)(p32;p10),der(19)t(5;9;19)ins(9;1),der(20)t(9;13;20)(p12;?;?),-22	Confirmed; p53 probe showed the presence of 1 copy only.
5	T.D. F/34	M4	DCA+BMT	7+	47,XX,inv(11)(p15q23-25),+mar [20]	47,XX,t(10;11)(p?;q23)inv(11)(p15q23),+der(21)(q21)	Confirmed; MLL probe showed split signals on 10p and inv(11q)

Pts.=no. treat: treatment; +: alive patient; SKY: spectral karyotyping; FISH: fluorescence in situ hybridization; DCA: daunomicine and cytosine arabinoside; CAT: cytosine arabinoside and thioguanine; BMT: allogeneic bone marrow transplantation; [ ]: number of metaphases.

Figure 1. SKY karyotype in patient 1 showing der(9) as resulted from a rearrangement involving chromosomes 3,9,13 and 14, der(11) as from t(11;12)(p12;q13), add(13) as from t(13;20)(q12;?), add(17) as from t(14;17)(q24;q25), and the four marker chromosomes as der(13)t(2;13)(?;p10), +del(11)(p11), +20 and +21. Numbers to the right of the classification color images indicate the chromosomal origin.

Moreover, other abnormalities, in particular t(1;17) with p53 loss, could be associated with rapid disease evolution.[9] Standard cytogenetic analysis of the fifth patient with an AML-M4 showed no inv/t(16;16) typically associated with this FAB subgroup.[1] A small marker chromosome was found and identified by SKY analysis as a der(21)(q22) in which the AML1 gene, on 21q22 and rearranged in different leukemias,[1] was lacking. Therefore AML1 appears to have had no role in the origin, progression and disease relapse after bone marrow transplantation in this patient. Following transplantation, by combining cytogenetic, SKY and FISH results, a complex rearrangement between chromosomes 10 and 11 was detected which resulted in disruption of the MLL gene. This mutation likely contributed to the rapid recurrence of the leukemia. In conclusion, the SKY approach allowed us to define the karyotype in all patients

studied by classifying complex rearrangements and detecting cryptic abnormalities overlooked by standard cytogenetics. In addition, SKY analysis resulted to be a relevant tool in disclosing the origin and mapping progression of disease in four of the five patients revealing AML specific gene and breakpoint rearrangements.

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Acute leukemia in Jehovah's Witnesses: a challenge for hematologists

With the aim of demonstrating the possibility of treating acute leukemia in Jehovah's Witnesses by protocols which include a marrow transplantation procedure without need of transfusions, we report our experience on 5 patients. Three patients had acute lymphocytic leukemia and two acute myeloid leukemia, two received autologous bone marrow transplants and one bone marrow transplantation without transfusions. One died because of anemia during induction. Our conclusion is that acute leukemia in Jehovah's Witnesses can be treated with protocols employed in transfused patients.

Sir,

Jehovah's Witnesses object to transfusions of blood products; thus, the management of acute leukemia in these patients is of great difficulty. Reports emphasize the lower rate of remissions and cure of Jehovah's Witnesses with acute leukemia, because of the reduced cytotoxicity of the regimens adopted in these patients.[1,2] Here we report on five recent cases of Jehovah's Witnesses with acute leukemia, trying to give an example that could be useful for physicians faced with a similar challenge. Table 1 summarizes the patients' data.

Case #1 was an 11-year old boy admitted to hospital October 1998 because of hyperleukocytosis and T-cell acute lymphocytic leukemia (T-ALL) ALL-T (blast cells $320x10^3/\mu L$). His hemoglobin was normal. He received induction chemotherapy according to the AIEOP 9503 protocol. Hematologic recovery occurred day +30 without him needing transfusions. Because of persistence of blasts he received a bone marrow transplant (BMT) from his brother with chemotherapy (CTX) and fractionated total body irradiation (TBI) as conditioning treatment without transfusions; he relapsed and died eight months following BMT.

Case #2 was a 24-year old man with acute myeloid leukemia (AML)-M2; he received a combination of idarubicin (10 mg/m^2) fludarabine (25 mg/m^2 days 1 to 5) and aracytin (2 g/m^2 days 1 to 5). Erythropoietin was started on day 5 but his hemoglobin dropped to 2.7 g/dL and the platelets became uncountable. Nevertheless, the patient recovered from cytopenia around day +20 and achieved CR. After a second course of therapy, he underwent autologous PBSC with busulfan 12 mg/kg and melphalan 120 mg/m^2 as conditioning therapy. He relapsed and died