Karyotype refinement by multicolor fluorescence *in situ* hybridization analysis in 18 patients with acute lymphoblastic leukemia

Eighteen patients with acute lymphoblastic leukemia (ALL) were investigated using spectral karyotyping (SKY), and multicolor fluorescence in situ hybridization (FISH) banding analyses. SKY analysis confirmed previous results in patients with G-banded diploid karyotype. Nevertheless in three out of five patients with two or more chromosomal abnormalities, SKY and multicolor banding methods refined the abnormal karyotypes and revealed cryptic anomalies.

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Fluorescence in situ hybridization (FISH) analysis with painting and locus/gene specific probes has greatly improved the detection of chromosome abnormalities in leukemia. However highly rearranged karyotypes and indecipherable marker chromosomes often remain difficult to identify.[1] Two recent approaches, spectral karyotype (SKY) analysis, based on the hybridization of 24 chromosome painting probes labeled with different fluorochromes, and the new multicolor FISH banding technique allow precise identification of subtle translocations, marker chromosomes, and complex chromosomal anomalies.[1-2] At present only a few results have been published for acute lymphoblastic leukemia (ALL) patients.[3-6]

Eighteen patients (age range: 1-78 years) were enrolled in the present study. Seven patients had a T-cell phenotype, seven a common phenotype, and four showed a pre-B cell phenotype. In agreement with recent reports, SKY analysis in five patients with two or more chromosomal abnormalities confirmed the same anomalies previously identified by standard G-banding in two cases (patients #1-2) and defined karyotypes more precisely in the other three (patients #3-5) (Table 1). Patient 3 with a T-cell ALL had two reciprocal translocations including a t(12;20)(p13;q12) which was redefined by SKY as t(11;20)(p13;q12) (Figure 1a). Rearrangements affecting 11p13 were found to involve genes with a central role in the regulation of the adult hematopoietic pathway. However no instance of t(11;20) has been reported so far.[7] In patient #4 with a common subtype two clones were observed, including one normal and one hyperdiploid with add(7) that SKY defined as a der(7)t(7;8)(q3;?) (Figure 1b). Rearrangements at 7q34-35 have been reported in a few patients with ALL, while partial or complete trisomy 8 has been described as an additional anomaly in ALL with complex karyotypes.[5] Moreover, using a multicolor banding probe for chromosome 7 (Zeiss-Metasystems, Milan, Italy), a deletion on 7q34-36 was also detected at the site of fusion with chromosome 8 material, revealing a cryptic, more complex rearrangement than previously thought in this patient (Figure 1b, inset). In patient #5, with a T-ALL phenotype, SKY analysis defined a derivative chromosome 1 as dic(1;9)(p36;p21) and FISH analysis with YAC clone 960C6 showed loss of the p15/p16 region on 9p (data not shown). Dicentric chromosome 9 with a breakpoint on the short arm has been reported in both B- and T-ALL, mostly rearranged with chromosome 12 or 20.[8] We are not aware of previous reports of chromosome 1 involvement in chromosome 9p dicentric formation. Dicentric chromosome 9 in T-ALL individuals over 10 years old has an unclear prognostic significance,[8] while chromosome 1p36 deletion harboring loss of tumor suppressor genes mapping to this region could be related to a short survival.[9] SKY analysis also revealed a previously undetected translocation t(10;11)(p12;q21). FISH analysis on metaphases using YAC clones for the AF10 gene (807D3), and for 5'CALM region (914D9) has shown fusion signals to be due to a CALM/AF10 rearrangement which results in a poor clinical outcome.[10]

A recent SKY analysis study in a series of ALL patients with normal karyotype showed the presence of cryptic translocations in two out of 20 individuals.[5] In the present study SKY analysis,

Table 1. Karyotype of six ALL patients with abnormal karyotypes as defined by GTG banding, FISH and SKY techniques.

Pt. n.	*Sex/age*	*Disease/diagnosis*	*Karyotype by*			*Survival (months)*
			GTG banding	*SKY*	*FISH*	
1	M/7	ALL common/07.90	46,XY [14] 47,XY,+i(1q),t(8;22)(q24; q13), t(14;18)(q32;q22) [6]	46,XY [12] 46,XY,+i(1)(q10),-6,t(8;22),t(14;18) (q32;q22) [6]	confirmed	5
2	F/31	pre B-ALL/05.00	46,XX [13] 47,XX,+X,t(9;22)(q34;q11) [7]	46,XX [10] 47,XX,+X,t(9;22)(q34;q11) [5]	confirmed	7
3	M/28	T-ALL /12.96	46,XY,t(4;10)(q21;q21),t(12;20) (p13;q12) [20]	46,XY,t(4;10)(q21;q21),t(11;20) (p13;q12) [10]	confirmed	14
4	M/2	ALL common/11.96	46,XY [10] 53,XY,+X,+Y,+6,add(7)(q3;?), +14,+17, +18,+21 [10]	46,XY [8] 53,XY,+X,+Y,+6,der(7)t(7;8)(q3;?), +14,+17,+18,+21 [8]	deletion at 7q34-36 by multiband paint 7 probe	34
5	M/20	T-ALL /07.99	45,XY,add(1)(p3?),-9 [20]	45,XY,dic(1;9)(p36;p21), t(10;11)(p12;q21) [12]	p15/p16 deletion, CALM/AF10 fusion gene	15
6	M/16	T-ALL/07.84	46,XY [17] 46,XY,del(17)(p12) [3]	46,XY [20]	TP53 deletion	+212*

*Pt. n: patient number; SKY: spectral karyotyping; FISH: fluorescence in situ hybridization; +alive patient; *bone marrow transplantation.*

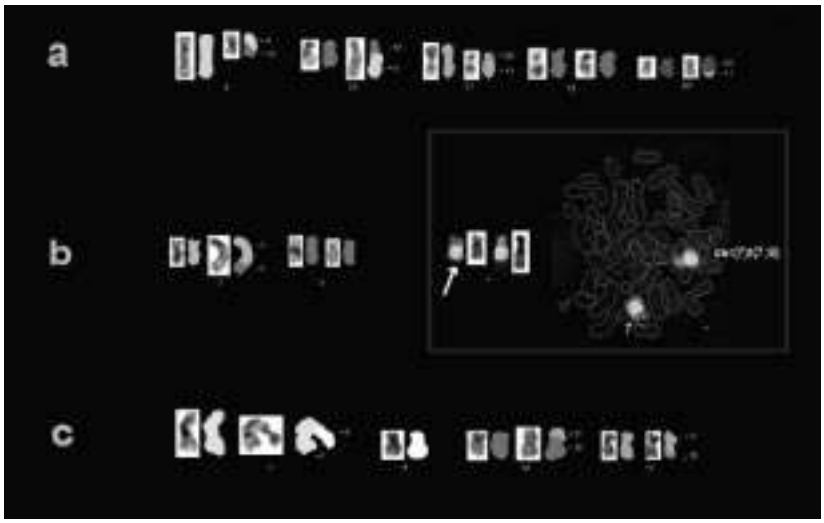

Figure 1. a) in patient #3, SKY karyotyping confirmed a t(4;10)(q21;q21) and allowed reclassification of a t(12;20) as a t(11;20)(p13;q12); b) in patient #4, SKY analysis showed a der(7) resulting from a t(7;8)(q3;?); *inset*: multicolor banding for chromosome 7 displayed a deletion of band 7q34-36 (arrow; orange signals) at the site of rearrangement with chromosome 8; c) in patient #5, SKY analysis classified a marker chromosome as dic(1;9)(p36;p21) and revealed a t(10;11)(p12;q21).

in 12 patients with a normal karyotype using standard G-banding, supported preliminary cytogenetic results in all cases. In a diploid patient (patient #6) with 15% cells with del(17)(p12) and TP53 gene loss as shown by FISH using a specific cosmid probe (Appligene/Resnova, Rome, Italy), SKY was unable to detect any additional anomaly, possibly because intrachromosomal rearrangements such as small deletions and inversions are not suitable for spectral investigation.[1] Indeed, Elghezal et al.[4] have recently reported that very subtle specific rearrangements in ALL patients, such as t(12;21), could be undetected by SKY analysis. Thus, larger series of patients are needed to clarify the usefulness of SKY analysis in ALL cases with normal standard cytogenetic karyotypes. In the present series the SKY method proved highly informative in defining karyotype in ALL patients with two or more chromosome rearrangements by G-banding. Moreover multicolor probe banding analysis, using the same spectral multifluorescence digital system, improved SKY definition of chromosome rearrangements at band level, resulting in a rapid cost-effective approach.

Giuseppe Calabrese,*° Tea Taraborelli,*° Donatella Fantasia,*° Paolo Guanciali Franchi,*° Antonio Spadano,# Giandomenico Palka*°

*Dip. Scienze Biomediche/Sez. Genetica Medica, Università di Chieti; °Serv. Genetica Umana, #Dip. Ematologia, Ospedale Civile , Pescara, Italy

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Correspondence: Giandomenico Palka, M.D., via B. Buozzi 93, 65121 Pescara, Italy. Phone: international +39.0871.3554136. Fax: international +39.0871.3554135.
E-mail: gdpalka@unich.it

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