



CORRESPONDENCE

Further characterization of complex chromosomal rearrangements in myeloid malignancies: spectral karyotyping adds precision in defining abnormalities associated with poor prognosis

TO THE EDITOR

In the last 30 years, information provided by cytogenetic analysis has become indispensable for the clinical management of patients with hematological malignancies. In acute myeloid leukemia (AML), the favorable prognostic subgroup is defined by the presence of leukemic blasts with t(15;17), t(8;21), or inv(16). The unfavorable cases are those with abnormalities involving more than two chromosomes, monosomy 5/5q- or 7/7q-, or rearrangements of the long arm of chromosome 3. The survival rate of this group is less than 20% at 5 years. These patients represent a considerable therapeutic challenge for whom no current treatment approach is satisfactory.¹ The patients with a normal karyotype or cytogenetic abnormalities that are not included in these other categories are characterized as having an intermediate risk of relapse.^{1,2} In myelodysplastic syndromes (MDS), abnormalities of 7q or a complex karyotype are also unfavorable prognostic factors.³ Improvements in treatment of AML, t-AML and MDS have resulted in high complete remission rates even in patients with unfavorable cytogenetics; however, the majority of patients relapse.^{1,3} Therefore, further genetic studies redefining the patients included in the poor prognostic group are necessary.

In order to characterize the karyotype more precisely, we analyzed 18 samples from patients with myeloid malignancies and a complex karyotype using three different techniques: G-banding, fluorescence *in situ* hybridization (FISH), and spectral karyotyping (SKY). The SKY probe mixture and hybridization reagents were obtained from Applied Spectral Imaging (Carlsbad, CA, USA). Slides for spectral karyotyping were hybridized as previously described.⁴ For analysis of cases that had complex markers or non-obvious chromosome rearrangements, FISH experiments were performed using the appropriate painting or centromere-specific probes (Vysis, Downers Grove, IL, USA).

All 18 cases were successfully analyzed using SKY. In the three cases with complete G-band analysis (cases 6, 8, 14), SKY confirmed the G-banding results. In the other 15 cases, the use of SKY substantially improved the precision of karyotype analysis of malignant cells, detecting several unexpected aberrations. The approach of combining three different cytogenetic techniques allowed the identification of hidden translocations and the reconstruction of complex rearrangements. The complete karyotypes after the combined analysis are given in Table 1. Deletions and unbalanced translocations in samples with complex aberrations were particularly prone to misinterpretation based on G-banding alone, especially when chromosomal regions that have a similar G-banding pattern were involved. In 13 cases (1, 2, 4, 5, 7, 9, 11, 12, 13, 15, 16, 17, 18), chromosome material from total or partial monosomies detected by G-banding was found in derivative or marker chromosomes. On the other hand, in seven cases (7, 9, 11, 13, 15, 16, 18) deletions were actually found to be translocations after SKY. This is especially important in cases with aberrations with prognostic significance. Thirteen cases (72%) had monosomy 5 or 7, or abnormalities of 3q, 5q or 7q. SKY confirmed the G-banding results in six of these cases and detected new abnormalities in seven others. Three of our cases were -5 (cases 7, 9 and 13); based on SKY, all three were changed to a deletion 5q, eg loss of chromosome 5, bands 5q31 to 5q33 (case 7); translocation of parts of 5 to a der(7) and a der(13), that resulted in loss of chromosome 5, bands 5q13 to 5q33, in case 9. In case 13, 5q was thought to be on chromosome 18, however, SKY demonstrated that a der(5), chromosome break in

5q13, was joined to 17q. SKY confirmed the del(5q) in four cases (2, 3, 4, 15) and clarified the nature of the 5;17 rearrangement in a fifth case (case 5). In case 11, del(5)(q15q33) was shown to involve a 5;6 translocation with a deletion of part of 5q in this process (Figure 1). In case 16, the add(5)(q13) was really a der(5)t(2;5)(p25;q13). Minus 5 is associated with a dismal prognosis.² Therefore modifying the analysis could move these patients from an extremely poor prognosis to one that is less poor. Eight patients had -7; SKY confirmed this in six patients and revealed that part of 7 was on a der(6) (case 9) or a der(15) (case 13). The analysis in each of the three cases (7,11,16) with a del(7q) or ring chromosome was modified based on SKY (Table 1).

Other studies using SKY have shown its power in resolving the full spectrum of chromosome abnormalities in tumors, as well as documenting new recurring breakpoints in lymphoproliferative disorders.⁴⁻⁶ Two recent papers have focused on the ability of this technique to detect hidden aberrations in myeloproliferative disorders,^{7,8} but this is the first report focusing on a group of myeloid malignancies with poor outcome. The results of Mohr *et al*⁷ and Zhang *et al*⁸ showed SKY could identify undetected cytogenetic aberrations in only two of 47 karyotypically normal AML. These results, and the fact this is a laborious and expensive technique, suggest that SKY is not likely to have a clinical impact for the study of normal G-banding cases. Although SKY did not detect any unexpected recurring aberrations in the cases we studied, our work confirms that this technique is a useful tool to resolve uncertainties of the G-banding analysis in cases with complex karyotypes. A recent report has shown yet again the importance of karyotype as a critical independent determinant of outcome in AML, especially in older patients.² Our study shows SKY could provide further precision to refine the poor prognostic groups in myeloid malignancies. As indicated by others, this technique should be used to complement G-banding analysis.^{4,5} The conventional karyotype provides an accurate description of the bands involved in the rearrangements and permits an analysis of a larger number of clones. In many rearrangements the breakpoints on the SKY-painted chromosomes were identified by comparing the corresponding DAPI banding and G-banded karyotype of the same tumor (Figure 1).

In conclusion, SKY can be an important tool to characterize aberrations in cases with complex karyotypes. The detection of subtle translocations in chromosomes thought to be deletions using G-banding shows that SKY can provide a focus for further FISH and molecular studies, especially in cases with 3q, 5q and 7q rearrangements. Moreover, SKY and other techniques of molecular cytogenetics, could help to identify specific subtypes of the disease with prognostic significance. Further studies in a larger number of cases are needed to show if any of the translocations found are recurrent, and to supply critical information on the correct implication of monosomy in myeloid malignancies.

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Table 1 Refinement of chromosome aberrations by G-banding with spectral karyotyping

Case	Sex/Age	State	Diagnosis	G-banding	Revised karyotype
1	F/74	Dx	AL-Biphenotypic	45,XX,der(1)(t(1;2)(q32;q31),add(2)(q31),der(9)(t(1;9)(q32;p21)inv(9)(p11q13)c,der(12)(7;12)(p11;p12),-13,add(16)(q22)[20]	45,X,t(X;9;16)(p22;p21;q22)t(1;2)(q32;q31)inv(9)(p11q13)c,der(12)(12;13)(p12;q?21),-13[8]
2	M/54	Dx	t-AML	43,X,dic(Y;22)(p11;p11),r(3)(p25q27),del(5)(q11q35),-7,der(12)(12;14)(p13;q11),-14[1]/45,XY,r(3)(p25q27),del(5)(q11q35),der(7)(7;18)(p?;q?12),-18[6]/46,XY[3]	43,X,dic(Y;22)(p11;p11),r(3)(p25q27),del(5)(q11q35),-7,der(12)(12;14)(p13;q11),-14[1]/45,XY,r(3)(p25q27),del(5)(q11q35),der(7)(7;18)(p?;q?12),-18[6]/45, idem, del(17)[2]
3	F/63	Dx	t-AML	NCA 1: 45,XY,r(73),del(5),del(17)(p11p13),-18[1] 46,XX,-3,del(5)(q13q33),der(6)(3;6)(q21;p21),+8,del(9)(q21q34),del(12)(p12p13),add(13)(q32),der(16)(?;16)(p21;p13)[13]/47, idem, +mar[4]/46,XX[2]	46,XX,-3,del(5)(q13q33),der(6)(3;6)(q21;p21),+8,del(9)(q21q34),del(12)(p12p13),dup(13)(q13q32),der(16)(6;16)(p21;p13)[6]/47, idem, +del(6)(11)/46,XX[1]
4	M/68	R	t-AML	NCA 1: 46, idem, -15, -18, +mar1, +mar2[1] 45,XY,del(5)(q13q33),-7,der(8)(3;8)(q21;q24),-15,add(16)(p13),der(19)(t(15;19)(q13;q13),-20,+mar1,+mar2[16]/ 45, idem, t(4;19)(q11;p13.3),der(6)(6;13)(p23;q14),-13,t(16;21)(p?;q22),-mar2,+mar3,+mar4[2]/ 45, idem, add(3)(q13),add(12)(p12)[3]	45,XY,del(5)(q13q33),-7,der(8)(3;8)(q21;q24),del(15)(q11),der(16)(15;16)(q11;p13),der(19)(19;20)(p13;q?13),+del(19)(q13),-20[6]/45, idem, der(4)(4;19)(q11;p13.3),del(13)(q14),del(15)(q11)[3]/45, idem, del(3)(p21),der(12)(3;12)(p21;p12)[2]
5	M/61	R	AML-M2	42,XY,dic(5;17)(q11;p11),-7,dic(11;12)(p13;p13),-18,der(19)?hsr(19)(q13)add(19)(q13)[17]/ 43, idem, +del(12)(p12p13)[3]/43, idem, +8[2]/ 46,XY,t(6;12)(p12;p12)[19]/46,XY[6], plus 5 related NCA 41,add(X)(q22),-Y,-5,del(7)(q11q36),inv(11)(p15q23),dic(12;?) (p12;?),-13,del(15)(q15q22),-16,add(17)(p13),-19,-20,-21,t(21)(q10),+mar1,+mar2[28], plus 2 related NCA	42,XY,der(5)(5;17)(q11;q11)-7,dic(11;12)(p13;p13),-17,-18,der(19)t(18;19)(q11;q13)[6]
6	M/34	R	AML-M2	46,XY,t(6;12)(p12;p12)[19]/46,XY[6], plus 5 related NCA	46,XY,t(6;12)(p12;p12)[9]/46,XY[1]
7	M/40	Dx	AML-M4	41,add(X)(q22),-Y,-5,del(7)(q11q36),inv(11)(p15q23),dic(12;?) (p12;?),-13,del(15)(q15q22),-16,add(17)(p13),-19,-20,-21,t(21)(q10),+mar1,+mar2[28], plus 2 related NCA	41,der(X)t(X;16)(q22;q22),der(Y)t(Y;15)(q11;q?),der(5)t(5;12)(q31;q13),der(7)t(7;15)(q11;q?),t(Y;13)(q12;p11)(13;20)(q21;q?12),-15,der(15)t(15;21)(p11;q11),-16,der(16)t(5;16)(q?33;p13),der(17)t(16;17)(q?;p13)(X;16)(q22;q?22),der(19)jins(19;7)(p13.1;q?)(7;19)(q22;p13.3),-20,-21,i(21)(q10)[6]
8	M/66	Dx	AML-M4 (CMML)	45,XY,del(3)(q21q2?6),-7,del(12)(p12p13.1),del(20)(q11q13)[32]	45,XY,del(3)(q21q2?6),-7,del(12)(p12),del(20)(q11q13)[6]
9	F/35	R	AML-M4 (CMML)	44,XX,-5,add(6)(p23),-7,add(7)(p15),del(10)(p12p15),der(12)(6;12)(p22;q24),del(13)(q12q22)[9]/44, idem, inv(X)(p11q23),add(6)(p23)[7]/44, idem, ?mv(X)(p11q23),add(19)(q13),-20[3], plus 2 related NCA	44,XX,-5,der(6)t(6;7)(p23;q22),-7,der(7)t(5;7)(q?13;p15)(5;13)(q?33;q?22),del(10)(p12p15),der(12)del(12)(p12p13)t(10;12)(p12;q24),der(13)t(5;13)(q?33;q22)[2]/43, idem, inv(X)(p11q23),der(6)t(6;15)(p21;q11),-15[7]
10	F/1	Dx	AML-M5	46,XX,der(22)add(22)(q13)[7]/47,XX,+9,der(12)(1;12)(q21;p12)[14]	46,XX,t(9;11)(p22;q23),del(17)(p13),der(22)t(17;22)(p13;q13)[2]/47,XX,t(9;11)(p22;q23),+der(9)t(9;11),der(12)t(1;12)(q21;p13)[5]

Table 1 Refinement of chromosome aberrations by G-banding with spectral karyotyping

Case	Sex/Age	State	Diagnosis	G-banding	Revised karyotype
11	M/66	Dx	AML-M6	45,XY,del(5)(q15q33),inv(9)(p11q13)c,-18[2]/46,idem,add(3)(p25),r(7)(p22q22),add(17)(p11),add(20)(q11),+mar1[7]/45,idem,add(3),-7,-17,add(20),+add(20)x2,-21,+mar1[5]/45,idem,add(3),del(3)(q13q27),-6,-7,-17,der(19)t(19;?21)(q13;q21),add(20),+add(20),-21,+mar1,+mar2,+mar3[12]/46,idem,add(3),r(7),+r(7),add(17),add(20)[3],plus 2 related NCA	43-45,XY,t(2;12)[1], der(3)t(3;7)(p25;q?) [8], der(3)t(3;19)(q13;q13) [5], der(5)t(5;6)(q15;p22) [10], der(6)t(5;6)(q?33;q21) [10], der(7)t(3;7)(q24;q14) [5], der(7)t(7;17)(q11;q21) [4],+ del(7) [2],inv(9)(p11q13)c,-17[10],-18[8], der(18)t(18;20)[1] , der(18)t(18;21)[1] , der(19)t(17;19)(q21;q13) [5],-20[2], der(20)t(20;21)(q11;q21) [9],+ der(20)t(20;21) [5],-21[5],del(21)(q21)[5][cp10] 48,XX, der(1)t(1;8)(p34;p12) +6,del(8)(p12p23), der(11)t(11;12)(q11;p11) t(1;12)(p34;p12) der(12)t(11;12)(q11;p11),del(13)(q12q14),+21[10] 43,XY, del(3)(q13) [4], der(3)t(3;12)(q13;p12) [1], der(5)t(5;17)(q13;q27) [6],-7[4], der(7)t(7;15)(q11;?) [2], del(11)(p15) , del(12)(p12p13) [5], der(15)t(15;17)(p11;q22)t(7;17)(q?;q?)t(7;22)(q?;q11) [5],-17[3], del(17)(q22) [3], der(18)t(3;18)(q?;q22) [5],-22[4], der(22)t(21;22) [2][cp6] 46,XX,der(2)inv(2)(q14q23)t(2;12)(q21;p13),inv(3)(q21q26),der(12)t(2;12)(q21;p13)inv(2)(q14q23)[7] 42,XX,t(1;11;17)(q25;?;p13),del(5)(q13q33),-7, der(12)del(12)(p12p13)t(12;13)(p13;q11) ,-13,-17,-18[6]/42,idem,t(9;22)(q34;q11)[1]/46,XX[1] 45,XY, der(5)t(2;5)(p25;q13) , der(7)t(7;13)(p13;q23)ins(7;13)(q22;q?) ,-13[2]/46,XY,t(2;5;12;17)(p25;q23;q31;p13;q12),der(5)t(2;5), der(7)t(7;?)q22 ,del(13)(q12q32)[6]/47,idem,+ del(13)(q11) [1] 46,XX,t(1;12)(p36;p13)t(9;11)(q34;q13)[10] 45,XY,t(6;12)(q25;p13), der(6)t(6;12) ,-7, del(20)(q12)[6]
12	F/2	Dx	AML-M7	48,XX,add(1)(p3?4),+6,del(8)(p12p23),del(8)(q24.1q24.3), der(11)t(1;?;11)(p3?4;q11) , der(12)t(11;12)(q11;p11) ,del(13)(q12q14),+21[20]/46,XX[2] 43,XY,-3,-5,-7,+der(9)t(9;15)(p13;q13),add(11)(p15), der(12)t(3;12)(q13;p12) ,+der(14)t(14;?)(p13;?)del(15),-17,der(18)t(5;18)(q14;q22),-22[cp14]	
13	M/65	R	MDS	46,XX,der(2)inv(2)(q14q23)t(2;12)(q21;p12),inv(3)(q21q26), der(12)t(2;12)(q21;p12)inv(2)(q14q23)[18] ,plus 2 related NCA 42,XX,del(1)(q25),del(5)(q13q33),-7,t(9;22)(q34;q11),der(11), der(12)t(12;?)(p13;?) ,-13,t(13;17)(p13;p13),-17,-18,-20,+mar[12]/46,XX[8] 46,XY,add(5)(q13),del(7)(q22q34),del(13)(q12q32)[6]/46,idem, der(2)del(2)(q12q21.3)t(2;2;5;12;17)(p25;q23;q31;p13;q12) ,t(2;5;12;17)[11]/47,idem,+mar[2] NCA 1: 49,idem,+21,+21,+22[1]	
14	F/38	R	MDS	46,XX,der(2)inv(2)(q14q23)t(2;12)(q21;p12),inv(3)(q21q26), der(12)t(2;12)(q21;p12)inv(2)(q14q23)[18] ,plus 2 related NCA 42,XX,del(1)(q25),del(5)(q13q33),-7,t(9;22)(q34;q11),der(11), der(12)t(12;?)(p13;?) ,-13,t(13;17)(p13;p13),-17,-18,-20,+mar[12]/46,XX[8] 46,XY,add(5)(q13),del(7)(q22q34),del(13)(q12q32)[6]/46,idem, der(2)del(2)(q12q21.3)t(2;2;5;12;17)(p25;q23;q31;p13;q12) ,t(2;5;12;17)[11]/47,idem,+mar[2] NCA 1: 49,idem,+21,+21,+22[1]	
15	F/69	Dx	MDS (RAEB)	46,XX,der(2)inv(2)(q14q23)t(2;12)(q21;p12),inv(3)(q21q26), der(12)t(2;12)(q21;p12)inv(2)(q14q23)[18] ,plus 2 related NCA 42,XX,del(1)(q25),del(5)(q13q33),-7,t(9;22)(q34;q11),der(11), der(12)t(12;?)(p13;?) ,-13,t(13;17)(p13;p13),-17,-18,-20,+mar[12]/46,XX[8] 46,XY,add(5)(q13),del(7)(q22q34),del(13)(q12q32)[6]/46,idem, der(2)del(2)(q12q21.3)t(2;2;5;12;17)(p25;q23;q31;p13;q12) ,t(2;5;12;17)[11]/47,idem,+mar[2] NCA 1: 49,idem,+21,+21,+22[1]	
16	M/46	Dx	MDS (RAEB)	46,XX,der(2)inv(2)(q14q23)t(2;12)(q21;p12),inv(3)(q21q26), der(12)t(2;12)(q21;p12)inv(2)(q14q23)[18] ,plus 2 related NCA 42,XX,del(1)(q25),del(5)(q13q33),-7,t(9;22)(q34;q11),der(11), der(12)t(12;?)(p13;?) ,-13,t(13;17)(p13;p13),-17,-18,-20,+mar[12]/46,XX[8] 46,XY,add(5)(q13),del(7)(q22q34),del(13)(q12q32)[6]/46,idem, der(2)del(2)(q12q21.3)t(2;2;5;12;17)(p25;q23;q31;p13;q12) ,t(2;5;12;17)[11]/47,idem,+mar[2] NCA 1: 49,idem,+21,+21,+22[1]	
17	F/66	R	MDS (RAEBT)	46,XX,del(1)(p36),t(9;11)(q34;q13)t(12;?)(p13;?) [20]	
18	M/59	R	MDS (RAEBT)	45,XY,del(6)(q25),-7,t(12;20)(p13;?q12)[20]	

Dx, at diagnosis; R, relapse; M, male; F, female; NCA, non-clonal abnormalities.

Patients 16, 17, and 18 are patients 5, 6, and 7, respectively, in Odero *et al.*⁹

Novel translocations or those redefined by the FISH and SKY analysis are highlighted in bold type.

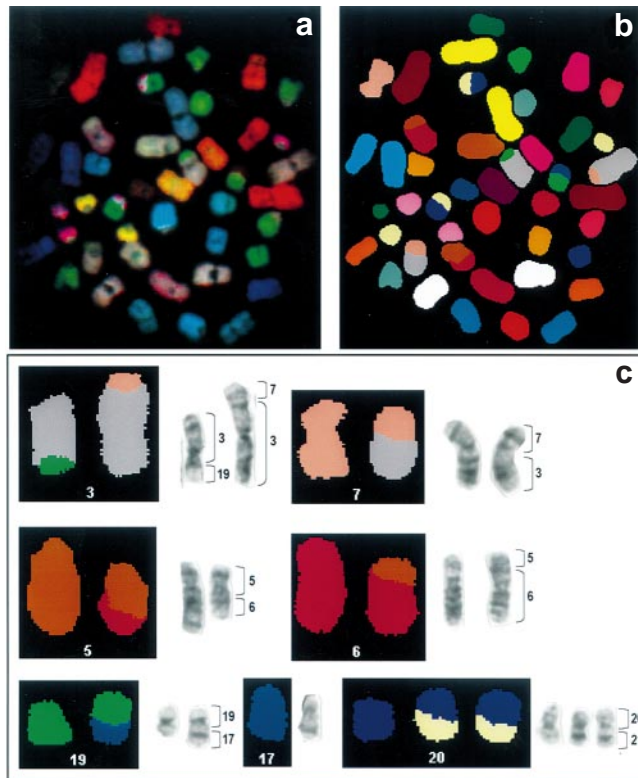


Figure 1 SKY analysis of case 11 (clone 4). (a) Spectral metaphase. (b) Classified metaphase. (c) Partial G-band and SKY karyotypes illustrating the power of the combined approach to clarify complex chromosome rearrangements. Normal homologues are included whenever possible to facilitate comparison. See text for detailed karyotype description.

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Abnormalities of the *p16^{INK4a}* gene in childhood B-precursor acute lymphoblastic leukemia without nonrandom translocations: analysis of seven matched pairs of primary leukemia and corresponding cell line

TO THE EDITOR

Progression of the cell cycle in eukaryotic cells is strictly and skillfully regulated by the sequential formation, activation, and subsequent inactivation of a series of cyclin and cyclin-dependent kinase (CDK) complexes. The activation of CDK4 and CDK6 by cyclin D elicits phosphorylation of retinoblastoma protein (pRB), which results in release of several pRB-associated transcription factors required for G1 to S phase progression. Among a family of proteins that bind and inhibit CDKs, *p16^{INK4a}* specifically blocks the activity of CDK4 and

CDK6 ultimately resulting in inhibition of cell division. The *p16* gene located on chromosomal region 9p21 appears to be inactivated in a wide variety of tumors, and mice carrying a targeted deletion of *p16* demonstrate development of spontaneous tumors at a high rate, suggesting that *p16* could be a tumor suppressor. The human *p16* gene locus encodes a second protein *p14^{ARF}* via transcription of an alternative first exon and common exon 2 in different reading frame. This protein does not directly bind and inhibit CDKs but appears to enhance the functional activity of wild-type p53. Because mice with disrupted *p19^{ARF}* (the mouse homologue of *p14^{ARF}*) but intact *p16* expression have a susceptibility to cancer, *p14^{ARF}* is considered as another tumor suppressor. Among mechanisms that inactivate *p16* in tumor cells, homozygous deletion may predominantly contribute to tumorigenesis or disease progression, because this inactivates both *p16* and *p14^{ARF}* that act upstream of pathways involving pRB and p53, respectively. However, there are some controversies regarding the sig-

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