

Spectral karyotyping: an unique technique for the detection of complex genomic rearrangements in leukemia

Bo Guo¹, Xiaoping Han², Zhanhe Wu³, Wanming Da², Hongli Zhu¹

¹Department of Hematology, ²Department of Hematology, General Hospital of PLA, Beijing 100853, China; ³Western Sydney Genomic Diagnostics, Western Sydney Genetics Program, The Children's Hospital at Westmead, NSW, Australia

Correspondence to: Hong-Li Zhu. Department of Hematology, General Hospital of PLA, Beijing 100853, China. Email: bjzhl202_cn@126.com or bjzhl202_cn@sina.com.

Abstract: Spectral karyotyping (SKY) is a novel cytogenetic technique, has been developed to unambiguously display and identify all 24 humans chromosomes at one time without a priori knowledge of any abnormalities involved. SKY can discern the aberrations that can't be detected very well by conventional banding technique and Fluorescent *in situ* hybridization (FISH). So SKY is hyper accurate, hypersensitive, and hyper intuitionist. We will review the elements and application of SKY in leukemia.

Keywords: Spectral karyotyping (SKY); chromosome analysis; leukemia

Submitted Dec 19, 2013. Accepted for publication Jan 09, 2014.

doi: 10.3978/j.issn.2224-4336.2014.01.02

View this article at: <http://www.thetp.org/article/view/3549/4412>

Introduction

Since the introduction of the conventional chromosomal banding techniques, clonal chromosome aberrations have been identified in more than 50,000 neoplasms, including malignant hematologic disorders, malignant lymphomas, and benign and malignant tumors and such detection has become essential for accurate diagnosis and treatment on leukemia, moreover, as independent prognostic factor, chromosomal changes are concerning the likelihood of remission induction and risk of relapse. In 1997, some of these aberrations detected have been used within the WHO classification to define specific subgroups of leukemia. With cytogenetic technical development, the abnormal detection rate on chromosome malignancies is increasing. Based on Fluorescent *in situ* hybridization (FISH), the Spectral karyotyping (SKY), Multicolor FISH (M-FISH) (1), comparative genomic hybridization (CGH) and some others techniques took chromosomal analysis to full colorful world. In 1996, Schröck E *et al.* (2) reported SKY application for the first time. Conquering weaknesses of conventional FISH, SKY can simultaneous and unambiguous display human and rat's all chromosomes in 24 deferent colors

without a priori knowledge of any abnormalities. So SKY is playing the important role in comprehensive analysis of chromosomes to help the study of leukemia (see *Figure 1*).

Elements of SKY

SKY combines Fourier spectroscopy, charge-coupled-device (CCD) imaging, and optical microscopy, and optical microscopy to measure simultaneously at all points in the sample emission spectra in the visible and near-infrared range. This allows the use of multiple spectrally overlapping probes. Hybridization, Image acquirement and results analysis are the major steps in the karyotype analysis with SKY technique.

Hybridization

SKY paint probe was hybridized with detected metaphases. SKY uses a combination of whole chromosome painting probes generated by Degenerate oligo-primed (DOP)-PCR amplification from flow-sorted chromosomes. The SKY probe was labeled with five different combinations of fluorescent dyes.

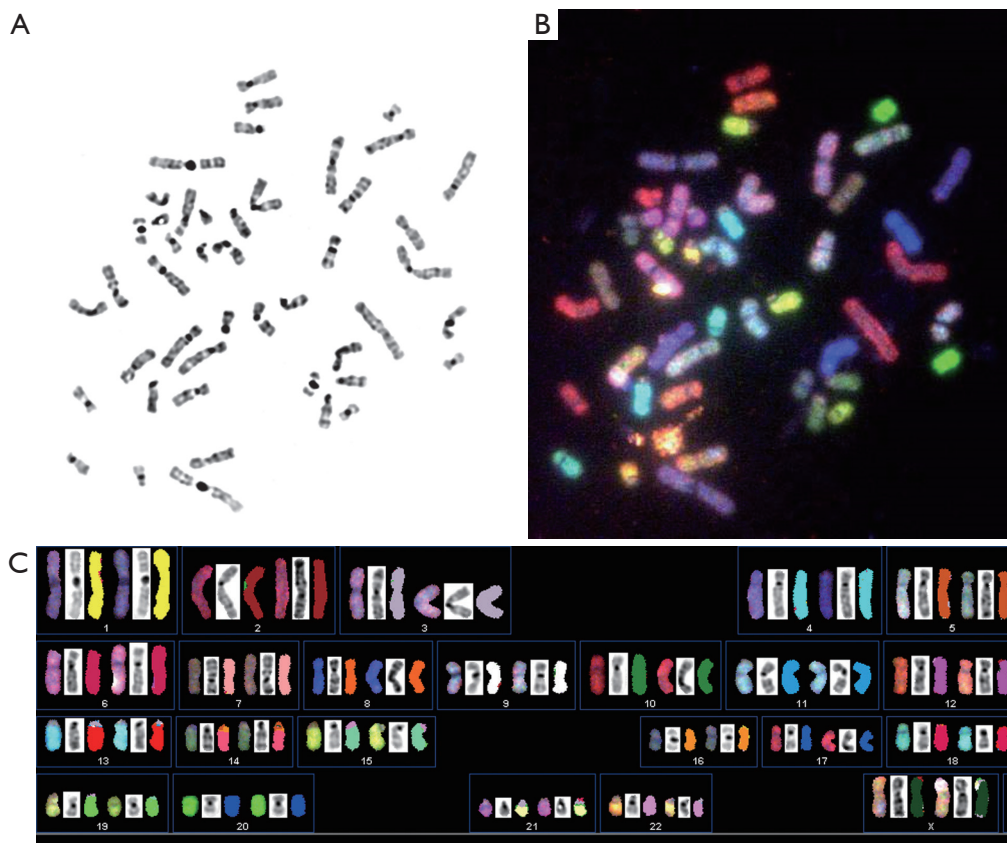


Figure 1 Spectral Karyotyping (SKY) karyotype of a normal female in SKY image, from left to right: (A) DAPI image; (B) SKY karyotype; (C) Karyotype analysis image of SKY.

Image acquirement

This step is the spectral imaging system measures the full visible light spectrum emitted from the sample in order to acquire a spectral image of the chromosomes. First, all the dyes in the chromosome painting probes are emitted by special lamp-house and cubes, through the spectral interferometer, spectrum-specific images are captured with a CCD camera. The conversion of emission spectra to the visualization of display colors is achieved by assigning blue, green, and red colors to specific spectral ranges, these three colors are named display colors. The display colors allow the assessment of the quality of the hybridization, e.g., uniformity of hybridization and signal brightness, and are used as a first evaluation of the hybridization directly after image acquisition and without further computation. But the display colors are similar because spectrally overlapping fluorochromes. Finally, the classification colors allow all chromosomes are distinguished based on measure the spectrum at every given pixel with Fourier transformation and computation soft. So each pair of chromosomes would

appear with unique distinct color.

Results analysis

SKY View, the analysis soft, analyzes the results of the spectral image and display the image so that chromosomal abnormalities are easily identified.

Characters of SKY

SKY are FISH based methods allowing for the simultaneous display of all chromosomes in different colors using five fluorochromes, alone and in combination in a single experiment. The techniques employ a different approach to image acquisition: the strength of SKY is the immediate identification of interchromosomal aberrations, for example, translocations that lead two color differences on the derivative chromosome, as well as the elucidation of complex rearrangements. They have their limitation in the identification of chromosomal changes that do not lead to

a discernable color change: small deletions, duplications and intrachromosomal inversions. The resolution of SKY for the detection of interchromosomal rearrangements has been shown to be between 500-2,000 Kb (3), but of course significantly depends on metaphase chromosome extension as well as hybridization quality (1,4,5). To achieve the most comprehensive karyotype description possible a combination of conventional band, FISH, CGH, and other techniques (6-9).

SKY in cell lines of leukemia

Mrózek *et al.* (10) analyzed the KG-1 and KG-1a cell line of erythroleukemia using SKY FISH and G-banding. The results revealed the lines shared several identical structural chromosomal aberrations, including der[4]t[4;8], del[7q], der[8]t[8;12], idic[8][p11], der[17]t[5;17], and der[20]t[12;20], but also differed with regard to other chromosome rearrangements. In contrast to KG-1, the KG-1a line lost one of the two copies of idic[8][p11] present in KG-1 cells and gained a der[8;22][q24;q13], an i[11][q10], and a der[19]t[14;19][q13;q13.4]. Notably, this study showed that the KG-1 cells harbor a partial hexasomy of the long arm of chromosome 8, which may related to AML1-ETO fusion gene in KG-1 cells that could be studied further to explain the differences in phenotypic properties between KG-1 and KG-1a cells.

Chronic myelogenous leukemia (CML) is a clonal bone marrow disease with progression from a chronic phase to an aggressive blast crisis. The cell line NALM-1 was originally established from the peripheral blood of a patient with CML blastic crisis. Cytogenetics of NALM-1 was analyzed with GTG banding, FISH, and SKY by Pelz AF *et al.* (11), who described the karyotype of NALM-1 cell line for the first time: 44,X,-X, der[7]t[7;9;15](q10;?:q15), der[9]t[9;9][p24;q33 approximately q34]t[9;22][q34;q11], der [15]t[7;9;15][?:?:q15], der[22]t[9;22][q34;q11]. In a research of comparing karyotypes between a new cell line of natural killer (NK) cell, IMC-1 and cells from an adult patient with aggressive NK cell leukemia. After 55 weeks culture of IMC-1, reported by Chen IM *et al.* (12) defined the karyotypes by G-banding and by SKY. They found complex structural and numerical abnormalities in both cells. Three apparently intact copies of chromosome 8 occurred in the diagnostic bone marrow specimen; the cell line also contains three copies of chromosome 8 but each was structurally altered. The development and detailed characterization of this new NK leukemic cell line by SKY

facilitated the biological and functional studies of NK cells and chromosomal aberrations potentially important in leukemic transformation investigation.

SKY application in leukemia

As SKY has appeared, this has been paid more attentions and researches. The first study on hematological malignancies was reported by Veldman *et al.* (13). Seven cases of AML and MDS were studied, and SKY confirmed the results of conventional banding based karyotyping (G-banding), and recognized previously unidentified material of the origin, thereby demonstrated its value for the analysis of such cases. After this, SKY was used in larger samples, and study level was higher and higher. SKY has become an important technique to improve the chromosome analysis.

SKY in cryptical chromosomal aberrations of leukemia

Ohsaka A *et al.* (8) reported a case of atypical chronic myeloid leukemia showed an abnormal clone, which was interpreted as 46, X,-Y, +der [?]t[?:1][?:q?1], and Philadelphia chromosome by G-banding but Reverse transcription-polymerase chain reaction(RT-PCR) but no BCR-ABL fusion genes were detected. After SKY and FISH analysis, the karyotype was refined to be 46,X, der[Y]t[Y;1][q11.1 or.2;q12]. Result demonstrated that SKY in combination with the conventional banding and FISH is useful and specific for the exploring of undefined chromosome abnormalities in hematological disorders.

About 25-45% of newly diagnosed adult AMLs and 10-30% of ALLs appear to be a karyotypically normal, with no clonal chromosomal aberrations detectable by conventional banding technique. Similarly, the great majority of these cases remained normal after SKY analysis, however, a part of these cases were detected critical aberrations by SKY which were showed normal by G-band or R-band (14-19). The cryptic t[11;19] and 5q- also indicating poor prognosis were found in another research of Hilgenfeld *et al.* (20). And t[11;19] was detected again by SKY in study of Kakazu *et al.* (14). These reports showed this translocation can be missed by conventional banding analysis.

About 75-90% of all children with leukemia showed evidence of clonal chromosomal aberration when they are first diagnosed, but 10-25% of which aberrations couldn't be identified by conventional band. Lu *et al.* (9) applied SKY to panel of 29 newly diagnosed pediatric pre B-cell ALL. They found a cryptic t[12;21][p14;q11] were in six cases, marker

chromosomes in two cases and identified the misidentified aberrations by G-band detected. They also identified five novel non-recurrent translocations t[2;9][p11.2;p13], t[2;22][p11.2;q11.2], t[6;8][p12;p11], t[12;14][p13;q32] and t[X;8][p22.3;q?]. Of these translocations, t[2;9], t[2;22] and t[12;14] were identified by G-banding analysis and confirmed by SKY. They characterized a t[12;14][p13;q32] translocation by FISH, and identified a fusion of TEL with IGH for the first time in ALL. These studies demonstrate the utility of using SKY in combination with G-banding and FISH to augment the precision with which chromosomal aberrations may be identified in tumor cells.

SKY in complex karyotypes of leukemia

Deletions on unbalanced translocations in part leukemia with complex aberrations were particularly prone to misinterpretation based on G-band alone, especially when chromosomal regions where they have a similar G-band pattern involved. In this condition, SKY has special advantages that it can correct or improve the detection to obtain more accurate karyotype changes.

Tchinda *et al.* (19) used SKY and FISH to further characterize chromosomal abnormalities in 35 patients with hematological disorders, that result confirmed 149 aberrations, refined 117, and detected 11 hidden changes. In contrast, 18 abnormalities were detected only by G-banding. Ten monosomies and two deletions described by G-banding were shown to be involved in translocations or ring chromosomes. These results demonstrate that SKY increases the accuracy of karyotype detection, which is important for proper diagnosis and management of hematological malignancies.

Since chromosomal changes are used both as independent prognostic factors and for therapy design in hematological disorders, it is necessary to elucidate chromosomal changes as accurately as possible. Therefore, SKY has important value for the therapy and prognosis for patients with leukemia could be re-judged.

Vaidya *et al.* (21) reported a novel 5-way translocation that it was 46,XY,t[9;11;13;19;22][9q34.12;11p11.12;13q21.31;19q13.12;22q11.21] using GTG banding, FISH, and SKY in a case of chronic-phase CML. Molecular analysis revealed the presence of two types of transcripts (b3a2, b2a2). The patient was responding to the imatinib treatment. However, the patient needs to be carefully monitored at various intervals.

Mrózek *et al.* (22) used SKY studied 29 adults with acute myeloid leukemia and a complex karyotype containing one to nine abnormalities that they were not fully identifiable

by G-banding. However, SKY showed the origin of rings and identified material origin in unbalanced translocations in all cases and the origin of markers in most, allowing reinterpretation of 136 aberrations and the discovery of these three aberrations hidden in normal chromosomes. SKY confirmed ten cases and refined the interpretation of three cases balanced aberrations recognized by G-banding and identified another nine balanced aberrations, including a novel translocation involving the RUNX1 gene. Eleven of 32 deletions found by G-banding were shown to be cryptic translocations or insertions, including t3 of 4 chromosome 3 deletions, 2 of three del[7q], and 2 of 12 del[5q]. The most frequently overrepresented was 21q, found in eight patients, including four with high-level 21q amplification.

SKY considerably enhances the accuracy of karyotype interpretation, and that amplification of chromosomal material may play a greater role in leukemogenesis than it has been recognized. Besides, a comprehensive karyotype in an adult ALL by SKY and R-banding. SKY confirmed and improved the abnormalities previously seen by R-banding and showed a comprehensive karyotype, 47,XY,+9, ins[1;5][q23;q23q34] t[6;7][q23;p13] (23).

Conclusions

SKY has proved to be a very useful cytogenetic tool, which is hyper accurate, hypersensitive, and hyper intuitionist comparing with other available techniques. SKY is playing an important role in the detection of complex chromosomal aberration and also in the identification of new leukemia subgroups. Improving itself and combining with other cytogenetic and molecular technique, SKY would be a very helpful tool to supplement conventional banding technique in the disease diagnosis, management and the prognosis.

Acknowledgements

Funding: This research was supported by the PLA General Hospital, four hundred projects funded.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Speicher MR, Gwyn Ballard S, Ward DC. Karyotyping

- human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 1996;12:368-75.
2. Schröck E, du Manoir S, Veldman T, et al. Multicolor spectral karyotyping of human chromosomes. *Science* 1996;273:494-7.
 3. Fan YS, Siu VM, Jung JH, et al. Sensitivity of multiple color spectral karyotyping in detecting small interchromosomal rearrangements. *Genet Test* 2000;4:9-14.
 4. Schröck E, Veldman T, Padilla-Nash H, et al. Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. *Hum Genet* 1997;101:255-62.
 5. Bayani J, Squire JA. Advances in the detection of chromosomal aberrations using spectral karyotyping. *Clin Genet* 2001;59:65-73.
 6. Saito H, Otsubo K, Kakimoto A, et al. Emergence of two unrelated clones in acute myeloid leukemia with MLL-SEPT9 fusion transcript. *Cancer Genet Cytogenet* 2010;201:111-5.
 7. Wong KF, Siu LL. Acute myeloid leukaemia with variant t(8;21)(q22;q22) as a result of cryptic ins(8;21). *Pathology* 2011;43:180-2.
 8. Ohsaka A, Hisa T. Spectral karyotyping refined the identification of a der(Y)t(Y;1)(q11.1 or.2;q12) in the blast cells of a patient with atypical chronic myeloid leukemia. *Acta Haematol* 2002;107:224-9.
 9. Lu XY, Harris CP, Cooley L, et al. The utility of spectral karyotyping in the cytogenetic analysis of newly diagnosed pediatric acute lymphoblastic leukemia. *Leukemia* 2002;16:2222-7.
 10. Mrózek K, Tanner SM, Heinonen K, et al. Molecular cytogenetic characterization of the KG-1 and KG-1a acute myeloid leukemia cell lines by use of spectral karyotyping and fluorescence in situ hybridization. *Genes Chromosomes Cancer* 2003;38:249-52.
 11. Pelz AF, Weillepp G, Wieacker PF. Re-analysis of the cell line NALM-1 karyotype by GTG-banding, spectral karyotyping, and whole chromosome painting. *Cancer Genet Cytogenet* 2005;156:59-61.
 12. Chen IM, Whalen M, Bankhurst A, et al. A new human natural killer leukemia cell line, IMC-1. A complex chromosomal rearrangement defined by spectral karyotyping: functional and cytogenetic characterization. *Leuk Res* 2004;28:275-84.
 13. Veldman T, Vignon C, Schröck E, et al. Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. *Nat Genet* 1997;15:406-10.
 14. Kakazu N, Taniwaki M, Horiike S, et al. Combined spectral karyotyping and DAPI banding analysis of chromosome abnormalities in myelodysplastic syndrome. *Genes Chromosomes Cancer* 1999;26:336-45.
 15. Stanchesu R, Betts DR, Rechavi G, et al. Involvement of der(12)t(12;21)(p13;q22) and as well as additional rearrangements of chromosome 12 homolog in ETV6/RUNX1-positive acute lymphoblastic leukemia. *Cancer Genet Cytogenet* 2009;190:26-32.
 16. Yamamoto K, Okamura A, Wakahashi K, et al. A novel unbalanced whole-arm translocation der(3;10)(q10;q10) in acute monocytic leukemia. *Cancer Genet Cytogenet* 2010;199:134-8.
 17. Mrózek K. Cytogenetic, molecular genetic, and clinical characteristics of acute myeloid leukemia with a complex karyotype. *Semin Oncol* 2008;35:365-77.
 18. Stanchesu R, Betts DR, Rechavi G, et al. Involvement of der(12)t(12;21)(p13;q22) and as well as additional rearrangements of chromosome 12 homolog in ETV6/RUNX1-positive acute lymphoblastic leukemia. *Cancer Genet Cytogenet* 2009;190:26-32.
 19. Tchinda J, Neumann TE, Volpert S, et al. Characterization of chromosomal rearrangements in hematological diseases using spectral karyotyping. *Diagn Mol Pathol* 2004;13:190-5.
 20. Hilgenfeld E, Padilla-Nash H, McNeil N, et al. Spectral karyotyping and fluorescence in situ hybridization detect novel chromosomal aberrations, a recurring involvement of chromosome 21 and amplification of the MYC oncogene in acute myeloid leukaemia M2. *Br J Haematol* 2001;113:305-17.
 21. Vaidya S, Joshi D, Ghosh K, et al. A novel 5-way translocation t(9;11;13;19;22) in a case of chronic-phase chronic myeloid leukemia. *Hum Pathol* 2013;44:2365-9.
 22. Mrózek K, Heinonen K, Theil KS, et al. Spectral karyotyping in patients with acute myeloid leukemia and a complex karyotype shows hidden aberrations, including recurrent overrepresentation of 21q, 11q, and 22q. *Genes Chromosomes Cancer* 2002;34:137-53.
 23. Guo B, Zhu HL, Li SX, et al. Spectral karyotyping reveals a comprehensive karyotype in an adult acute lymphoblastic leukemia. *EXCLI Journal* 2012;11:89-92.

Cite this article as: Guo B, Han X, Wu Z, Da W, Zhu H. Spectral karyotyping: an unique technique for the detection of complex genomic rearrangements in leukemia. *Transl Pediatr* 2014;3(2):135-139. doi: 10.3978/j.issn.2224-4336.2014.01.02