

Case report:

SPECTRAL KARYOTYPING REVEALS A COMPREHENSIVE KARYOTYPE IN AN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT

Cytogenetic abnormalities are frequently detected in patients with acute lymphoblastic leukemia (ALL). Comprehensive karyotype was related to poor prognosis frequently in ALL. We present a comprehensive karyotype in an adult ALL by spectral karyotyping (SKY) and R-banding. SKY not only confirmed the abnormalities previously seen by R-banding but also improved comprehensive karyotype analysis with the following result 47,XY,+9,ins(1;5)(q23;q23q34) t(6;7)(q23;p13). Our report demonstrated that SKY is able to provide more information accurately for prediction of disease prognosis in adult ALL with comprehensive karyotype.

Keywords: SKY, comprehensive karyotype, acute lymphoblastic leukemia

INTRODUCTION

Cytogenetic abnormalities are frequently detected in patients with acute lymphoblastic leukemia (ALL). Comprehensive karyotype was related to poor prognosis frequently in ALL (Lu et al., 2002). SKY can simultaneously and unambiguously display human and rat ALL chromosomes in 24 different colours without a priori knowledge of any abnormalities (Schröck et al., 1996). SKY has been shown to provide additional chromosome information by improving the characterization of marker chromosomes, cryptic translocations, and complex chromosomal rearrangements that cannot be fully defined by conventional cytogenetics (Betts et al., 2008; Cohen et al., 2004; Karst et al., 2006; Mrózek et al., 2002). In this report, we demonstrate the role of SKY in resolving complex cytogenetic abnormalities in an adult ALL with comprehensive karyotype.

CASE REPORT

A 33-year-old man was admitted to General Hospital of People's Liberation Army of China, because of fever and cough for 10 days. The clinical laboratory findings are summarized as follows: hemoglobin was 44 g/L, white blood cells were $1.9 \times 10^9/L$ with 33 % blast cells, and a platelets were $103 \times 10^9/L$. Bone marrow examination found hypercellularity with 92 % cells, and chemical staining revealed POX (peroxidase) (-), a periodic acid Schiff (PAS) positive rate of 87 %, and an α -naphthyl acetate esterase (ANAE) positive rate of 35 %. Immunophenotyping studies with flow cytometry indicated that the blasts expressed the following antigens: CD19, CD22, CD7, CD33, CD14 and HLA-DR, and part cells expressed CD5, CD3, and CD84. The diagnosis of T/B double expression lymphoblastic leukemia was established according to the World Health Organization classification. R-band cyto-

netic analysis on bone marrow aspirates was performed by using standard methods. Karyotypic analysis was made in accordance with ISCN 1995 (Shaffer and Tommerup, 2005).

Slides for SKY analysis were prepared from fixed suspension material stored at -20°C . The SKY kit probe cocktail (Applied Spectral Imaging, USA) was hybridized to metaphase slides according to the manufacturer's protocol. After hybridization, chromosomes were counterstained with DAPI. Image acquisition was performed with an SKY View 1.6 system of Nikon E600 microscope. Automatic identification of chromosomes was based on the measurement of the spectrum for each chromosome.

Cytogenetic analysis of bone marrow cells revealed a $47, \text{XY}, \text{t}(1;5)(\text{q}32;\text{q}21), 7\text{p-}, +\text{mar}1$ karyotype in 20 metaphases analyzed (Figure 1A). SKY analysis confirmed the abnormalities previously seen by R-

banding. SKY analysis also showed a complex karyotype involving chromosomes 1, 5, 6, 7 and 9 together with the $47, \text{XY}, +9, \text{ins}(1;5)(\text{q}23;\text{q}23\text{q}34) \text{t}(6;7)(\text{q}23;\text{p}13)$ (Figure 1B).

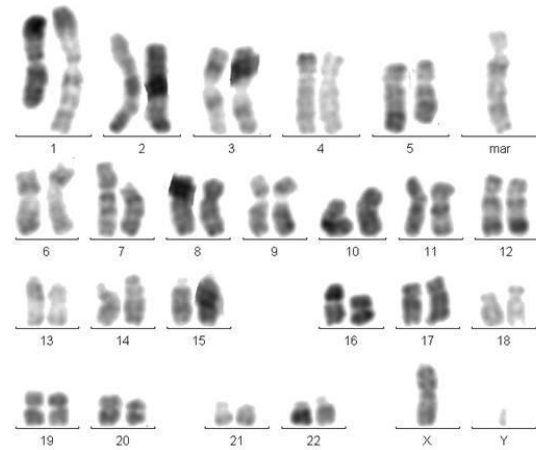


Figure 1A: R-band karyotype showing $47, \text{XY}, \text{t}(1;5)(\text{q}32;\text{q}21), 7\text{p-}, +\text{mar}1$

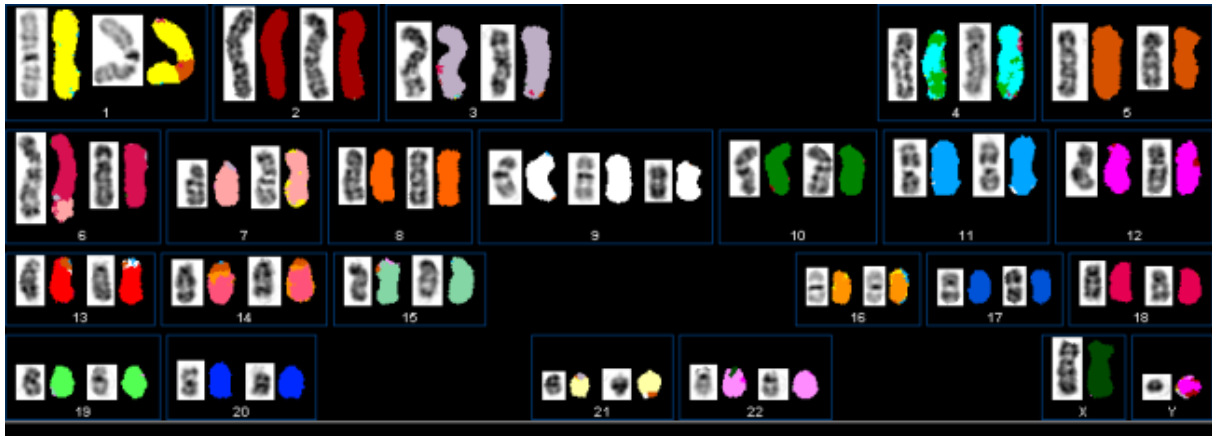


Figure 1B: SKY karyotype from a leukemia cell at the time of diagnosis showing the inverted DAPI (left) and the classified (right) profiles for each chromosome. Translocation $\text{t}(6;7)(\text{q}23;\text{p}13)$, insertion $\text{ins}(1;5)(\text{q}23;\text{q}23\text{q}34)$, and the chromosome 9 are trisome.

DISCUSSION

The accurate detection of chromosome 6q aberrations by R-banding presents a major challenge to the cytogeneticist. The main reason is that the chromosome arm lacks major landmarks, and it is difficult to make exact breakpoint determination. A problem of the frequent poor morphology in ALL metaphases is further accentuated (Karst et al., 2006; Lu et al., 2002; Stanchesu et al. 2009).

In this case, conventional karyotype was applied and results showed translocation between chromosomes 1 and 5, 7p deletion. In addition, marker chromosome was indicated. Moreover, SKY exactly identified the aberration as insertion between chromosomes number 1 and 5. The supernumerary marker chromosome was identified by SKY as chromosome number 9 based on the colour specificity. Thus, reorganization and abnormalities of chromosomes and their origin can be demonstrated by SKY

R-banding detected 7p- but not the aberration in chromosome number 6 while SKY indicated the karyotype as t(6;7)(q23;p13). It can be concluded that SKY identified the breakpoints in marker chromosomes which cannot be characterized by R-banding, as well as cryptic rearrangements missed by R-banding.

Abnormalities with chromosome 47, XY, +9, ins(1;5) (q23;q23q34), t(6;7) (q23;p13) were rarely observed by conventional cytogenetic technique. In adult ALL, del 6q occurs somewhat less frequently than in children and seems to preferentially correlate with a T cell phenotype; this abnormality does not appear to be associated with an unfavorable prognosis (Betts et al., 2008; Burkhardt et al., 2006; Mancini et al., 2002). It has been proposed that a tumor suppressor gene may be located in this region, whose absence through deletion may contribute to malignant transformation or proliferation reference (Crowley et al., 2005).

In this report, SKY revealed unbalanced translocations. The patient's comprehensive karyotype indicated poor prognosis, which

was in accordance with the clinical status. The patient received DOLP regimen (Daunorubicin 40 mg, d1-3, Vincristine 2 mg, d1, 8, L-asparaginase 1wu, d6-16, Prednisone 60 mg, d1-28), without remission, and died after 20 days. From our study, SKY revealed the accurate identification of cytogenetic breakpoints, which is prone to be missed by conventional cytogenetics. It is suggested to use SKY for improving the diagnostic quality of conventional banding to gain more accurate karyotypes.

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REFERENCES

- Betts DR, Stanchesu R, Niggli FK, Cohen N, Rechavi G, Amariglio N et al. SKY reveals a high frequency of unbalanced translocations involving chromosome 6 in t(12;21)-positive acute lymphoblastic leukemia. *Leuk Res* 2008;32:39-43.
- Burkhardt B, Bruch J, Zimmermann M, Strauch K, Parwaresch R, Ludwig WD et al. Loss of heterozygosity on chromosome 6q14-q24 is associated with poor outcome in children and adolescents with T-cell lymphoblastic lymphoma. *Leukemia* 2006; 8:1422-9.
- Cohen N, Betts DR, Tavori U, Toren A, Ram T, Constantini S et al. Karyotypic evolution pathways in medulloblastoma/ primitive neuroectodermal tumor determined with a combination of spectral karyotyping, G-banding, and fluorescence in situ hybridization. *Cancer Genet Cytogenet* 2004;149: 44-52.

Crowley JA, Butler MS, Ronnenburg MJ, Ament CN, Meekins JS, Ning Y. Development of a dual-color fluorescence in situ hybridization probe set on chromosome 6q to improve cytogenetic diagnosis of lymphoid malignancies. *Cancer Genet Cytogenet* 2005;157:78-81.

Karst C, Gross M, Haase D, Wedding U, Höffken K, Liehr T et al. Novel cryptic chromosomal rearrangements detected in acute lymphoblastic leukemia detected by application of new multicolor fluorescent in situ hybridization approaches. *Int J Oncol* 2006;28:891-7.

Lu XY, Harris CP, Cooley L, Margolin J, Steuber PC, Sheldon M et al. The utility of spectral karyotyping in the cytogenetic analysis of newly diagnosed pediatric acute lymphoblastic leukemia. *Leukemia* 2002;16:2222-7.

Mancini M, Vegna ML, Castoldi GL, Mecucci C, Spirito F, Elia L et al. Partial deletions of long arm of chromosome 6: biologic and clinical implications in adult acute lymphoblastic leukemia. *Leukemia* 2002;16:2055-61.

Mrózek K, Heinonen K, Theil KS, Bloomfield CD. 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.

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA et al. Multicolor spectral karyotyping of human chromosomes. *Science* 1996;273:494-7.

Shaffer LG, Tommerup N (eds). *ISCN (2005): an international system for human cytogenetic nomenclature*. Basel: Karger, 2005.

Stanchesu R, Betts DR, Rechavi G, Amarglio N, Trakhtenbrot L 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.