MedCrave
Step into the World of Research

MOJ Proteomics & Bioinformatics

Successful identification of complex rearrangements involving multiple chromosomes in Burkitt-type/mature B-cell acute lymphoblastic leukemia: further emphasis on spectral karyotyping

Abstract

We report a case of Burkitt-type/mature B-cell acute lymphoblastic leukemia harboring complex chromosomal rearrangements involving t(8;14)(q24;q32) and *IGH/MYC* fusion. Multiple chromosome aberrations, where a precise karyotype was not established employing G-banding, were observed at presentation, disappeared in remission, but reappeared on the recurrence of the disease. Spectral karyotyping (SKY) analysis in combination with G-banding revealed eight common aberrations, including t(8;14)(q24;q32). We performed triple-color fluorescence *in situ* hybridization (FISH) analysis, and identified *IGH/MYC* fusion signals in 95% of the interphase nuclei analyzed. SKY and FISH analyses may be useful for determining complex karyotypes that were not identified employing conventional cytogenetic alone.

Keywords: Burkitt-type leukemia, mature B-cell acute lymphoblastic leukemia, complex karyotype, spectral karyotyping, fluorescence *in situ* hybridization

Case Report

Volume 7 Issue 1 - 2018

Atsushi Kakimoto,[1,2] Kaori Otsubo,[2] Hajime Saito,[3] Norio Komatsu,[4] Akimichi Ohsaka[1]

[1]Department of Transfusion Medicine and Stem Cell Regulation, Juntendo University Graduate School of Medicine, Japan
[2]Center for Genetic and Chromosomal Analysis, Japan
[3]Mito-chuo Hospital, Japan
[4]Department of Hematology, Juntendo University Graduate School of Medicine, Japan

Correspondence: Akimichi Ohsaka, Department of Transfusion Medicine and Stem Cell Regulation, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan, Tel +81 3 5802 1109, Fax +81 3 3811 2724,
Email ohsaka@juntendo.ac.jp

Received: February 14, 2018 | **Published:** February 26, 2018

Introduction

Mature B-cell acute lymphoblastic leukemia (ALL) or Burkitt-type ALL is a rare entity and can be defined as the leukemic manifestation of Burkitt lymphoma (BL).[1] BL is a highly aggressive B-cell malignancy and can be endemic, sporadic, or associated with immunodeficiency.[2,3] Sporadic BL accounts for 1-2% of all adult lymphoma in Western Europe and the United States.[2] Mature B-cell ALL is characterized by the expression of pan-/mature B-cell antigens (e.g., HLA-DR, CD19, cyCD22, and CD79α), together with surface immunoglobulin (sIg) accompanying light chain restriction, the association of an L3 morphology according to the FAB classification, and the presence of 8q24/*MYC* rearrangement.[1-3] The *MYC* gene is most frequently found to be translocated into the Ig heavy chain locus (IGH), resulting in t(8;14)(q24;q32), whereas the less frequently observed variant translocations, t(2;8)(p12;q24) or t(8;22)(q24;q11), juxtapose *MYC* to the light chain kappa or lambda locus, respectively.[1-3] However, there are often discrepancies between the morphology, immunophenotype, and genotype, leading to a heterogenous disease spectrum.

Additional recurrent chromosome aberrations other than t(8;14)(q24;q32) or its variants have been described, with chromosomes 1,6,13,17, and 22 most commonly involved.[4] However, unlike acute myeloid leukemia,[5] the clinical characteristics of mature B-cell ALL with a complex karyotype (five or more aberrations) remain to be fully elucidated. In this paper, we report a case of mature B-cell ALL harboring complex chromosomal rearrangements, involving t(8;14)(q24;q32) and *IGH/MYC* fusion, which were successfully identified employing multicolor spectral karyotyping (SKY) and fluorescence *in situ* hybridization (FISH) analyses.

Case Report

The patient was a 70-year-old male who presented with flu-like symptoms in April 2010. Hematologic findings upon admission were: hemoglobin (Hb), 8.4 g/dL; platelets, $34x10^9$/L; and white blood cells (WBC), $21.8x10^9$/L with 24% blasts, 1% myelocytes, 4% band-form neutrophils, 14% segmented neutrophils, 1% basophils, 11% monocytes, 45% lymphocytes, and 3 erythroblasts per 100 WBC. Laboratory tests showed 2,356 IU/L lactate dehydrogenase (normal range in our hospital: 106-230). A bone marrow aspirate showed hypercellularity with 93.2% blasts characterized by medium-sized cells with a modest amount of cytoplasm and a few blasts with basophilic cytoplasm and prominent cytoplasmic vacuoles. Immunophenotypic analysis of bone marrow cells at diagnosis by flow cytometry revealed that the blasts were positive for CD5 (57%), CD10 (96%), CD19 (99%), CD20 (96%), CD38 (96%), HLA-DR (100%), and the Ig kappa chain (98%), but negative for CD34, cytoplasmic myeloperoxidase, and cytoplasmic TdT (terminal deoxynucleotidyl transferase), consistent with a mature B-cell phenotype except for CD5 positivity.[2] The patient was diagnosed with mature B-cell ALL

and treated with the hyper-CVAD plus rituximab regimen,[6] the former being modified with a 70% dose. He achieved complete remission (CR) after receiving the first course of the hyper-CVAD phase of the regimen, and was discharged after completing 6 alternating courses of the regimen. In September 2010, the patient was readmitted to the hospital because of recurrence of the disease. A bone marrow aspirate showed normocellularity with 9.8% blasts. The immunophenotype of blasts was positive for CD10 (69%), CD19 (58%), CD38 (98%), and HLA-DR (97%), but negative for CD20 and sIg, suggesting a phenotypic change to precursor B cells. The patient received salvage chemotherapy, but became refractory to it. He died of disease progression and sepsis in January 2011.

The cytogenetic analysis of bone marrow cells at diagnosis by G-banding showed multiple chromosome aberrations, suggestive of a hypodiploid karyotype in 7 out of 10 metaphase cells analyzed (45 chromosomes in 5 cells and 43 chromosomes in 2 cells), and the remaining 3 cells were normal. The tentative karyotype by G-banding was 45,XY,-4,-4,-6,-7,-8,-8,-11,-14,-14,-15,-15,-17,-18,-19,-20,+14mar. However, the complex karyotypes in 7 metaphase cells were not completely identical, and a precise karyotype was not established employing conventional cytogenetics alone. Thus, we performed SKY analysis using a SkyPaint™ kit (Applied Spectral Imaging, Migdal Haemek, Israel), as described previously.[7] SKY analysis showed complex rearrangements involving multiple chromosomes (Figure1). Although SKY analysis was performed on only two cells available, spectral karyotypes were not identical, as in G-banding. The final representative karyotype, combining the results of SKY and G-banding, was 45,XY, der(1)t(1;14)(p13;q13) t(1;17)(q44;q21), -4, der(4)t(4;6) (p11;?), -6,-8, der(11)t(1;11)(?;p15), der(11)t(11;15)(q23;q15), del(13)(q?), der(14)t(1;14)(p13;q13), der(14)t(8;14)(q24;q32),15,der(15)t(11;15)(q13;q11.2),der(17) t(1;17)(?;p13)t(1;15)(?;q15),der(17)t(8;17)(?;p13)t(3;17)(?;q23),-21, der(21)t(3;21)(?;q22),+4mar (Figure 2). The complex chromosome aberrations disappeared in remission but reappeared on the recurrence of the disease. The complex karyotypes at relapse were not completely identical, as at diagnosis. As shown in Table 1, eight common aberrations were identified in each karyotype, as follows: der(1)t(1;14) (p13;q13)t(1;17)(q44;q21),-8, der(11)t(1;11)(?;p15),der(11)t(11;15) (q23;q15),der(14)t(8;14)(q24;q32),der(15)t(11;15)(q13;q11.2), der(17)t(8;17)(?;p13)t(3;17)(?;q23) and der(21)t(3;21)(?;q22).

Table 1 Changes in Chromosomal Aberrations during the Course of the Disease in the Patient

Chromosomal Aberrations	4/22/2010	5/14/2010	6/7/2010	9/6/2010	9/27/2010	12/1/2010
der(1)t(1;14)(p13;q13)t(1;17)(q44;q21)	Y	N	N	Y	Y	Y
-8	Y	N	N	Y	Y	Y
der(11)t(1;11)(?;p15)	Y	N	N	Y	Y	Y
der(11)t(11;15)(q23;q15)	Y	N	N	Y	Y	Y
der(14)t(8;14)(q24;q32)	Y	N	N	Y	Y	Y
der(15)t(11;15)(q13;q11.2)	Y	N	N	Y	Y	Y
der(17)t(8;17)(?;p13)t(3;17)(?;q23)	Y	N	N	Y	Y	Y
der(21)t(3;21)(?;q22)	Y	N	N	Y	Y	Y
Others	*1	N	N	*2	*3	*4

Each karyotype was defined by combining the results of SKY and G-banding analyses (Y: yes; N: no).

*1: –4,der(4)t(4;6)(p11;?),-6,del(13)(q?),der(14)t(1;14)(p13;q13),der(17)t(1;17)(?;p13)t(1;15)(?;q15),-21,+4mar.

*2: add(2)(q21),add(2)(q21),-4,del(13)(q?),der(14)t(1;14)(p13;q13),-15,-16,-17-18,-18,-21,+7mar.

*3: add(2)(q21),-4,-4,add(7)(q22),-13,-13,-14,-16,-17,add(18)(q21),add(19)(q13.1),-21,+8mar.

*4: add(4)(q31),del(13)(q?),der(14)t(1;14)(p13;q13),der(15)t(13;15)(q14;q11.2),t(16;18)(p13.1;q21),der(17)t(1;17)(?;p13)t(1;15)(?;q15), der(21)t(17;21)(?;p11.2)t(3;17)(q25;?).

To confirm the presence of t(8;14)(q24;q32) and 11q23 abnormality, the later is related to *MLL* (Mixed Lineage Leukemia) gene rearrangement, we performed triple-color FISH analysis using the LSI IGH/MYC, CEP 8 Tri-Color, Dual Fusion Translocation Probe and LSI MLL Dual-Color, Break Apart Rearrangement Probe (Abbott Laboratories, Abbott Park, IL, USA), as described previously.[8] The expected pattern for a nucleus hybridized with the LSI IGH/MYC, CEP 8 probe in a cell harboring the reciprocal t(8;14) with the 8q24 breakpoint is one orange, one green, two (orange/green) fusions, and two aqua signals. As shown in Figure 3, yellow (orange/green) fusion signals of the IGH and MYC probes were detected in 95% of the interphase nuclei analyzed at diagnosis, indicating the presence of t(8;14)(q24;q32). After achieving CR, the karyotype became normal in all metaphase cells analyzed based on G-banding, and FISH analysis identified no *IGH/MYC* fusion signals (data not shown). Furthermore, FISH analysis using the LSI MLL probe showed yellow (orange/green) fusion signals of the MLL probe, which were displayed by normal chromosome 11, in 80% of the interphase nuclei analyzed (data not shown), suggesting that the 11q23 region, i.e., t(11;15)(q23;q15), in the patient was not involved in *MLL* gene rearrangement.

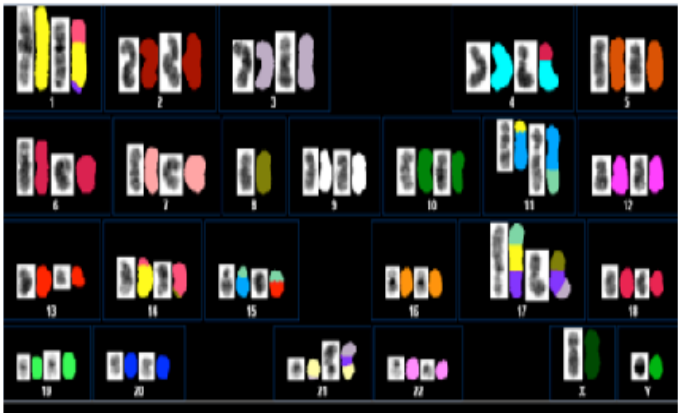

Figure 1 A representative classified spectral karyotype of bone marrow cells at diagnosis showing complex rearrangements involving multiple chromosomes. Reversed DAPI-banded imaging appears on the left of the color display in each box.

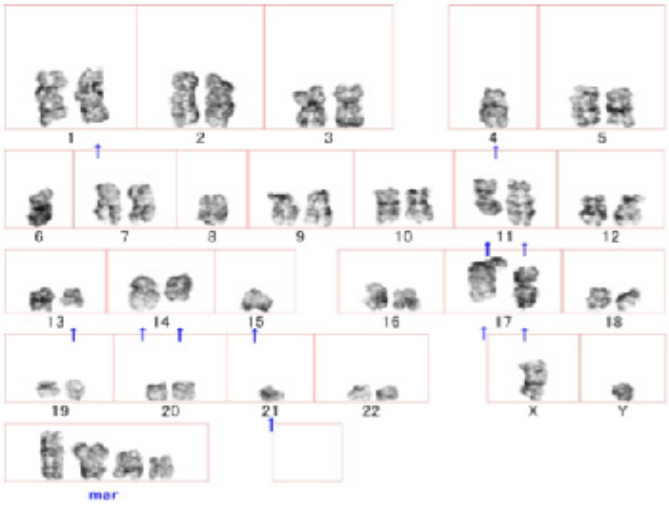

Figure 2 A representative karyotype of bone marrow metaphase cells at diagnosis by combining the results of SKY and G-banding analyses, showing a complex karyotype involving t(8;14)(q24;q32). The arrows represent the derivative chromosome, and the normal chromosome is on the left.

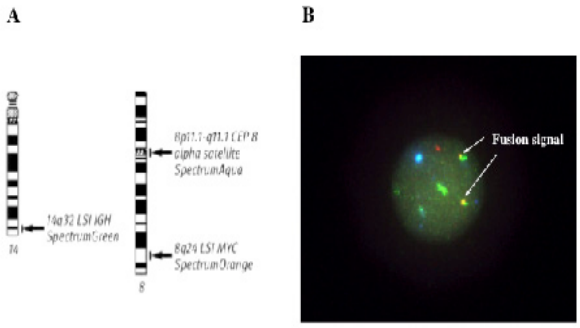

Figure 3 Triple-color FISH analysis of bone marrow interphase cells at diagnosis using probes specific for the *IGH* and *MYC* genes. (A) Schematic representation of the FISH probes currently used. (B) Yellow (green/orange) fusion signals of the IGH and MYC probes were detected in 95% of the interphase nuclei analyzed.

Discussion

The cytogenetic hallmark of BL is t(8;14)(q24;q32) or its variants, t(2;8)(p12;q24) or t(8;22)(q24;q11), resulting in constitutive deregulation of the *MYC* gene expression driven by the Ig gene enhancer.[1-4] Approximately 60 to 70% of sporadic BL cases in adults have additional chromosomal abnormalities, more complex than those found in the uniformly Epstein-Barr virus-positive endemic BL, suggesting potentially more diverse mechanisms of malignant transformation and disease progression in the former.[9] When excluding the Burkitt-type translocations, the chromosomes most frequently involved in abnormalities were 1, 6, 13, 17, and 22,[4,9]. Boerma et al.[10] reviewed the 'Mitelman Database of Chromosome Aberrations in Cancer' for defining a cytogenetic profile of 'true' BL, where lymphomas were diagnosed on a morphological basis, contained an *IGH-MYC* translocation, and did not harbor chromosomal translocations of the *BCL2*, *BCL6*, or cyclin D1 gene (*CCND1*) loci, and found that additional recurrent abnormalities included gains at chromosome 1q, 7, and 12, and losses of 6q, 13q32-34, and 17p. Poirel et al.[11] reported a large cytogenetic study performed on an international trial in children and adolescents with mature B-cell lymphoma, in which the main BL-associated secondary chromosomal aberrations were +1q (29%) and +7q and del(13q)(14% each). The relationship between karyotypic abnormalities and outcomes showed that +7q and del(13q) were independently associated with a significantly inferior event-free survival.[10] As for BL with a complex karyotype, Onciu et al.[9] reported an analysis for secondary chromosomal abnormalities in sporadic BL comparing pediatric and adult patients, in which approximately half of the patients had a complex karyotype (>3 chromosome abnormalities) in both groups, and the presence of a complex karyotype was associated with a poor prognosis on univariate analysis in children but not in adults.[9] In this study, complex karyotypes including t(8;14)(q24;q32) and *IGH/MYC* fusion were revealed by combining the results of SKY and G-banding. Although the patient achieved CR with the first cycle of the hyper-CVAD plus rituximab regimen, he relapsed with a short CR duration. The short clinical course of the patient may have been, in part, due to the complex chromosomal aberrations.

The characteristic cytogenetic finding of the patient was complex rearrangements involving multiple chromosomes. The complex karyotypes were observed at presentation, disappeared in remission, but reappeared on the recurrence of the disease, being presumably derived from the leukemic clone of the patient. Although the complex karyotypes were not completely identical during the course of the disease, eight common aberrations were identified in each karyotype detected using SKY analysis in combination with G-banding. Of these, the t(8;14)(q24;q32) abnormality may play a central role in the progression of the disease. FISH analysis using probes specific for the *IGH* and *MYC* genes revealed the *IGH/MYC* fusion. Clonal heterogeneity during the course of the disease may result from chromosomal instability.[12] Further studies are needed to clarify the issue.

With regard to the immunophenotype of mature B-cell ALL, leukemic cells express sIg and B-cell-specific antigens (i.e., CD19 and CD20), and are negative for CD5, CD23, and TdT.[2] They have a germinal center phenotype expressing CD10 and BCL6 but BCL2.[2,3] Unexpectedly, leukemic cells of the patient were positive for CD5, which is expressed on all mature T cells and in some B-cell

malignancies, such as mantle cell lymphoma (MCL).[13] The t(11;14)(q13;q32) is the cytogenetic hallmark of MCL, leading to the over expression of *CCND1*.[14] Although the complex karyotype of the patient included the 11q13 region, which is related to the *CCND1* locus, the characteristic t(11;14)(q13;q32) abnormality in MCL was not observed in the patient. Finally, immunohistochemistry of the bone marrow clot section at diagnosis showed *CCND1* negativity (data not shown). In addition, the immunophenotype of leukemic cells at relapse was changed into precursor B cells, presumably resulting from the eradication of a mature B-cell clone expressing CD20 by rituximab containing chemotherapy. Interestingly, it has been reported that rare ALL cases with t(8;14)(q24;q32) and an FAB-L3 morphology are associated with a B-precursor immunophenotype.[15]

Conclusion

In conclusion, we report an elderly patient with mature B-cell ALL harboring a complex karyotype involving t(8;14)(q24;q32) and *IGH/MYC* fusion. Our case showed the usefulness of SKY and FISH analyses for determining complex rearrangements involving multiple chromosomes that were not identified employing conventional cytogenetics alone.

Acknowledgements

None.

Conflict of interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1) Burmeister T, Schwartz S, Horst HA, et al. Molecular heterogeneity of sporadic adult Burkitt–type leukemia/lymphoma as revealed by PCR and cytogenetics: correlation with morphology, immunology and clinical features. *Leukemia.* 2005;19(8):1391–1398.

2) Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. *Blood*. 2004;104(10):3009–3020.

3) Linch DC. Burkitt lymphoma in adults. *Br J Haematol*. 2012;156(6):693–703.

4) Miles RR, Arnold S, Cairo MS. Risk factors and treatment of childhood and adolescent Burkitt lymphoma/leukaemia. *Br J Haematol*. 2012;156(6):730–743.

5) Mrózek K. Cytogenetic, molecular genetic, and clinical characteristics of acute myeloid leukemia with a complex karyotype. *Semin Oncol*. 2008;35(4):365–77.

6) Thomas DA, Faderl S, O'Brien S, et al. Chemoimmunotherapy with hyper–CVAD plus rituximab for the treatment of adult Burkitt and Burkitt–type lymphoma or acute lymphoblastic leukemia. *Cancer*. 2006;106(7):1569–1580.

7) Ohsaka A, Otsubo K, Yokota H, et al. Spectral karyotyping and fluorescence in situ hybridization analyses identified a novel three–way translocation involving inversion 16 in therapy–related acute myeloid leukemia M4eo. *Cancer Genet Cytogenet*. 2008;184(2):113–118.

8) 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(2):111–115.

9) Onciu M, Schlette E, Zhou Y, et al. Secondary chromosomal abnormalities predict outcome in pediatric and adult high–stage Burkitt lymphoma. *Cancer*. 2006;107(5):1084–1092.

10) Boerma EG, Siebert R, Kluin PM, et al. Translocations involving 8q24 in Burkitt lymphoma and other malignant lymphomas: a historical review of cytogenetics in the light of todays knowledge. *Leukemia*. 2009;23(2):225–234.

11) Poirel HA, Cairo MS, Heerema NA, et al. Specific cytogenetic abnormalities are associated with a significantly inferior outcome in children and adolescents with mature B–cell non–Hodgkin's lymphoma: results of the FAB/LMB 96 international study. *Leukemia*. 2009;23(2):323–331.

12) Holland AJ, Cleveland DW. Boveri revisited: chromosomal instability, aneuploidy and tumorigenesis. *Nat Rev Mol Cell Biol*. 2009;10(7):478–487.

13) Matolcsy A, Chadburn A, Knowles DM. De novo CD5–positive and Richter's syndrome–associated diffuse large B cell lymphomas are genotypically distinct. *Am J Pathol*. 1995;147(1):207–216.

14) Navarro A, Royo C, Hernández L, et al. Molecular pathogenesis of mantle cell lymphoma: new perspectives and challenges with clinical implications. *Semin Hematol*. 2011;48(3):155–165.

15) Navid F, Mosijczuk AD, Head DR, et al. Acute lymphoblastic leukemia with the (8;14)(q24;q32) translocation and FAB L3 morphology associated with a B–precursor immunophenotype: the Pediatric Oncology Group experience. *Leukemia*. 1999;13(1):135–141.