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Double minute chromosomes containing MYB gene and NUP214-ABL1 fusion gene in T-cell leukemia detected by single nucleotide polymorphism DNA microarray and fluorescence *in situ* hybridization

Norihiko Kawamata[1,*], **Ling Zhang**[2,*], **Seishi Ogawa**[3], **Yasuhito Nannya**[3], **Azadeh Dashti**[1], **Daning Lu**[1], **Stephen Lim**[1], **Rhona Schreck**[2], and **H. Phillip Koeffler**[1]

[1] Hematology/Oncology, Cedars-Sinai Medical Center/David Geffen School of Medicine at UCLA, Los Angeles, CA

[2] Pathology/Laboratory Medicine & Medical Genetics Institute, Cedars-Sinai Medical Center, Los Angeles, CA

[3] Regeneration Medicine of Hematopoiesis, University of Tokyo, School of Medicine, Tokyo, Japan

A 57-year old female was referred to our hospital because of flu like symptoms, and extensive bilateral pleural and pericardial effusion. However, the patient showed neither lymphadenopathy nor hepatosplenomegaly. Examination of cells in her pleural effusion revealed atypical cells with convoluted nuclear contour, dense chromatin, and bizarre mitotic figures. Numerous atypical cells had “flower shape” nuclei (Figure 1A).. She was negative for HTLV-I virus. Similar abnormal cells were also detected in the peripheral blood and bone marrow.

Flow cytometry analysis revealed that the abnormal cells were TdT−, CD2 +, CD4+, CD5+, CD7+, CD10−, CD20−, CD33− and CD34−. Loss of CD3 and CD8 was also noted. She was diagnosed as T-cell acute lymphoblastic leukemia (T-ALL), although the differential diagnosis, based on morphologic and immunophenotypical features of the leukemic cells, included T-cell prolymphocytic leukemia (T-PLL).

The karyotype of her leukemic cells was pseudotetraploid with a deleted copy of chromosome 1, numerous marker chromosomes and the presence of multiple double minute chromosomes: 89,XXXX,+del(1)(q25), +2, +3, +4×2, +5×2, +7×2, +8×4, +9, −10, +11, +12×2, +13, +14, 15×3, +16×2, +17×2, +18×2, +19×2, +20×2, +21×2, +22×2, +Mar1,+Mar2, +Mar3, +Mar4, +Mar5, +Mar6, +dms (Fig. 1B). To further characterize the double minutes, we performed single nucleotide polymorphism DNA microarray (SNP-chip) analysis on the leukemic cells using Affymetrix GeneChip Nsp 250K as previously reported [1]. The analysis demonstrated high copy number gene amplification of several chromosomal regions, including 6q and 9q (Figs. 1C and 2A). One of the amplified regions contained the *NUP214* and *ABL1* genes located at 9q34.1 (Fig. 1C). Rapid cloning of 5’end (RACE) was used to clone the gene fused to

Corresponding author: Norihiko Kawamata, M.D. Hematology/Oncology, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA90048, U.S.A. Tel -1-310-423-7736 Fax -1-310-423-0443, e-mail: kawamatan@cshs.org.
*These two authors equally contributed to this work as the first authors.

ABL1 and demonstrated that exon 31 of *NUP214* was fused to exon 3 of *ABL1* gene (Fig. 1D). Furthermore, we determined that the *MYB* gene was involved in one of the amplified region on chromosome 6 (Fig. 2A). FISH using *ABL1*, *MYB*, and *BCR* probes (Abbott Molecular Des Plaines, IL). showed that the *ABL1* and *MYB* genes were extensively amplified with co-localization in the nucleus (Fig. 2B). Consistent with the amplification of *MYB*, real-time RT-PCR showed that the *MYB* gene was highly expressed in the leukemic cells (Fig. 2C). FISH and SNP-chip analysis also revealed a homozygous deletion of the p15/p16 genes at 9p21 (not shown).

Double minute chromosomes, which are cytological manifestations of gene amplification, are rare abnormalities in leukemic cells. It can be difficult to define the genomic regions involved in their formation. In this study, SNP-chip analysis provided insight into the composition of the double minute chromosomes, while FISH analysis provided confirmation. *NUP214-ABL1* gene fusion has been reported in about 6% of T-acute lymphoblastic leukemia (ALL), usually presenting as cryptic episomes, and has been associated with a poor prognosis [2]. The present case is the first in which *NUP214-ABL1* fusion was found in larger double minute chromosomes. The increased size of the amplified structures may reflect the presence of other genomic material included in the double minutes. SNP-chip analysis had revealed amplification of *MYB* which was also confirmed by FISH. Co-localization of *NUP214-ABL1* and *MYB* was observed by the FISH analysis. Translocations and duplications leading to increased expression of the *MYB* gene have recently been reported in about 8% of T-ALL patients [3,4]. It has been reported that homozygous deletion of p15/p16 genes (9p21), as seen here, are detected in at least 65% of T-ALL [5]. The molecular features of this case, including presence of *NUP214-ABL1* fusion, over expression of *MYB* and deletion of the p16/p15 genes, supported the diagnosis of T-ALL.

There have been several reports of amplification of large genomic regions associated with complex translocations (complicons), especially in B-cell malignancies, involving IGH/MYC, IGH/BCL2, and IGH/CCND1 [6–8]. This is the first report of a complicon in a T-cell disorder, and also the first demonstration of nonsyntenic co-amplification of a complicon with two distinct oncogenes (*NUP214-ABL1* and *MYB*). This complex phenomenon would not have been suspected without the information provided by the SNP-chip analysis.

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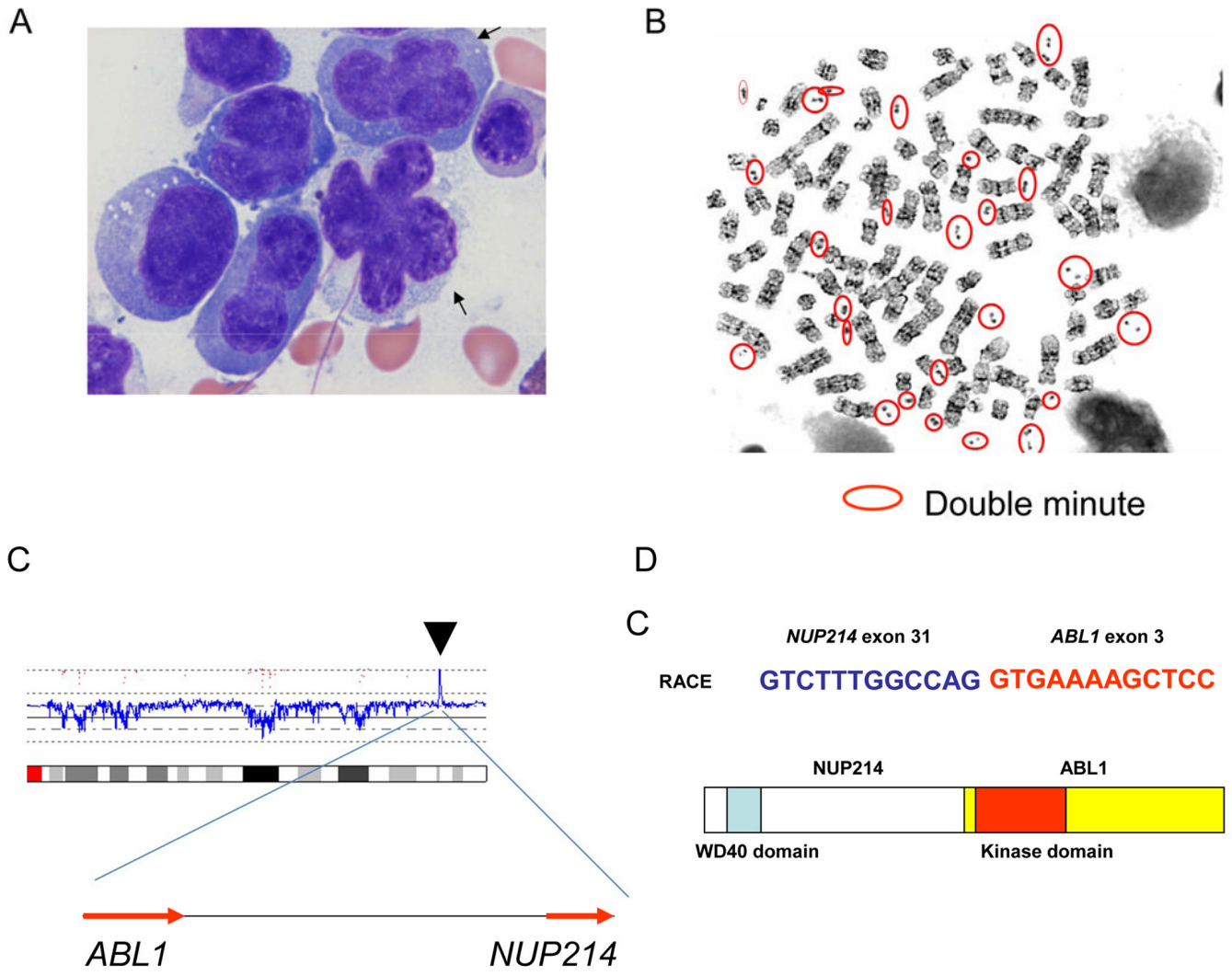

Fig. 1. *NUP214-ABL1* gene in T-cell leukemic cells

A: Morphology of leukemic cells. Atypical cells show convoluted nuclear contour, dense chromatin, and bizarre mitotic figures. Cells with “flower shape” nuclei are also observed (arrows).

B: Metaphase spreads. Double minute chromosomes are circled.

C: High copy number amplification of *NUP214* and *ABL1*. The result of SNP-chip analysis (chromosome 9) revealed high copy number amplification of chromosome 9q34 region (arrow head) which contained *NUP214* and *ABL1* genes (arrows indicate the direction of transcription of the genes).

D: *NUP214-ABL1* fusion was detected in T-cell leukemia. Rapid amplification of cDNA ends (RACE) method and nucleotide sequencing showed the fusion of exon 31 of *NUP214* and exon 3 of *ABL1*. Schematic representation of the structure of NUP214-ABL1 fusion protein is shown.

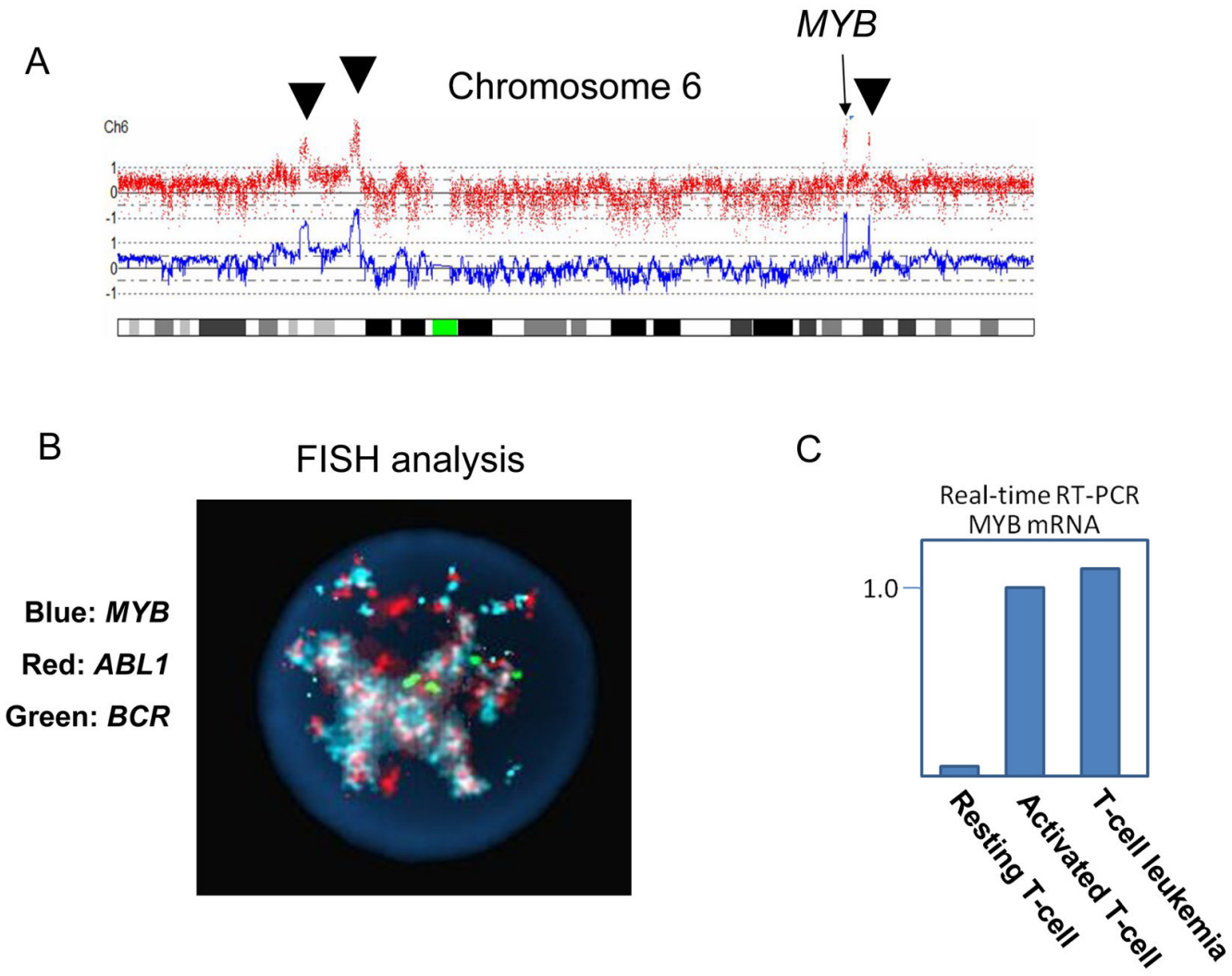

Fig. 2. *MYB* and *NUP214-ABL1* genes are components of double minute chromosomes in T-cell leukemic cells

A: The result of SNP-chip analysis (chromosome 6). High copy number amplifications of four chromosomal regions (arrow heads and an arrow) are detected. One of the amplified regions contains *MYB* gene (arrow).

B: FISH analysis. *MYB* (aqua), *ABL1* (red) and *BCR* (green) genes were examined by FISH analysis. *MYB* and *ABL1* show co-amplification. BCR shows four signals in this cell, consistent with four copies of chromosome 22 (see text).

C: *MYB* gene is over expressed in the leukemic cells. The expression levels of *MYB* gene were measured in resting and activated normal T-cells (stimulated with PHA) as well as the patient's leukemic cells by real-time RT-PCR.