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Case report

RUNX1 rearrangement in mature B-cell acute lymphoblastic leukemia with non-L3 morphology

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Mature B-cell acute lymphoblastic leukemia (ALL) is defined by the expression of light chain-restricted surface immunoglobulin (sIg) and usually has features of the leukemic phase of Burkitt lymphoma including FAB-L3 morphology and *MYC* rearrangement. Recently, another distinct entity in childhood mature B-cell ALL has been characterized as non-L3 morphology and *KMT2A* rearrangement. Here we report an unusual case of mature B-cell ALL that presented with *RUNX1* rearrangement. A 65-year-old male was admitted to our department for thorough examination of leukocytosis and thrombocytopenia. The patient's bone marrow was hypercellular and infiltrated with 97.8% myeloperoxidase-negative, medium-to-large-sized blasts without cytoplasmic vacuoles. Immunophenotypes were characterized by the presence of light chain-restricted sIg and the lack of immature markers, indicating a diagnosis of mature B-cell ALL with L2 morphology: sIg-κ+, CD19+, CD20+, CD22+, CD79a+, TdT−, and CD34−. G-banding combined with spectral karyotyping showed the following complex karyotype: 45,X,der(Y;10)(p10;q10),del(13)(q?),inv(21)(p13q22.1). Fluorescence *in situ* hybridization revealed separated signals of *RUNX1* at 21q22.1, whereas rearrangements of *MYC* and *KMT2A* were not found. To our knowledge, inv(21)(p13q22.1) involving *RUNX1* is a novel cytogenetic aberration and this is the first case of mature B-cell ALL that presented with *RUNX1* rearrangement. Thus, *RUNX1* may be implicated in the pathogenesis of mature B-cell ALL showing non-L3 morphology without *MYC* rearrangement.

Keywords: mature B-cell acute lymphoblastic leukemia, fluorescence *in situ* hybridization, *RUNX1* rearrangement, non-L3 morphology

INTRODUCTION

B-lineage acute lymphoblastic leukemia (ALL) is defined by the expression of early B-cell markers (CD19, CD22, and CD79a) and is classified into four categories according to the degree of differentiation of B-lymphoblasts: pro-B-ALL, common ALL, pre-B-ALL, and mature B-ALL.[1] Among these, mature B-cell ALL is characterized either by the appearance of surface immunoglobulin (sIg) or cytoplasmic expression of one of the immunoglobulin light chains. In contrast to the other three subtypes, most cases of mature B-cell ALL do not express the immature marker, terminal deoxynucleotidyl transferase (TdT). Mature B-cell ALL is an uncommon type accounting for 1–2% of all ALL cases and usually shows a morphology of L3-type ALL cells, which contains abundant vacuoles in the basophilic cytoplasm, according to the French–American–British classification.[2,3] It is now classified as a leukemic stage of Burkitt lymphoma (Burkitt leukemia variant) in the World Health Organization (WHO) classification and International Consensus Classification (ICC).[4-6] Most cases of Burkitt lymphoma have the nonrandom chromosomal translocation t(8;14)(q24;q32), or less commonly its variants t(8;22)(q24;q11) or t(2;8)(p12;q24). The *MYC* gene at 8q24 and one of the immunoglobulin genes are involved in these translocations.

Recently, another entity has been reported in childhood mature B-cell ALL. This distinct subset of B-lineage ALLs is characterized by a mature B-cell phenotype (sIg-λ+, CD10−, CD19+, TdT−, and CD34−), non-L3 morphology without *MYC* rearrangement, and poor clinical course.[7-13] Instead of *MYC*, the *KMT2A* gene at 11q23 was involved in the translocations, and t(9;11)(p21;q23) forming a *KMT2A*::*MLLT3* fusion was predominantly observed.

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However, genetic abnormalities other than *MYC* and *KMT2A* have not been fully elucidated in mature B-cell ALL. Here we report an unusual case of mature B-cell ALL harboring a novel cytogenetic aberration inv(21)(p13q22.1), which led to a rearrangement of the *RUNX1* gene at 21q22.1.

CASE REPORT

A 65-year-old male was admitted to our department for thorough examination of leukocytosis and thrombocytopenia. He had no lymphadenopathy or hepatosplenomegaly. Peripheral blood values were hemoglobin 129 g/L, platelets 33 × 10^9/L, and leukocytes 26.7 × 10^9/L with 2% band forms, 27% segmented neutrophils, 17% lymphocytes, 7% monocytes, and 47% blasts. Blood chemistry revealed elevated lactate dehydrogenase (4271 U/L, normal range 124–222) and uric acid (9.3 mg/dL, normal range 4.0–7.8). The bone marrow of the patient was hypercellular and infiltrated with 97.8% blasts. These cells were medium-to-large in size, and presented with fine nuclear chromatin, a few small nucleoli, and scarce basophilic cytoplasm. No cytoplasmic vacuoles were found (Fig. 1A). Bone marrow blasts were negative for myeloperoxidase staining (Fig. 1B). Flow cytometry analysis demonstrated that cells delimited by CD45/side scatter gating (95.5% of bone marrow cells) were positive for CD10 (23.7%), CD19 (95.4%), CD20 (45.5%), CD22 (95.7%), CD38 (99.1%), CD79a (98.2%), HLA-DR (87.1%), and sIg κ light chain (68.8%). In contrast, they were negative for CD34 (0.0%), TdT (1.6%), λ light chain (2.5%), and other T-lymphoid and myeloid markers (Fig. 1C). Molecular screening revealed no expression of major and minor *BCR*::*ABL1* fusion transcripts. Based on these morphological and immunophenotypic findings, the patient was diagnosed with mature B-cell ALL showing L2 morphology. Pathological diagnosis from bone marrow clot sections also confirmed B-lymphoblastic leukemia.

Induction therapy using a hyper-CVAD/MA regimen was administered, and the patient achieved complete remission. However, after 8 months, the disease relapsed with 68% lymphoblasts in the bone marrow. Subsequently, the patient

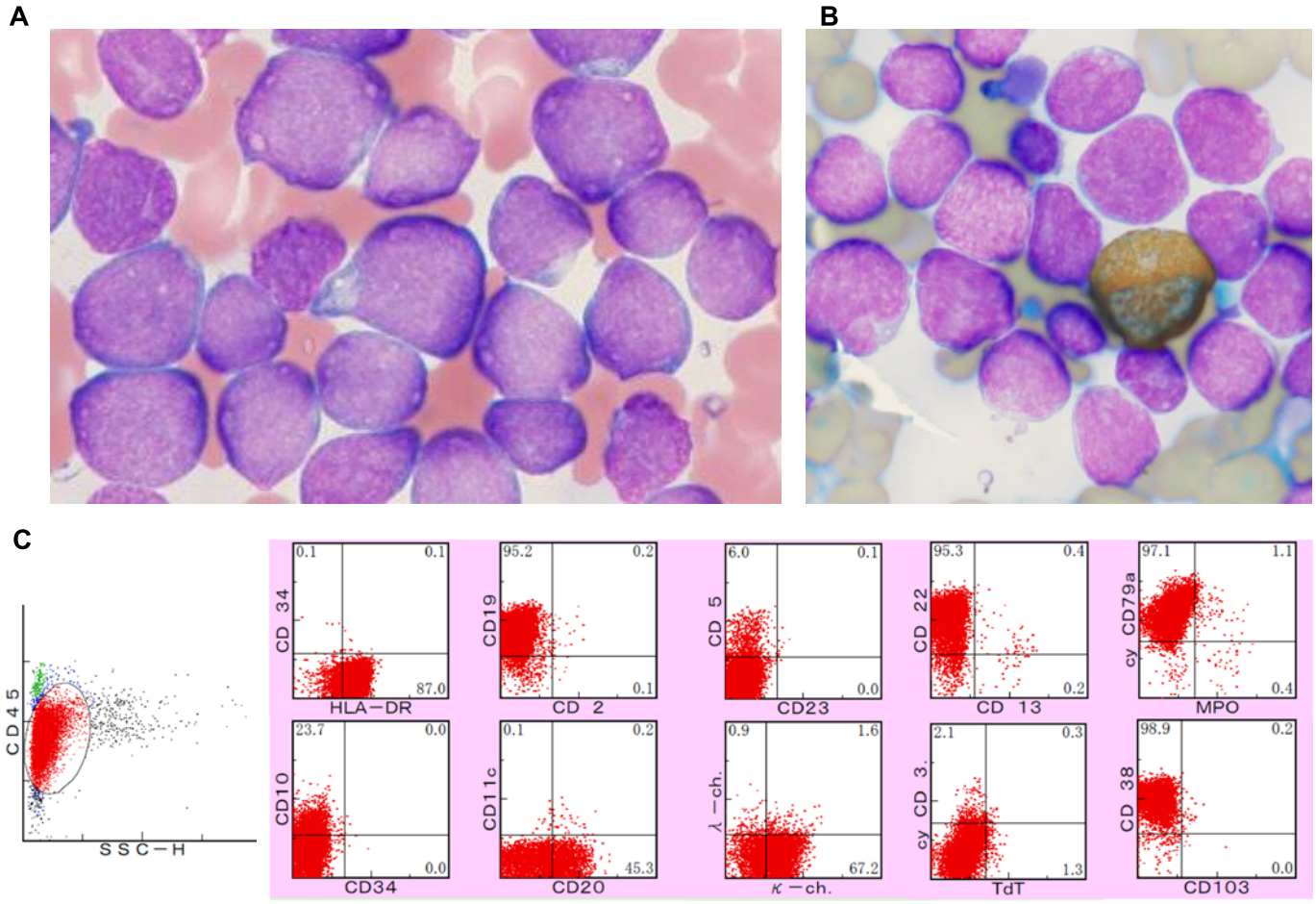

Fig. 1. Morphologic and immunophenotypic findings of leukemia cells.
A Bone marrow smears show medium-to-large lymphoblasts having fine nuclear chromatin, basophilic cytoplasm, and a few nucleoli. Cytoplasmic vacuoles are not seen (May–Grünwald–Giemsa stain, ×1000).
B Bone marrow lymphoblasts are negative for myeloperoxidase staining, while myeloid cells are positive.
C Flow cytometry analysis of bone marrow lymphoblasts. The percentage of cells delimited by CD45/side scatter gating is 95.5%. The results of two-color analyses for the indicated markers are demonstrated. Lymphoblasts are positive (>20%) for CD19, CD22, CD79a, CD10, CD20, CD38, HLA-DR, and sIg κ-chain.

received further treatment with blinatumomab followed by re-induction therapy using JALSG (Japan Adult Leukemia Study Group)-ALL97 protocol including vincristine, daunorubicin, cyclophosphamide, prednisolone, and L-asparaginase with no noticeable effect. He did not wish to undergo stem cell transplantation. Eleven months after the first visit, the patient died due to progressive disease.

CYTOGENETIC STUDIES

At initial diagnosis, the G-band karyotype of bone marrow cells was 45,X,-Y,add(10)(p11.2),del(13)(q ?),-21,+mar1[20] (Fig. 2A). Next, spectral karyotyping (SKY) was performed on metaphase spreads using Sky Paint Probes (Applied Spectral Imaging, Carlsbad, CA, USA). SKY combined with DAPI (4', 6-diamidino-2-phenylindole) banding revealed that the marker chromosome was derived from the inversion between the long and short arms of chromosome 21: inv(21)(p13q22.1). Furthermore, add(10)(p11.2) was found to be an unbalanced translocation with the Y chromosome: der(Y;10)(p10;q10) (Fig. 2B). Accordingly, the karyotype was revised to 45,X,der(Y;10)(p10;q10),del(13)(q?),inv(21)(p13q22.1)[5]. The karyotype reverted to 46,XY[20] after induction therapy. At the time of recurrence, the karyotype was 46,X,der(Y;10)(p10;q10),add(1)(q21),add(4)(q21),del(13)(q?),+18,inv(21)(p13q22.1),add(22)

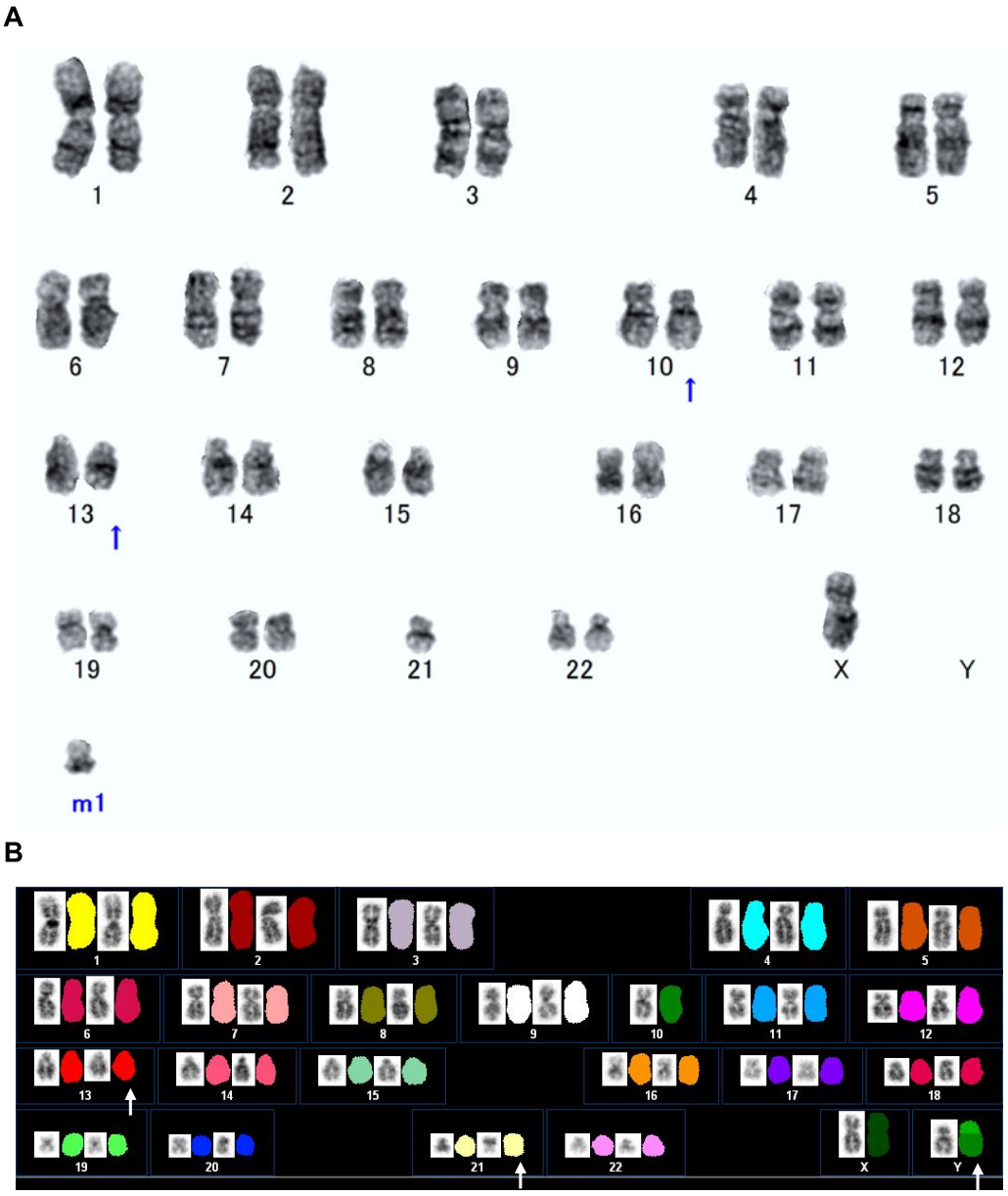

Fig. 2. Cytogenetic findings of leukemia cells.
A G-banded karyotype of leukemia cells: 45,X,-Y,add(10)(p11.2),del(13)(q ?),-21,+mar1. Arrows point to rearranged chromosomes.
B Spectral karyotyping (SKY) (right, SKY; left, reverse DAPI). The karyotype is corrected to 45,X,der(Y;10)(p10;q10),del(13)(q?),inv(21)(p13q22.1). Arrows point to rearranged chromosomes.

(p11.2)[7]/46,XY[1].

To examine whether the *RUNX1* gene was involved in the inv(21)(p13q22.1) breakpoint, we performed fluorescence *in situ* hybridization (FISH) analyses on metaphase spreads using a CytoCell AML1 (RUNX1) Breakapart Probe (Cytocell, Cambridge, UK). FISH revealed that a part of the green signal (5' *RUNX1*) remained at 21q22.1, whereas the rest of the green signal (5' *RUNX1*) and a red signal (3' *RUNX1*) moved to 21p13 and formed a yellow (fusion) signal, indicating *RUNX1* rearrangement (Fig. 3A). The breakpoint was supposed to be located in the region covered by the 5' *RUNX1* probe (Fig. 3C). Furthermore, FISH analysis using interphase nuclei also confirmed the green, green/red, and normal yellow (5'/3' *RUNX1* fusion) signals (Fig. 3B). In this interphase FISH, at least 13 of 100 cells were confirmed to show *RUNX1* rearrangement, suggesting a clonal genetic event. These cytogenetic changes are depicted in Fig. 3D. To exclude rearrangements of *MYC* and *KMT2A*, we also performed FISH with the Vysis LSI MYC Dual Color, Break Apart Rearrangement Probe Kit or the Vysis LSI MLL Dual Color, Break Apart Rearrangement Probe Kit (Abbott, Abbott Park, IL, USA). Of the 100 cells with interphase nuclei examined, none showed a split of *MYC* or *KMT2A* signals (data not shown).

DISCUSSION

We detected *RUNX1* rearrangement in an adult ALL patient with inv(21)(p13q22.1). Immunophenotypes of lymphoblasts were characterized by the presence of light chain-restricted sIg and the lack of the immature marker TdT, indicating a mature B-cell phenotype: sIg-κ+, TdT−, CD19+,

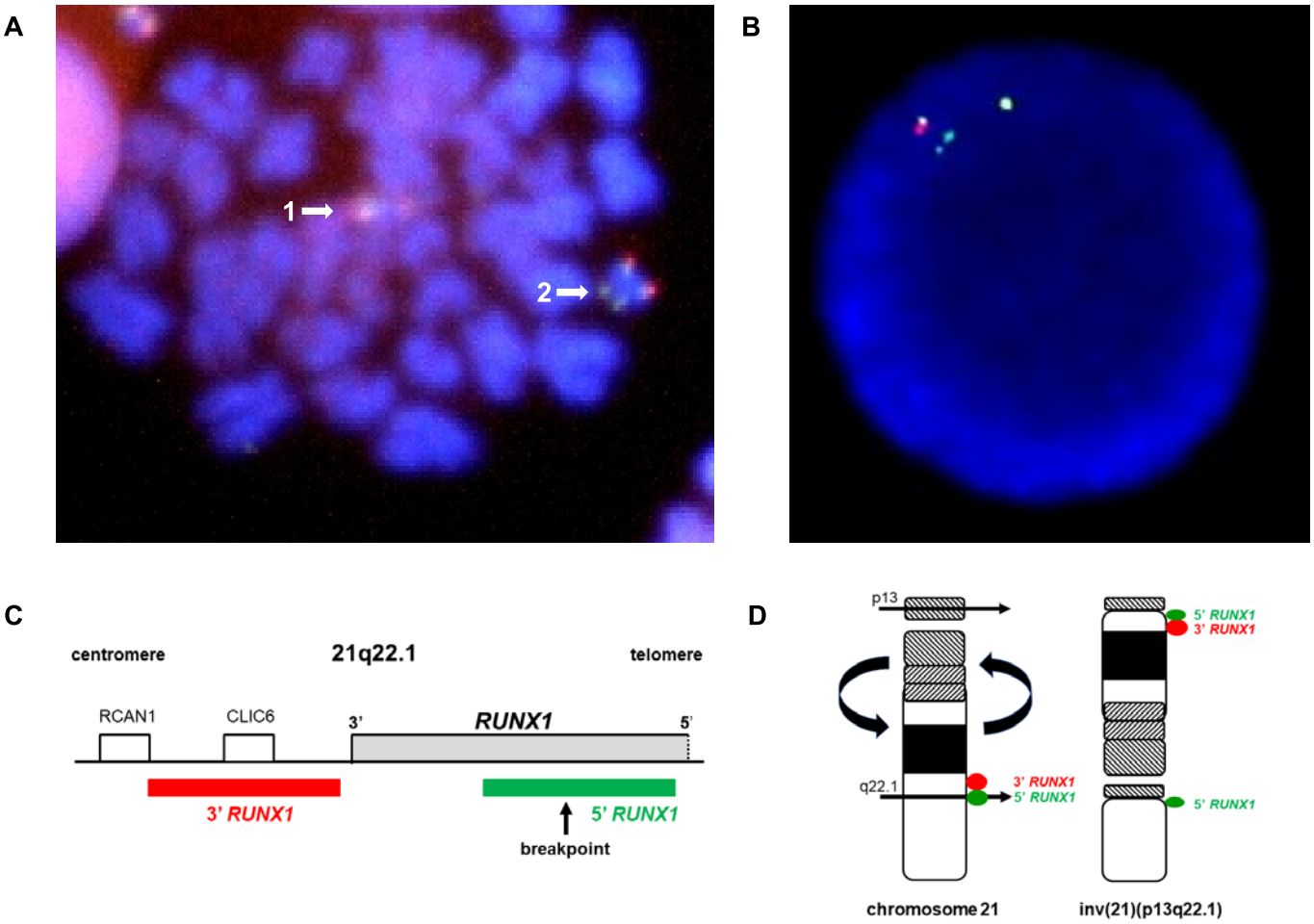

Fig. 3. Fluorescence *in situ* hybridization (FISH) analyses of leukemia cells.
A FISH using CytoCell RUNX1 breakapart probe on metaphase spreads. The arrows indicate 1) a normal 5' *RUNX1*/3' *RUNX1* fusion signal (yellow) on a normal chromosome 21, and 2) a faint 5' *RUNX1* signal (green) at 21q22.1, and a 5' *RUNX1* signal (green) and 3' *RUNX1* signal (red) at 21p13 on the inv(21)(p13q22.1).
B FISH using a CytoCell RUNX1 breakapart probe on interphase nuclei. Red/green, green, and yellow signals are observed on an interphase cell.
C Schematic representation of the *RUNX1* gene and assumed breakpoints in the 21q22.1 region. The position of a CytoCell RUNX1 probe (5' *RUNX1* and 3' *RUNX1* probes) covering the *RUNX1* gene on a normal chromosome 21 is presented. The vertical arrow indicates the assumed breakpoint; that is, middle of the region covered by the 5' *RUNX1* probe.
D Idiograms showing G-banding patterns of normal chromosome 21 (left) and inv(21)(p13q22.1) (right) at 300-band levels. Chromosome breakpoints are shown by horizontal arrows. The locations of 5' *RUNX1* (green) and 3' *RUNX1* (red) signals are indicated on the right sides of the normal and inverted chromosomes.

CD20+, CD22+, CD79a+, and CD34−. However, medium- to large-sized lymphoblasts had no vacuoles in the basophilic cytoplasm. These results indicate a diagnosis of mature B-cell ALL showing non-L3 morphology. Furthermore, G-banding combined with SKY demonstrated a novel cytogenetic aberration inv(21)(p13q22.1). FISH revealed rearrangement of *RUNX1* at 21q22.1, whereas no rearrangements of *MYC* and *KMT2A* were found. To our knowledge, this is the first case of mature B-cell ALL that presented with *RUNX1* rearrangement. Thus, *RUNX1* may be implicated in the pathogenesis of mature B-cell ALL showing non-L3 morphology without *MYC* rearrangement.

Mature B-cell ALL with sIg expression is typically Burkitt lymphoma presenting with L3 morphology and *MYC* translocations, but three types of variations are recognized: 1) L3 morphology but no *MYC* rearrangement, 2) *MYC* rearrangement but non-L3 morphology, and 3) non-L3 morphology and absence of *MYC* rearrangement.[2] In mature B-cell ALL showing non-L3 appearance without *MYC* rearrangement, there seems to be various cytogenetic abnormalities including complex karyotypes. One of these abnormalities has been shown to be t(9;11)(p21;q23) with *KMT2A* rearrangement.[7,8,11-13] Our results suggest that, following *KMT2A* rearrangement, *RUNX1* rearrangement may also represent a novel genetic aberration in this disease entity.

Clinically, it is important to distinguish Burkitt lymphoma/leukemia (mature B-cell ALL) and precursor B-lymphoblastic leukemia/lymphoma (pre-B ALL, common ALL, and pro-B ALL) since these two entities respond to different treatment regimens.[14] Based on the genetic abnormalities and morphology instead of immunophenotypes, it is considered appropriate to categorize and manage mature B-cell ALL showing non-L3 morphology without *MYC* translocations as precursor B-lymphoblastic leukemia/lymphoma rather than Burkitt lymphoma.[14] We also treated the patient using a protocol designed for precursor B-lymphoblastic leukemia/lymphoma.

With regard to the diagnosis, we classified the present case into mature B-cell ALL, which was included within a previous category. This is because the immunophenotype was apparently that of mature B-cell neoplasms, although morphologically and clinically this case appears to belong to B-lymphoblastic leukemia/lymphoma. Recently, the revised 5th edition of the WHO classification and the ICC 2022 were published.[5,6] Mature B-cell ALL usually corresponds to Burkitt leukemia variant in both classifications, but in the present case, the diagnosis of Burkitt leukemia variant could not be made because of the non-L3 morphology and the absence of *MYC* rearrangement. This discrepancy was also observed in childhood mature B-cell ALL with *KMT2A* rearrangement.[7-13] Therefore, it is difficult to diagnose this case as one of the subtypes of lymphoid neoplasms defined by both classifications. If we dare to classify, given the absence of *MYC* rearrangement, it is probably appropriate to classify this case as a leukemic presentation of high-grade B-cell lymphoma (HGBL), not otherwise specified (NOS).[5,6] However, to describe the immunophenotypic and morphologic features of this case more accurately, we considered that a diagnosis of mature B-cell ALL would be more applicable than HGBL-NOS.

The *RUNX1* gene was initially identified in acute myeloid leukemia (AML) M2 with t(8;21)(q22;q22) and shown to form the *RUNX1*::*RUNX1T1* fusion gene. *RUNX1* encodes a transcription factor containing a runt homology domain (RHD) accountable for DNA-binding and heterodimerization with CBFβ and is involved in the establishment of definite hematopoiesis.[15,16] It has been shown that *RUNX1* is implicated in leukemogenesis by reciprocal translocations, point mutations and amplification. Over 50 chromosomal translocations involving *RUNX1* have been documented in ALL as well as AML.[15,16] Among these, an inversion of chromosome 21 involving *RUNX1* at 21q22.1 has been reported in only one AML case: 46,XX,inv(21)(q11.2q22).[17,18] Namely, inv(21)(p13q22.1) involving *RUNX1* is a novel cytogenetic aberration. It is suggested that the rearranged *RUNX1* was fused to its partner gene (PG) at 21p13, although this *RUNX1* rearrangement could also be a non-specific genetic change that causes loss of RUNX1 function.

The organization of *RUNX1* fusion genes is usually 5' *RUNX1*-3' PG in AML and myelodysplastic syndrome harboring translocations such as t(1;21)(p36;q22), t(3;21)(q26;q22), and t(8;21)(q22;q22). Exceptionally, t(12;21)(p13;q22), which is specifically found in B-cell ALL of childhood, results in 5' *ETV6*-3' *RUNX1* fusion gene.[16] Both types of *RUNX1* fusions include RHD. In a case of AML with inv(21)(q11.2q22), a 5' *RUNX1* exon 8-*LINC00478* exon 5 fusion gene was generated.[18] In the present case, inv(21)(p13q22.1) likely resulted in the two fusion genes: 5' *RUNX1*-3' PG at 21q22.1 and 5' PG-3' *RUNX1* at 21p13. However, at present, it is unknown which fusion gene is critical in leukemogenesis. Identification of PG will be helpful to elucidate the pathological role of *RUNX1* in mature B-cell ALL showing non-L3 appearance.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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