***De novo* transformed follicular lymphoma that carried a double t(14;18)(q32;q21) chromosomal translocation**

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A 72-year-old man presented with a tentative diagnosis of acute leukemia. His white cell count was $7.59 \times 10^3/\mu\text{L}$, including 15.0% leukemic cells with immature morphology. Lactate dehydrogenase value was 4,243 IU/L. The bone marrow biopsy showed 'packed' marrow infiltration of leukemic cells. The cells were CD5⁺, CD10⁺, CD19⁺, CD20⁺, and surface immunoglobulin $\gamma\lambda^+$ by flow cytometry, and BCL2⁺, BCL6⁺, and MUM1⁺ by immunohistochemistry. ¹⁸Fluorodeoxyglucose-positron emission tomography combined with computed tomography showed heterogeneous tracer uptake throughout the bone marrow space. G-banding of leukemia cells revealed a hyperdiploid karyotype with two copies of t(14;18)(q32;q21), and fluorescence *in situ* hybridization of interphase nuclei confirmed the double t(14;18)(q32;q21). There was no 8q24/MYC rearrangement. Long-distance polymerase chain reaction generated a 2.6 kilobase-pair product, encompassing the major breakpoint cluster of BCL2, N-like segments, and the J5 segment of the immunoglobulin heavy chain gene. Although, the patient initially responded to chemotherapy, he relapsed shortly and died of disease progression. This case represents a rare presentation of follicular lymphoma, in which transformation to aggressive disease occurred *de novo* without a preceding indolent phase. It is possible that the double t(14;18)(q32;q21) may have generated a higher amount of BCL2 protein, thereby accounting, in part, for the progressive/refractory behavior of this case.

Keywords: follicular lymphoma, transformation, double t(14;18)(q32;q21), long-distance PCR, FISH

INTRODUCTION

Follicular lymphoma (FL) is a well-defined subtype of B-cell non-Hodgkin's lymphomas. The disease predominantly involves lymph nodes and manifests indolent clinical behavior for several years.¹ However, a significant proportion of cases, ranging from 10% to 60%, exhibit progression into high-grade histopathology with an

aggressive clinical course, followed by treatment failure and a rapidly fatal outcome.² On the other hand, over 80% of cases of FL carry the t(14;18)(q32;q21) chromosomal translocation.¹ The translocation juxtaposes the BCL2 gene at chromosomal band 18q21 to the immunoglobulin heavy chain gene (IGH) at 14q32 in the same transcriptional orientation, resulting in the deregulated expression of the anti-apoptotic BCL2 protein.¹ The breakpoints involved in the translocation have been extensively investigated at the molecular level, and nearly

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70% of the breakpoints have been found to be clustered within a 150 base-pair segment at the untranslated end of *BCL2* exon 3, designated as the major breakpoint region (MBR).³

We describe here a man who exhibited circulating immature cells and bone marrow replacement. Although the disease was initially diagnosed as acute lymphoid leukemia, the cells were found to express the surface immunoglobulins and carry the hallmark translocation of FL, t(14;18)(q32;q21), in duplicate. These findings demonstrate a case of transformed FL that developed *de novo*.

CASE REPORT

A 72-year-old man was admitted to hospital with a tentative diagnosis of acute leukemia. He exhibited

general fatigue and progressive loss of appetite 1 month before admission, and developed marked night sweats 2 weeks prior to admission. On admission, his body temperature was 38.2°C, blood pressure was 94/55 mmHg, heart rate was 90 beats per minute, and oxygen saturation was 94% while breathing ambient air. There was no surface lymphadenopathy. The liver and spleen were not palpable. His performance status was ECOG 3. His hemoglobin level was 10.3 g/dL, white cell count was $7.59 \times 10^3/\mu\text{L}$, and platelet count was $66 \times 10^3/\mu\text{L}$. The white cell differential was 6.0% lymphocytes, 8.5% monocytes, 0.5% eosinophils, 62.5% segmented neutrophils, 7.0% banded neutrophils, 0.5% metamyelocytes, and 15.0% leukemic cells with immature appearance (Figure 1A). The level of lactate dehydrogenase (LDH)

Figure 1. Appearance and immunophenotype of leukemic cells in peripheral blood. (A) Wright-stained peripheral blood smear (original magnification, $\times 100$ objective lens). The cells had irregular nuclear contours with immature chromatin and a modest amount of basophilic cytoplasm. Nucleoli were not conspicuous. (B) Flow cytometry. The mononuclear cell fraction was separated by dual-color flow cytometry based on the expression of the antigens indicated. The cells of interest were: CD5⁻, CD10⁺, CD19⁺, CD20⁺, CD21⁻, CD22⁺, CD23^{dim}, CD38⁺, and Smlg λ ⁺. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

was 4,243 IU/L, aspartate aminotransferase was 108 IU/L, alanine aminotransferase was 43 IU/L, and uric acid was 8.2 mg/dL. C-reactive protein was 14.8 mg/dL and soluble interleukin-2 receptor was 1,758 U/mL. He was seropositive for hepatitis B (HBV) surface antigen.

Flow cytometry revealed that leukemic cells were CD5⁻, CD10⁺, CD19⁺, CD20⁺, CD21⁻, CD22⁺, CD23^{dim}, and CD38⁺, and expressed $\gamma\lambda$ surface immunoglobulins (Figure 1B). Bone marrow aspiration was dry tap. The bone marrow biopsy specimen showed 'packed' marrow infiltration of leukemic cells with irregular nuclear contours and eosinophilic nucleoli (Figure 2). The cells were positive for CD10, CD20, CD79a, and BCL2, and negative for CD5, BCL6, and MUM1. The positivity of Ki-67 immunostaining was below 50% (Figure 2B).

¹⁸F-fluorodeoxyglucose-positron emission tomography combined with computed tomography (FDG-PET/CT) showed heterogeneous tracer uptake within the bone marrow space in the central skeleton, extending into the long bones, and the level of splenic uptake was indicative of leukemic infiltration (Figure 3A). FDG-accumulated lymph nodes were detectable in the right inguinal region. The maximum standardized uptake value (SUV-max) of the lumbar vertebrae was 11.87.

To prevent reactivation of HBV and tumor lysis syndrome, entecavir and rasburicase were initiated immediately after admission. The patient was then treated with the dose-adjusted (DA) EPOCH (etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin) regimen in combination with rituximab (R),⁴ leading to resolution of the constitutional symptoms. The bone marrow was replaced with necrotic cells one month after treatment, the cell membrane of which was stained by CD20 immunostaining. A second cycle of DA-EPOCH-R was performed uneventfully; however, the patient declined further treatment and was lost to follow-up.

Eleven months after the end of initial treatment, the patient relapsed with widespread infiltration of the soft tissues of the left hemi-trunk (Figure 3B). A biopsy of

the skin and subcutaneous tissues revealed perivascular infiltration of lymphoma cells throughout the dermis, extending to the underlying subcutaneous fat tissues (Figure 4A). The immunophenotype of the cells was identical to that of primary leukemic cells, but the Ki-67 positivity was close to 100% (Figure 4B). The bone marrow included 65% leukemic cells. Although the disease transiently responded to chemotherapy, the patient died of disease progression 22 months after initial presentation.

MOLECULAR CYTOGENETIC STUDIES

Metaphase spreads were obtained from a short-term culture of peripheral blood mononuclear cells. The chromosome number was within the hyperdiploid range. Structural abnormalities included del(6)(q13q21), dup(12)(q13q22), and t(14;18)(q32;q21), the latter two of which were duplicated. The karyotype, according to the ISCN⁵, was 53~54,XY,+X,+6,del(6)(q13q21),+8,+12,dup(12)(q13q22)×2,+14,t(14;18)(q32;q21)×2,+18,+19,+20,+1~2mar[cp7]/46,XY[1] (Figure 5). Fluorescence *in situ* hybridization (FISH) applied to the peripheral blood smear slide using the Vysis LSI *IGH/BCL2* dual-color, dual-fusion translocation probe showed the leukemic cell nuclei carried one red (*BCL2*), one green (*IGH*), and 4 yellow (*IGH-BCL2* fusion) signals in agreement with double t(14;18)(q32;q21) (Figure 6A). FISH using the Vysis LSI *MYC* dual-color, break-apart rearrangement probe showed 3 yellow (germline *MYC*) signals, matching trisomy 8 (Figure 6B); no rearrangement was detected by the Vysis LSI *BCL6* (ABR) dual-color, break-apart rearrangement probe (not shown). The karyotype obtained from the bone marrow during relapse was 51~52,XY,+1,add(1)(p32),+7,+8,+12,dup(12)(q13q22)×2,+14,t(14;18)(q32;q21)×2,+18,+19,+mar[cp5], in which add(1)(p32) and +7 appeared as additional abnormalities.

To confirm the t(14;18)(q32;q21) translocation at the nucleotide level, we performed long-distance polymerase chain reaction (LD-PCR) using primers designed for the MBR and the enhancer region of *IGH*.³ As shown

A

B

Figure 2. Histopathology of the bone marrow biopsy at presentation. (A) Hematoxylin and eosin (H&E) staining. The bone marrow space was replaced by lymphoma cell infiltrates. (B) Immunohistochemistry. The lymphoma cells were positive for CD10, CD20, and BCL2, and negative for BCL6 and MUM1. The Ki-67 proliferation index was below 50%. CD5 and CD79a immunostaining are not shown.

Figure 3. FDG-PET/CT images at presentation (A) and relapse (B). The anterior view of the maximum intensity projection images (*left*) and representative axial images of the body (*right*) are shown. In A, the spleen showed a higher degree of activity than that in the liver (*middle*), and an inguinal lymph node was FDG-avid (*bottom*). In B, para-aortic lymph nodes (*middle*) and the scrotum (*bottom*) were considered to be involved. The SUVmax value of the subcutaneous tumor was 10.41.

in Figure 7A, the LD-PCR generated a 2.6 kilobase-pair product, and nucleotide sequencing revealed that the product encompassed the MBR, N-like segments, and the J5 segment of *IGH* in the 5' to 3' orientation (Figure 7B).

DISCUSSION

The current case was unusual for the presentation of FL because initial clinical behavior and laboratory data suggested aggressive leukemia/lymphoma, the BM was replaced by leukemic cells with immature morphology, instead of the typical paratrabecular pattern of BM involvement of FL, and lymph node involvement was minimal. The disease initially responded to therapy, but quickly progressed into refractory disease. Nevertheless, the cells had the mature B-cell immunophenotype and carried the t(14;18)(q32;q21) translocation, but lacked 8q24/*MYC* rearrangement. The alignment of the *BC2-IGH* fusion gene was identical to that of FL.³ These find-

ings suggest that this case represents a rare presentation of FL, in which transformation to aggressive disease occurred *de novo* without a preceding indolent phase. Alternatively, leukemic manifestation of FL at presentation may be considered. However, this condition, as nodal FL, exhibits indolent cytomorphology and clinical behavior,⁶ and the level of SUVmax of the BM with biopsy-proven involvement is at most 6.0 with a mean value of 3.7.⁷ Thus, we propose that the term “*de novo* transformed FL” most suitably represents the clinical features of this case.

In most cases of FL transformation, the histopathology is consistent with diffuse large B cell lymphoma (DLBCL).^{1,2} Less commonly, FL evolves into Burkitt-like lymphoma, which is currently referred to as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; cytogenetic studies often show both *BCL2* and *MYC* translocation, or so-called double-hit disease.^{1,2,8} Other rare forms of

Figure 4. Histopathology of the cutaneous biopsy at relapse. (A) H&E staining. The dermis was infiltrated with lymphoma cells showing perivascular proliferation and extending into the subcutaneous fat tissue layer. (B) Immunohistochemistry. The lymphoma cells were positive for CD10, CD20, CD79a, and BCL2, and negative for BCL6. The Ki-67 proliferation index was close to 100%.

Figure 5. *Top*, G-banding karyotype obtained from the peripheral blood. Two copies of der(14)t(14;18)(q32;q21) and der(18)t(14;18)(q32;q21) chromosomes are indicated by arrows. +X is not present in this karyotype. *Bottom*, partial karyotype showing +X, del(6)(q13q21), and dup(12)(q13q22)×2 (arrowheads).

Figure 6. FISH of the peripheral blood smear slide using *IGH/BCL2* dual-color, dual-fusion translocation probe (A) and *MYC* dual-color, break-apart rearrangement probe (B). Hybridization signals are indicated by arrowheads of each color. Asterisks indicate diploid cell nuclei.

transformation include acute lymphoblastic leukemia, in which terminal deoxynucleotidyl transferase is positive and surface immunoglobulin expression is lost,⁹⁻¹¹ and blastic/blastoid transformation that retains mature B-cell immunophenotype.^{12,13} Although the last form of

transformation is not defined clearly, the morphology of circulating lymphoma cells and BM histopathology, described previously by others, appear to match those of our present case.¹²

A characteristic feature of this case is the presence

Figure 7. Amplification and sequencing of the *BCL2*-*IGH* junction. (A) Long-distance PCR using the MBR/02 and Eμ/01 primer pair.³ (B) Nucleotide sequence of the 5' *BCL2* and 3' *IGH* junction.

of two copies of t(14;18)(q32;q21), determined by G-banding and confirmed by FISH of interphase nuclei. In review of the Mitelman's database,¹⁴ in which a total of 1,638 t(14;18)(q32;q21) cases are registered, we found only two cases that carried t(14;18)(q32;q21)×2 with hyperdiploid-range chromosome number, both of which were categorized into DLBCL.^{15,16} It is therefore suggested that t(14;18)(q32;q21)×2 is rare but associated with aggressive histopathology. In the present case, because the LD-PCR generated the single species of the 5' *BCL2* and 3' *IGH* fusion gene (Figure 6), the two copies of the translocation do not represent the occurrence of two independent t(14;18)(q32;q21)s, but rather duplication of a t(14;18)(q32;q21). An additional copy of der(18)t(14;18)(q32;q21) [+der(18)t(14;18)(q32;q21)] has been shown to be one the most common secondary abnormalities in t(14;18)(q32;q21)-positive FL,^{17,18} giving rise to the gain of gene copies localized on this particular derivative chromosome. With regard to the *BCL2* gene copy, however, the der(18)t(14;18)(q32;q21) contains the reciprocal 5' *IGH* and 3' *BCL2* fusion gene that lacks the body of *BCL2*-coding sequences. In contrast, duplication of t(14;18)(q32;q21) theoretically leads to the duplication of the 5' *BCL2* and 3' *IGH* fusion gene

that encodes the *BCL2* protein. Thus, it is possible that the double t(14;18)(q32;q21) may have generated a higher amount of *BCL2* protein, thereby accounting, in part, for the progressive/refractory behavior in the present case.

From the point of view of genomic imbalance, the karyotype of this case is characterized by a gain of whole chromosomes X, 6, 7 (at relapse), 8, 12, 14, 18, 19, and 20, in addition to del(6q). In one study to reveal clonal evolution in t(14;18)(q32;q21)-FL, +X, +7, +8, +12, +19, and +20 were segregated into a single cluster, suggesting association of these numerical abnormalities.¹⁸ On the other hand, duplication of a segment of 12q was first described in two cases of t(14;18)(q32;q21)-negative FL.¹⁹ Nevertheless, dup(12q) has been identified in a variety of B-cell tumors including t(14;18)(q32;q21)-FL and is listed in secondary chromosomal abnormalities in t(14;18)(q32;q21)-FL.^{13,20} In the present case, +12 and dup(12)(q13q22)×2 lead to pentasomy for 12q13-22, which includes the commonly duplicated segment 12q13-15.²⁰ These cytogenetic observations show that this case carried many numerical and structural abnormalities identified in t(14;18)(q32;q21)-FL, providing persuasive evidence for transformed FL.

REFERENCES

1. Harris NL, Nathwani BN, Swerdlow SH, et al. Follicular lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008:220-226.
2. Lossos IS, Gascoyne RD. Transformation of follicular lymphoma. *Best Pract Res Clin Haematol* 2011;24:147-163.
3. Akasaka T, Akasaka H, Yonetani N, et al. Refinement of the *BCL2*/immunoglobulin heavy chain fusion gene in t(14;18)(q32;q21) by polymerase chain reaction amplification for long targets. *Genes Chromosomes Cancer* 1998;21:17-29.
4. Dunleavy K, Pittaluga S, Shovlin M, et al. Low-intensity therapy in adults with Burkitt's lymphoma. *N Engl J Med* 2013;369:1915-1925.
5. ISCN (2013): An International System for Human Cytogenetic Nomenclature, Schaffer LG, McGowan-Jordan J, Schmid M, editors. Basel: S. Karger; 2013: 140.
6. Maeshima AM, Taniguchi H, Tanioka K, et al. Clinicopathological characteristics of follicular lymphoma with peripheral blood involvement. *Leuk Lymphoma* 2015;56:2000-2004.
7. Perry C, Lerman H, Joffe E, et al. The value of PET/CT in detecting bone marrow involvement in patients with follicular lymphoma. *Medicine (Baltimore)* 2016;95:e2910.
8. Kluin PM, Raphael M, Harris NL, et al. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC; 2008:265-266.
9. Gauwerky CE, Hoxie J, Nowell PC, et al. Pre-B-cell leukemia with a t(8; 14) and a t(14; 18) translocation is preceded by follicular lymphoma. *Oncogene* 1988;2:431-435.
10. Kroft SH, Domiati-Saad R, Finn WG, et al. Precursor B-lymphoblastic transformation of grade I follicle center lymphoma. *Am J Clin Pathol* 2000;113:411-418.
11. D'Achille P, Seyr JF, Campbell LJ. Translocation (14;18)(q32;q21) in acute lymphoblastic leukemia: a study of 12 cases and review of the literature. *Cancer Genet Cytogenet* 2006;171:52-56.
12. Natkunam Y, Warnke RA, Zehnder JL, et al. Blastic/blastoid transformation of follicular lymphoma: immunohistologic and molecular analyses of five cases. *Am J Surg Pathol* 2000;24:525-534.
13. Mohamed AN, Palutke M, Eisenberg L, et al. Chromosomal analyses of 52 cases of follicular lymphoma with t(14;18), including blastic/blastoid variant. *Cancer Genet Cytogenet* 2001;126:45-51.
14. Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (2016). Mitelman F, Johansson B and Mertens F (Eds.). Available at: <http://cgap.nci.nih.gov/Chromosomes/Mitelman>.
15. Aamot HV, Torlakovic EE, Eide MB, et al. Non-Hodgkin lymphoma with t(14;18): clonal evolution patterns and cytogenetic-pathologic-clinical correlations. *J Cancer Res Clin Oncol* 2007;133:455-470.
16. Cerretini R, Noriega MF, Narbaitz M, et al. New chromosome abnormalities and lack of *BCL-6* gene rearrangements in Argentinean diffuse large B-cell lymphomas. *Eur J Haematol* 2006;76:284-293.
17. Höglund M, Sehn L, Connors JM, et al. Identification of cytogenetic subgroups and karyotypic pathways of clonal evolution in follicular lymphomas. *Genes Chromosomes Cancer* 2004;39:195-204.
18. d'Amore F, Chan E, Iqbal J, et al. Clonal evolution in t(14;18)-positive follicular lymphoma, evidence for multiple common pathways, and frequent parallel clonal evolution. *Clin Cancer Res* 2008;14:7180-7187.
19. Wlodarska I, Mecucci C, Vandenberghe E, et al. dup(12)(q13----qter) in two t(14;18)-negative follicular B-non-Hodgkin's lymphomas. *Genes Chromosomes Cancer* 1992;4:302-308.
20. Dierlamm J, Wlodarska I, Michaux L, et al. FISH identifies different types of duplications with 12q13-15 as the commonly involved segment in B-cell lymphoproliferative malignancies characterized by partial trisomy 12. *Genes Chromosomes Cancer* 1997;20:155-166.

Double t(14;18)(q32;q21) translocation を認め、*de novo* transformed follicular lymphoma と考えられた 1 例

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症例：72 歳男性。1 か月前から全身倦怠感と盗汗を自覚し次第に増悪した。かかりつけ医での血液検査で異常細胞と LDH 高値を認めたため、急性白血病を疑われて紹介受診した。体温 38.2℃。表在性リンパ節腫脹なし，肝脾腫なし。

検査結果：Hb 10.3 g/dL, WBC $7.59 \times 10^3/\mu\text{L}$ (leukemic cells 15.0%), PLT $66 \times 10^3/\mu\text{L}$, LDH 4,243 IU/L, sIL-2R 1,758 U/mL。骨髓穿刺はドライタップ，骨髓生検では骨梁間に CD5⁻, CD10⁺, CD20⁺, BCL2⁺, BCL6⁻, MUM1⁻ の腫瘍細胞が充満していた。FDG-PET/CT で全身の骨髓に不均一な集積を認めた。

染色体・遺伝子検査：末梢血から得られた核型は高二倍体で，t(14;18)(q32;q21) 転座が重複していた。FISH では *IGH-BCL2* 融合シグナルが 4 個認められた。8 番染色体は 3 本に増加していたが，8q24/*MYC* の再構成は認めなかった。Long-distance PCR で *BCL2*-MBR, N-like segment, *IGH-J5* から構成される fusion gene が増幅された。

治療経過：R-DA-EPOCH 療法を 2 サイクル実施し寛解に至ったが，約 1 年後に広範な軟部腫瘍で再燃した。再燃後は，化学療法に反応することなく短期間で死亡した。

考案：本症例は indolent な段階を経過することなく *de novo* 発症した transformed follicular lymphoma と考えられた。Double t(14;18) による BCL2 蛋白の発現亢進が，難治性・治療抵抗性の原因となった可能性が示唆された。

キーワード：濾胞性リンパ腫，トランスフォーメーション，double t(14;18)(q32;q21) 転座，long-distance PCR，FISH