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

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Received 2016/4/15; accepted 2016/5/19; released online 2016/7/15

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.3 The breakpoints involved in the translocation have been extensively investigated at the molecular level, and nearly...
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 × 10⁹/µL, and platelet count was 66 × 10⁹/µL. 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)
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⁺, CD23dim, and CD38⁻, and expressed γλ 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 (SUVmax) 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), 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
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**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.
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 findings 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%.
transformation include acute lymphoblastic leukemia, in which terminal deoxynucleotidyl transferase is positive and surface immunoglobulin expression is lost, and blastic/blastoid transformation that retains mature B-cell immunophenotype. 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.

A characteristic feature of this case is the presence
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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, 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. 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, 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. On the other hand, duplication of a segment of 12q was first described in two cases of t(14;18) (q32;q21)-negative FL. 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. In the present case, +12 and dup(12)(q13q22)×2 lead to penta-somy for 12q13-22, which includes the commonly duplicated segment 12q13-15. 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.

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


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℃、表在性腫脹なし、肝脾腫なし。

検査結果: Hb 10.3 g/dL, WBC 7.59 × 10^3/µL (leukemic cells 15.0%), PLT 66 × 10^3/µL, LDH 4,243 IU/L, sIL-2R 1,758 U/mL。骨髄穿刺はドライタップ、骨髄生検では骨梁間に CD5−, CD10+, CD20+, BCL2+, BCL6−, MUM1−の腫瘍細胞が充満していた。FDG-PET/CT で全身の骨髄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 が增幅された。

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