

Development of diffuse large B-cell lymphoma in a patient with Waldenström's macroglobulinemia/lymphoplasmacytic lymphoma: clonal identity between two B-cell neoplasms

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Abstract

Waldenström's macroglobulinemia (WM)/lymphoplasmacytic lymphoma (LPL) is an indolent mature B-cell neoplasm. In rare cases of WM/LPL, diffuse large B-cell lymphoma (DLBCL) develops as a result of histologic transformation. In this report, we present a case of DLBCL developing in a patient with WM/LPL. Combination chemotherapy for DLBCL was effective and complete remission was eventually achieved. We attempted to determine the clonal relatedness between WM/LPL and DLBCL in the patient by analyzing complementarity-determining region 3 (CDR3) in the immunoglobulin heavy chain gene. A common CDR3 sequence was found in tumor cells of DLBCL and those of WM/LPL, indicating that tumor cells of DLBCL are clonally identical to those of WM/LPL. Therefore, in the present case, DLBCL is developed from WM/LPL cells by clonal evolution.

Introduction

Waldenström's macroglobulinemia (WM), a rare B-cell neoplasm characterized by lymphoplasmacytic cell proliferation in bone marrow with IgM monoclonal gammopathy, is classified as a subtype of lymphoplasmacytic lymphoma (LPL) in WHO classification.¹⁻³ Although WM/LPL is an indolent disorder and the median overall survival of patients with WM/LPL is in the range of 5-10 years, diffuse large B-cell lymphoma (DLBCL) develops as a result of histologic transformation in approxi-

mately 5-10% of patients with WM/LPL.⁴⁻⁶ Once DLBCL occurs in patients with WM/LPL, the disease course turns more aggressive. In general, DLBCL developing in a patient with WM/LPL is resistant to chemotherapy and the prognosis is poorer than that of de novo DLBCL. Median survival from the development of DLBCL is only 2-5 months even when combination chemotherapy for aggressive lymphoma is applied.⁶

It has been debated whether DLBCL co-occurring with WM/LPL develops by clonal evolution or as a secondary malignancy. To address this issue, the clonal relatedness between tumor cells of WM/LPL and those of DLBCL have been investigated by different procedures. Now, the clonality of mature B-cell neoplasm can be determined more precisely by analyzing complementarity determining region 3 (CDR3) in the immunoglobulin heavy chain (*IgH*) gene by a PCR-based molecular biological technique.⁷ To date, three groups have independently reported on the clonal relatedness between tumor cells of WM/LPL and those of co-occurring DLBCL by analyzing CDR3 sequences.⁸⁻¹⁰ One group showed clonal identity between tumor cells of WM/LPL and those of co-occurring DLBCL,⁸ while two other groups showed that tumor cells of DLBCL are not clonally identical to those of WM/LPL tumor cells.^{9,10} In this report, we present a case of DLBCL developing in a patient with WM/LPL. We attempted to determine clonal relatedness between tumor cells of WM/LPL and those of DLBCL by analyzing CDR3 sequences.

Case Report

A 63-year-old man was diagnosed with WM/LPL based on findings including increased serum IgM (4823 mg/dL) with a monoclonal component and lymphoplasmacytic cell proliferation in bone marrow. He received chemotherapy consisted of ranimustine, vindesine, melphalan, and prednisolone. After completion of the first cycle of therapy, serum IgM level was decreased to 3058 mg/dL. Three years after diagnosis of WM/LPL, supraclavicular lymph node swelling was found on physical examination, and an abnormal nodular lesion in lung field was observed on chest X-ray. Supraclavicular lymph node biopsy was performed for pathological diagnosis. Histopathological studies on supraclavicular lymph node revealed diffuse proliferation of large B-cells positive for CD20, CD79a and lambda chains by immunohistochemistry, leading to a pathological diagnosis of DLBCL (Figure 1A and B). Chemotherapy by a THP-COP regimen consisting of cyclophosphamide, epirubicin, vincristine, and prednisolone was initiated.

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Treatment was effective and lung tumor disappeared and lymph node swelling was improved. Subsequently, complete remission was achieved after the completion of six cycles of THP-COP therapy. Five years after completion of THP-COP therapy, when he was the age of 71-year-old, disease status was re-evaluated. There was no sign of relapse of DLBCL, while serum IgM level was still high, and bone marrow showed proliferation of lymphoplasmacytic cells (11.7% of all nucleated cells) positive for CD79a, cytoplasmic IgM, and lambda chain but not for CD20, without large B-cell proliferation (Figure 1C and D)

Materials and Methods

Samples and DNA extraction

Written informed consent regarding genetic analysis of tumor cells was obtained from the patient. Mononuclear cells were separated from the bone marrow sample as described previously.¹¹ Genomic DNA was extracted from bone marrow mononuclear cells using QIAamp

DNA Blood Mini kit (QIAGEN Sciences, Hilden, Germany) and from the paraffin-embedded lymph node sample by using DEPAX kit (TAKARA BIO., Ohtsu, Shiga, Japan) according to the manufacturers' instructions.

Polymerase chain reaction

Semi-nested polymerase chain reaction (PCR) was carried out to amplify CDR3 sequences in the *IGH* gene as described previously with minor modifications, using primer sets Fr3A (5'-ACACGGC[C/T][G/C]TGTATTACTGT-3') and LJH (5'-TGAGGAGACGGTGACC-3') for first round amplification, and Fr3A and VLJH (5'-GTGACCAGGGT[A/G/C/T] CCTTGGCCCCAG-3') for second round amplification.⁷ First round PCR was carried out using 100 ng of genomic DNA as a template in 50 μ L of a reaction mixture containing 2 μ L of 10X buffer, 1.5 mM MgCl₂, 250 nM of each deoxynucleotide, 0.2 units of rTaq DNA polymerase (TAKARA BIO.), and 300 nM of each primer. Forty cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 60 s were performed. Second round PCR was carried out using purified first round PCR product as a template in 50 μ L of the same reaction mixture as that for first round PCR except for the primer sets. Forty cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s were performed.

Subcloning of polymerase chain reaction products and DNA sequencing

Sequences of PCR products of CDR3 were determined after subcloning as described previously.¹¹ Briefly, PCR products were purified with the QIAquick PCR purification kit (QIAGEN Sciences), and cloned into pGEM-T vector (Promega, Madison, WI, USA). After bacterial transformation, plasmids were purified and subjected to sequence determination. Both strands of each PCR product were sequenced using DYEnamic ET Dye Terminator sequencing kit (Amersham, Buckinghamshire, UK) and the MegaBase sequence system (Amersham) according to the manufacturer's instructions.

Results

We analyzed sequences of CDR3 by PCR from the lymph node biopsy specimen, by which the diagnosis of DLBCL was made, and from the bone marrow sample, in which lymphoplasmacytic cell proliferation was observed. PCR products were cloned into pGEM-T vector, and then nucleotide sequences of PCR products were determined after subcloning. For lymph node specimen, eight bacterial colonies were isolated

and subjected to sequence analysis. Six of the eight bacterial colonies contained the same nucleotide sequence (Figure 2), and the remaining two did not contain any CDR3 sequence. For the bone marrow specimen, 16 bacterial colonies were isolated and subjected to sequence analysis. Six out of the 16 bacterial colonies did not contain any CDR3 sequences. The remaining ten bacterial colonies contained seven different CDR3 sequences (A-G), and three colonies contained "A" sequence, which is identical to the CDR3 sequence identified in lymph node (Table 1). Subsequently, we found a common CDR3 sequence in DLBCL cells (lymph node) and WM/LPL cells (bone marrow).

Discussion

The results of analysis of the CDR3 sequence in the *IGH* gene shows clonal identity between tumor cells of WM/LPL and those of co-occurring DLBCL, indicating that DLBCL are originated from WM/LPL by clonal evolution in the present case. It has been debated whether tumor cells of co-occurring DLBCL are clonally identical to those of WM/LPL. To date, three groups have independently reported on the clonal relatedness between tumor cells of WM/LPL and those of co-occurring DLBCL by analyzing CDR3. Similar to the present result, Nakamura *et al.* showed clonal identity between tumor cells of WM/LPL and those of co-occurring DLBCL.⁸ On the other hand, Shimizu *et al.* and Tojo *et al.* showed clonal dif-

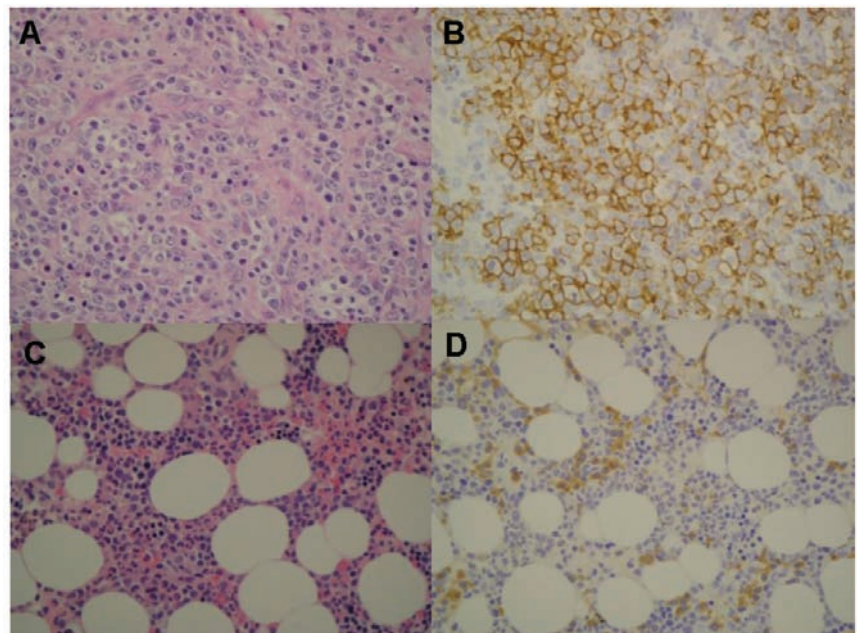


Figure 1. Histopathology of lymph node and bone marrow tissues. A) In lymph node, proliferation of large tumor cells with diffuse pattern is observed (HE staining X400); B) and tumor cells are positive for CD20 (Immunohistochemistry, X400). C) In bone marrow, proliferation of lymphoplasmacytic cells is observed (HE staining, X400); D) and these cells are positive for CD79a (Immunohistochemistry, X400).

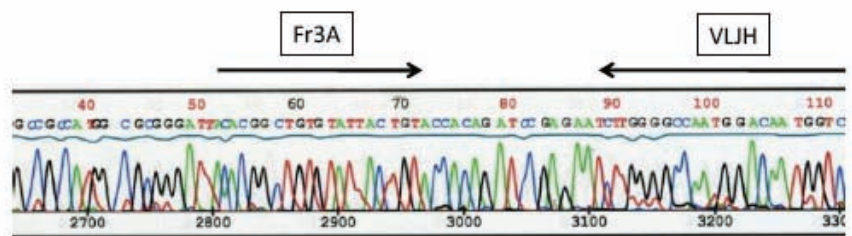


Figure 2. Sequence analysis of CDR3 in lymph node. Arrows indicate primer sequences of Fr3A and VLJH.

ference between tumor cells of DLBCL and those of WM/LPL, suggesting that DLBCL develops independently as a second neoplasm in patients with WM/LPL.^{9,10} Collectively, these discordant results suggest that there are two different pathways of development of DLBCL in patients with WM/LPL: clonal evolution or the development of a second neoplasm. Histologic transformation occurs in other types of indolent B-cell neoplasms, including chronic lymphocytic leukemia (CLL). Clonal relatedness between tumor cells of co-occurring DLBCL and those of CLL has been investigated more extensively, and the results indicate that DLBCL can develop either by clonal evolution or as a secondary neoplasm.^{12,13} In the majority of patients with CLL, DLBCL occurs by clonal evolution, suggesting that clonal evolution is a major pathway of the development of DLBCL in cases of CLL.^{12,13} Similar to CLL, in cases of WM/LPL, DLBCL develops by these two pathways, although it is not clear which of two pathways is dominant in the development of DLBCL in WM/LPL.

The molecular mechanism of the development of DLBCL by clonal evolution in WM/LPL has not been elucidated. In the case of CLL, several different events, including additional genetic alterations, and viral infection, may trigger clonal evolution.¹³⁻¹⁶ For instance, the acquisition of *p53* tumor suppressor gene mutation and/or chromosome 17p13 deletion, and *c-MYC* alterations frequently occur in tumor cells during histologic transformation to DLBCL.^{13,14,17} Epstein-Barr virus infection has been identified in lymphoma cells in a few patients with co-occurring aggressive lymphoma with CLL.¹⁸ Similar events may occur during clonal evolution in DLBCL development in patients with WM/LPL. Further investigation is necessary.

It is also important to clarify whether there are any differences in biological behaviors of tumor cells and clinical features between DLBCL developed by clonal evolution and that developed as a secondary neoplasm. Once DLBCL develops in WM/LPL patients, the disease course turns more aggressive. In general, the response to chemotherapy is not good and median overall survival is only 3-5 months, even when multi-agent chemotherapy is applied.⁶ The introduction of rituximab to chemotherapy may improve the treatment outcome of DLBCL developed in WM/LPL patients.^{19,20} When the patient in the present case received therapy for DLBCL, rituximab had not been available in Japan. Combination chemotherapy (THP-COP therapy) without rituximab was, however, effective for DLBCL, and the patient eventually achieved complete remission and maintained it for approximately five years. Interestingly, as described in report by Nakamura *et al.*, a patient with co-occurring

Table 1. Summary of CDR3 sequences observed in bone marrow.

	CDR3 sequences	Number * of subclones
A	5'-(Fr3A)-ACCACAGATCCGAGAAT-(VLJH)-3'	3
B	5'-(Fr3A)-GCGAGACAACGCGAGTCATTGTAGTGTACCAGCTGCTATACGT CGCTGCTTTTGATTTTGATAT-(VLJH)-3'	1
C	5'-(Fr3A)-GCGAGACAACGCGAGTCATTGTAGTAGTACCAGCTATACGTCG GTGCTTTTGATAT-(VLJH)-3'	1
D	5'-(Fr3A)-TGTCGAAAAGCTTCATTAGTATGGTTCGGGGAGATTGACTA-(VLJH)-3'	1
E	5'-(Fr3A)-GCGAGTACGTATTACGATTTTGGAGTGGTTATTATCGGAAATT GACTA-(VLJH)-3'	1
F	5'-(Fr3A)-ACCACCAAGGGCCCCACCCCGATATTTGACTGGTTATTA CCCCTTATCATTGACTA-(VLJH)-3'	2
G	5'-(Fr3A)-GCAAAAAGCTTCATTACTATGGTTCGGGGAGATTGACTA-(VLJH)-3'	1

* Subclones that did not contain any CDR3 sequences are excluded.

DLBCL caused by tumor cells clonally identical to WM/LPL achieved complete remission by combination chemotherapy.⁸ In contrast, as described in reports by Shimizu *et al.* and Tojo *et al.*, patients with DLBCL caused by different clones from those of WM/LPL showed poorer prognosis, and died within several months.^{9,10} However, the number of analyzed cases is too small to clarify clinical implication of clonal relatedness. Currently, Rossi *et al.* performed comprehensive molecular study including analysis of clonal relatedness of tumor cells of CLL and co-occurring DLBCL, indicating that clonally unrelated cases are clinically and biologically distinct from clonally related cases, and are characterized by better clinical outcomes.¹³ It is not clear whether clinical significance of clonal relatedness between tumor cells of co-occurring DLBCL and original tumor cells in WM/LPL is different from that in CLL. It is necessary to accumulate cases of DLBCL co-occurring with WM/LPL and to analyze correlation between clinicopathological features and clonal relatedness.

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