Case report

Pulmonary extranodal marginal zone lymphoma that presented with macroglobulinemia and marked plasmacytic cell proliferation carrying the t(14;18)(q32;q21)/MALT1-immunoglobulin heavy-chain fusion gene in pleural fluid

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An 80-year-old man presented with the accumulation of pleural fluid in the right thoracic cavity. Serum electrophoresis revealed an M-component and immunofixation confirmed IgM/λ. The level of IgM was 1,526 mg/dL. Imaging studies showed an infiltrative condition of the ipsilateral lung parenchyma. The fluid contained abundant neoplastic cells with the morphological and immunophenotypic features of plasma cells, which expressed IgM/λ monoclonal immunoglobulins on the cell surface and in the cytoplasm. The karyotype was 48,XY,+3,add(9)(p13),+12,add(14)(q32),del(16)(q22),−18,−mar, and a series of fluorescence in situ hybridization studies demonstrated that the add(14) chromosome represented der(14)t(14;18)(q32;q21), at which the MALT1-immunoglobulin heavy-chain (IGH) fusion gene was localized. A long-distance polymerase chain reaction amplified the fragment encompassing the two genes, showing that the junction occurred at the J6 segment of IGH and 3.7-kb upstream of the MALT1 breakpoint cluster. We propose that this case represents an extreme form of the plasmacytic differentiation of extranodal marginal zone lymphoma that developed in the lung.

Keywords: macroglobulinemia, extranodal marginal zone lymphoma, plasmacytic differentiation, t(14;18)(q32;q21)/MALT1-IGH fusion gene

INTRODUCTION

Waldenström macroglobulinemia (WM) is a distinct clinicopathological disease entity showing lymphoplasmacytic lymphoma (LPL) in bone marrow and the IgM monoclonal protein in serum (i.e., macroglobulinemia) at any concentration.1,2 WM/LPL is characterized by the proliferation of small lymphocytes admixed with variable numbers of plasma cells and plasmacytoid lymphocytes.2 Patients with WM/LPL present with a number of symptoms attributable to tumor cell proliferation and/or an excess of macroglobulin. However, macroglobulinemia is observed not only in WM/LPL, but also a wide range of B-cell lymphoproliferative disorders, including extranodal marginal zone lymphoma (EMZL) of mucosa-associated lymphoid tissue (MALT lymphoma).3,5 EMZL/MALT lymphoma is composed of morphologically heterogeneous small B cells and the plasmacytic differentiation of lymphoma cells is common.4 Thus, the distinction between WM/LPL and EMZL/MALT lymphoma showing plasmacytic differentiation and associated with the serum IgM monoclonal protein is not necessarily clear, even though >90% cases of the former disease have the MYD88 P265P somatic mutation.2,6

We herein describe a patient who presented with macroglobulinemia and the accumulation of pleural fluid in the unilateral thoracic cavity. Neoplastic cells in the fluid showed the cytormorphological and immunophenotypic features of plasma cells, indicating the differential diagnosis between WM/LPL and EMZL/MALT lymphoma. Cytogenetic and molecular studies demonstrated a translocation and fusion gene, which was exclusively associated with the latter disease.
CASE REPORT

Case presentation

An 80-year-old man, who had been treated in another hospital for the recurrent accumulation of pleural fluid in the right thoracic cavity for 2 years, was referred to our department because a cytological examination of the fluid suggested lymphoma cells. On examination, breath sounds were decreased in the right hemithorax. There was no surface lymphadenopathy or hepatosplenomegaly. Oxygen saturation was 96% in room air. A chest radiograph showed prominent pleural effusion (Figure 1A). His hemoglobin level was 12.5 g/dL, white blood cell count was 6.95 × 10^3/μL, and platelet count was 289 × 10^3/μL. Total serum protein was 6.7 g/dL, albumin 3.2 g/dL, globulin 3.5 g/dL, lactate dehydrogenase 307 IU/L, higher than the normal range of 124 to 222 IU/L, creatinine 0.6 mg/dL, and C-reactive protein 2.25 mg/dL. Serum protein electrophoresis revealed an M-component migrating in the γ globulin area and immuno- fixation confirmed the IgM/λ M protein. No urinary Bence Jones protein was detected. The level of IgG was 723 mg/dL, IgA was 83 mg/dL, and IgM was 1,526 mg/dL (normal range, 33 to 183 mg/dL). Serum free light-chain κ (FLC-κ) was 16.1 mg/L, FLC-λ was 20.5 mg/L, and the κ/λ ratio was 0.79. Soluble interleukin 2 receptor was 3,051 U/mL.

Computed tomography (CT) of the chest revealed the accumulation of pleural fluid in the right thoracic cavity in association with atelectasis of the middle and lower lobes, where the marked accumulation of the tracer was demonstrated by 18F-fluorodeoxyglucose-positron emission tomography (FDG-PET) combined with CT (Figure 1B), suggesting that the atelectatic lesion contained an infiltrative condition.

Thoracentesis yielded exudative fluid containing abundant neoplastic cells with the morphological features of plasma cells at a concentration of 6.13 × 10^3 cells per micro-liter (Figure 2A). These cells expressed IgM/λ monoclonal immunoglobulins both on the cell surface and in the cytoplasm, and showed a wide range of expression levels of CD20, CD22, CD323, CD38, and CD138. CD19 and CD45RA were positive, and CD5, CD10, CD21, CD24, CD56, and surface IgD were negative (Figures 2B and C). The DNA index was 1.06 from normal diploid cells. Bone marrow showed hypercellularity containing CD20-positive plasma cells, and flow cytometry revealed a lymphoplasmacytic population with an identical immunophenotype to that of pleural fluid cells. Tests for the MYD88 L265P somatic mutation were negative (Supplementary Figure S1).

Cytogenetic studies

The G-banding of metaphase spreads obtained from a short-term culture of pleural fluid cells demonstrated numerical and structural abnormalities, including a 14q+ chromosome and uncharacterized small marker chromosome; the karyotype according to the ISCN 2016 was 48,XY,+3,add(9)(p13),+12,add(14)(q32),del(16)(q22),−18,+mar (Figure 3A). Fluorescence in situ hybridization (FISH) of interphase nuclei with the MYC, BCL2, and BCL6 dual-color, break-apart probes detected no rearrangement of these 3 genes, while nuclei carried 3 hybridization signals of BCL2 and BCL6 (Supplementary Figure S2). The hybridization of metaphase cells with the BCL2-IGH dual-color dual-fusion probe showed that the add(14) chromosome was marked by the IGH and BCL2 probes (Figure 3B); however, these probes did not generate a fusion signal (Supplementary

Fig. 1. Imaging studies showing pleural effusion of the right thoracic cavity and an infiltrative condition of the lung parenchyma. (A) Postero-anterior chest X-ray. (B) FDG-PET/CT. The anterior view of a maximum intensity projection image (left) and representative axial images of the thorax (right) are shown. The maximum standardized uptake value of the pulmonary lesion was 17.89.
We then performed hybridization with the MALT1 dual-color, break-apart probe and found that add(14) was marked by the green-labeled telomeric probe, representing 3' MALT1, while the red-labeled centromeric MALT1 signal was missing, and, accordingly, interphase nuclei showed the one green and two yellow signal pattern (Figure 3B). The uncharacterized marker chromosome was marked by unrearranged BCL2 and MALT1 probes, but not of IGH.

These cytogenetic studies indicated that the add(14) chromosome represented der(14)t(14;18)(q32;q21), at which the MALT1-IGH fusion gene was localized, while the reciprocal der(18)t(14;18)(q32;q21) was deleted.

Amplification and sequencing of the t(14;18)(q32;q21)/MALT1-IGH junction

Since the t(14;18)(q32;q21)/MALT1-IGH fusion gene was suggested, DNA extracted from pleural fluid cells was subjected to a long-distance polymerase chain reaction (LD-PCR) in order to amplify the junction encompassing the two genes. PCR primers were designed for MALT1 exon 1 and for the Eμ enhancer as well as the Cμ, Cγ, and Cα constant genes of IGH; the MALT1 probe and IGH probes were oriented in the opposite direction (Figures 4A and B, Supplementary Table S1). As shown in Figure 4C, we obtained LD-PCR products by the MALT1-Eμ and MALT1-Cγ primer combination, indicating Cμ to Cγ class switching. Nucleotide sequencing of the MALT1-Eμ product revealed that the junction occurred at the J6 segment of IGH and the nucleotide position −5,399 of adenine of the ATG start codon of MALT1 at the position +1, and a fragment of 9 base pairs (bp) in length was inserted at the junction (Figures 4A and D). As a result of translocation, the MALT1 and IGH Eμ-Cγ segments were aligned in the divergent transcriptional orientation. Reverse-transcriptase PCR confirmed the expression of MALT1 in pleural fluid cells, even though the level was lower than those of in vitro cultured lymphoma cell lines (Supplementary Figure S3).

We then applied LD-PCR using the MALT1-Eμ primer combination to another case with t(14;18) reported previously by us. The sequences encompassing the MALT1-IGH
junction were composed of the J4 segment, a 46-bp insertion fragment, and the upstream sequences of MALT1 at the position −1,738 of the start codon (Figures 4A and D).

**Treatment course**

The patient was treated with 6 cycles of the DRP (dexamethasone, rituximab, and cyclophosphamide) regimen every 21 days. This led to the resolution of pleural effusion, the disappearance of lymphoma cell invasion in the bone marrow, and accumulation of the tracer within the lung lesion by FDG-PET/CT. However, the serum IgM/λ M protein remained detectable by immunofixation and the level of serum IgM remained higher than the normal range.

**DISCUSSION**

We herein described an elderly man who presented with macroglobulinemia and the unilateral accumulation of pleural
fluid. Lymphoma cells in the fluid showed a plasma cell cytomorphology and expressed CD38 and CD138 antigens, but lacked myelomatous antigen aberrations. Most importantly, cytogenetic studies suggested t(14;18)/IGH, and the MALT1-IGH junction was confirmed by LD-PCR, which we developed to amplify long DNA targets. Since t(14;18)/MALT1-IGH has been described in a fraction of cases with MALT lymphoma arising in the lung, and because an infiltrative condition of the ipsilateral lung parenchyma was suggested by imaging studies, we propose that this case represents an extreme form of the plasmacytic differentiation of pulmonary EMZL/MALT lymphoma.

**Fig. 4.** LD-PCR of the MALT1-IGH fusion gene. (A) Genomic structure of the MALT1 gene, which is located at the sub-band 18q21.32 and is oriented from centromere to telomere. The positions of 8 reported breakpoints are indicated by arrows, demonstrating the breakpoint cluster. Open and closed arrows indicate the breakpoint of the current case and that of Gomyo et al., respectively. (B) Schematic diagram of LD-PCR of the MALT1-IGH junction. The sequences of the primers are described in Supplementary Table S1. (C) Ethidium bromide-stained gel electrophoresis of LD-PCR, showing 7.2- and 11-kb products. (D) Nucleotide sequences of the t(14;18)(q32;q21)/MALT1-IGH junction of the present case (top) and those of Gomyo et al. (bottom). Vertical lines indicate nucleotide identity. The J6 and J4 segments of IGH are boxed and de novo nucleotide additions are underlined.
present, as in WM/LPL, to very uncommon, as in MCL. Furthermore, the extent of plasmacytic differentiation may vary from minimal to very extensive, resulting in a resemblance to plasmacytoma in extreme cases. Thus, plasmacytic differentiation does not define any specific type of small B-cell lymphoma. Since the MYD88 L265P somatic mutation is found in most patients with WM/LPL, it has had a significant impact on the differential diagnosis of small B-cell lymphomas; however, this mutation is not entirely specific to WM/LPL and not required for a diagnosis. In contrast, translocations involving MALT1, BCL10, FOXP1, and GPR34 are exclusively associated with EMZL/MALT lymphomas,10,11,13,14 therefore, the detection of these translocations by G-banding, FISH, or appropriate molecular methods is of value for a diagnosis.15 However, the frequencies of each translocation markedly vary with the primary site of disease and not all tumors carry a translocation; the frequency of t(14;18)(q32;q21) in EMZL/MALT lymphoma arising in the lung ranges between 6 and 10%.4,11 Thus, cytogenetic/molecular studies are currently insufficient to effectively discriminate each small B-cell lymphoma category, and the combination of cytomorphological, phenotypic, and sometimes clinical findings is still required in the differential diagnosis of small B-cell lymphomas.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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