Multiple Brain Tumors of Diffuse Large B Cell Lymphoma in a Patient with Waldenström’s Macroglobulinemia/Lymphoplasmacytic Lymphoma: PCR and DNA Sequence Analysis Show Evidence of Differences in Clonality of the Two B Cell Malignancies

Kana Tojo, Takeshi Hattori, Toshiro Ito*, Naoko Asano*, Kenji Sano**, Takefumi Suzuki** and Keiko Maruyama

Abstract

Multiple brain tumors of diffuse large B cell lymphoma (DLBL) were observed in a 75-year-old man with Waldenström’s macroglobulinemia (WM). Paravertebral and multiple subcutaneous nodules occurred in succession and he died 4 months after onset. We investigated B cell monoclonality by means of polymerase chain reaction (PCR) analysis and sequencing of the immunoglobulin heavy chain of paraffin-embedded sections. The PCR product of the brain tumors showed a different rearrangement pattern from those of the other sections. The co-occurrence of DLBL with WM is rare, and some investigators have examined the clonality of the two malignancies. This case is important because DLBL brain tumors co-occurred with WM, enabling us to prove that DLBL and WM have different clonality.

Case Report

A 75-year-old man consulted our hospital in September 2001 because of general fatigue. The results of physical examination were unremarkable. Hematological and blood chemical laboratory data showed that hemoglobin was 9.6 mg/dl, total protein 9.7 g/dl with 3.5 g/dl of albumin, and IgM 4,740 mg/dl. Serum protein immunoelectrophoresis detected the presence of IgM-lambda gammopathy, while urine immunoelectrophoresis did not show any Bence-Jones protein. Bone marrow biopsy demonstrated nodular infiltration by small lymphoid cells and plasmacytoid cells (Fig. 1, A

Key words: Waldenström’s macroglobulinemia/lymphoplasmacytic lymphoma, diffuse large B cell lymphoma, brain tumor, clonality, immunoglobulin heavy chain complementarity determining region III, polymerase chain reaction

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Figure 1. A: bone marrow (HE stain, ×100), B: bone marrow (HE stain, ×1,000), C: brain tumor (HE stain, ×1,000), D: parathoracic vertebral tumor (HE stain, ×1,000), E: parathoracic vertebral tumor (immunohistochemical stain with CD20, ×400), F: parathoracic vertebral tumor (immunohistochemical stain with immunoglobulin (Ig) M, ×400). Bone marrow and parathoracic vertebral tumor biopsy specimens showing infiltration with numerous small lymphoid cells and some plasmacytoid cells. Brain tumor biopsy specimen showing infiltration by diffuse large cells with an oval nucleus. Immunohistochemical study showing that the surface of almost all abnormal cells expressed CD20, and many of those cells also expressed IgM.
and B), with lymphocytes accounting for 35%. Immunohistochemical examination showed that all of the lymphoid cells stained positively for CD20, but it was difficult to conclude whether or not those abnormal cells stained positively for IgM and lambda light chain because the background around the abnormal cells was also stained. Immunohistochemical staining by IgG, IgA and kappa light chain was negative. Flow cytometry immunophenotypic studies performed on the same bone marrow aspirate demonstrated the cells to be positive for CD19, CD20 and negative for CD10. The patient was diagnosed as WM/LPL, and treatment was started from November 2001 with 50 mg daily of cyclophosphamide.

In the middle of January 2002, he showed signs of hypersonnia, left hemiparesis and ataxic gait, and was admitted to our hospital on January 28, 2002. The general physical examination indicated anemic change in the conjunctiva, but no lymphadenopathy or other abnormalities. He was drowsy, but showed no signs of meningeal irritation. Mild muscle weakness of the left upper and lower limbs was observed. Coordinative movement was disturbed on the left side and his gait was ataxic. Hematological and chemical laboratory data showed an erythrocyte sedimentation rate of 60 mm/hour, WBC of 6.2×10^9/l with normal differential count, RBC of 2.96×10^12/l, hemoglobin of 9.9 g/dl, platelets of 337×10^9/l, total protein of 8.1 g/dl with 3.1 g/dl of albumin, and IgM of 4.120 mg/dl. The cerebrospinal fluid (CSF) contained 35 large lymphoma cells per cubic millimeter, 190 mg/dl of total protein, and 25 mg/dl of IgM. Cranial computed tomography (CT) scan showed multiple abnormally high and low density areas in the left frontal lobe, right basal ganglia and right cerebellar hemisphere. A cranial magnetic resonance imaging (MRI) scan demonstrated high intensity regions in the T2-weighted sequence in the same areas. These lesions were strongly enhanced by gadolinium-diethylenetriaminepenta-acetic-acid (Gd-DTPA) (Fig. 2, A–C). Chest and abdominal CT scan findings were normal. A 67Ga-citrate scintigram revealed no abnormal accumulations. A stereotactic biopsy was performed on the left frontal lobe of the cerebral. The biopsy specimen showed diffuse infiltration to the cerebral parenchyma by large lymphoma cells with an oval nucleus (Fig. 1C). Immunohistochemical examination showed that all lymphoma cells stained positively for CD20, 30% of lymphoma cells for IgM, and 70% of lymphoma cells for lambda light chain. Immunohistochemical staining by IgG, IgA and kappa light chain was negative. The brain tumors were diagnosed as DLBL. Whole-brain irradiation therapy (RT) with 30 Gy was started on February 25, and consisted of 10 fractions given over 2 weeks. Intrathecal therapy with methotrexate (15 mg) and prednisolone (10 mg) was administered on February 25 and March 5. RT resulted in a reduction in the lymphoma cell count in CSF from 142 to 4 cells per cubic millimeter. A Gd-DTPA enhanced MRI scan 3 weeks after RT showed no abnormally enhanced region (Fig. 2D).

In the middle of March, the patient developed pneumocystis carinii pneumonia. Although the pneumonia went into remission as a result of methylprednisolone pulse therapy and oral sulfamethoxazole-trimethoprim, the cyclophosphamide and intrathecal therapy was stopped.

Early in April, right hemiparesis was seen for the first time. Chest CT and MRI scan showed a right parathoracic vertebral tumor, which had infiltrated the thoracic spinal cord, as well as the presence of right pleural effusion (Fig. 3). A specimen obtained with transcutaneous biopsy from the tumor showed infiltration by small, atypical lymphoid cells. Immunohistochemical staining showed that all of these cells were positive for CD20 and 70–80% of them for IgM and lambda light chain (Fig. 1, D–F). Immunostaining by IgG, IgA and kappa light chain was negative. The bloody pleural effusion contained many small, atypical lymphoid cells. RT with 3 Gy per 1 fraction for treatment of the parathoracic vertebral tumor was started on April 22. In early May, multiple abnormal nodules were observed in abdominal and inguinal subcutaneous tissue. These nodules gradually increased in number and size. Subcutaneous nodule biopsy was not performed because of the patient’s severely deteriorated condition. RT was stopped on May 7, 2002 after a total irradiation of 24 Gy. The patient died on May 23, 2002, 9 months after the onset of WM/LPL and 4 months after the DLBL brain tumors were first observed. No autopsy was performed.

To determine the clonal relationship between bone marrow, brain tumors and paravertebral tumor, we examined the IgH CDR III of paraffin-embedded sections by means of PCR analysis. DNA extraction and PCR were performed as previously described by Trainor et al (13) and by Wan et al (14). For both PCR rounds Fr3A (the third framework) primer (5’-ACACGGC(C/T)(G/C)TGTATTACTCT-3’) was used as the sense primer. Two different JH primers, LJH (5’- TAGAGGACGCTGACC-3’) and VLJH (5’-GTGACCA GGGT(A/G/C/T)CTTTGGCCACAG-3’) were used as the respective anti-sense primers in the first and second round. The PCR products were electrophoresed on a 10% polyacrylamide gel and stained with ethidium bromide. We obtained the same PCR products around 60 bp and 80 bp from bone marrow and paravertebral tumor, but the product of brain tumors showed a different band around 75 bp (Fig. 4A). To determine the clonal relationship between bone marrow, paravertebral tumor and brain tumors in more detail, we examined the DNA sequence of all the PCR products. The reamplified PCR products were purified and subcloned with a GENECLEAN II Kit (BIO 101, Vista, CA) and a TA Cloning Kit (Invitrogen, Carlsbad, CA). These products were sequenced with a BigDye Terminator v1.1 Cycle Sequence Kit (Applied Biosystems, Foster City, CA) and analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). We obtained two different PCR products (81 bp and 64 bp) from the bone marrow and paravertebral tumor, and one PCR product (76 bp) from the brain tumor. The DNA sequence proved that the clonality of the brain tumor was independent (Fig. 4B).
Discussion

The case reported here was characterized by multiple brain tumors of DLBL co-occurring with WM/LPL, and the difference in the origin of DLBL and WM/LPL was demonstrated by PCR and DNA sequence analysis.

WM/LPL is considered to be a chronic lymphoproliferative disorder which produces monoclonal IgM, which makes the blood circulation sluggish and causes hyperviscosity syndrome. WM/LPL is classified as a low-grade malignant lymphoma, and the median survival of patients with WM/LPL is 5 years (3). Older age (>65 years), male sex, marked or remarkable lymphadenopathy, severe anemia (Hb <10g/dl), hypoalbuminemia (Alb <3.5g/dl), and severe bone marrow infiltration (>50%) have been reported as poor prognostic factors for WM/LPL (2, 15, 16), and many of these were observed in the present case.

In 1936, Bing and Neel first reported a case of central
nervous system (CNS) infiltration in WM/LPL (17), which became known as the Bing-Neel syndrome. A few cases with tumor formation in the CNS which was histologically proven to be WM/LPL have been reported (18–21). The present case was different from the Bing-Neel syndrome because the biopsy specimen showed diffuse infiltration of large lymphoma cells. Different B cell malignancy is known to rarely occur in patients with WM/LPL, and most cases are DLBL of the WHO Classification (2–12). The frequency of occurrence of different B cell malignancy in WM/LPL is reportedly about 5–10% (2, 4), and median survival after the occurrence of DLBL is 2–5 months (4, 5). Two hypotheses

Figure 3. Tumor of the right parathoracic vertebrae. A: enhanced chest computed tomography (CT) scan; B: axial Gd-DTPA-enhanced MRI scan of thoracic vertebrae; C: sagittal T2-weighted MRI of thoracic vertebrae. Enhanced chest CT scan showing strong enhancement of right parathoracic vertebrae and right pleural effusion. Axial enhanced MRI scan showing that the enhanced region has infiltrated the thoracic spinal cord. Sagittal T2-weighted MRI scan showing abnormally long low-intensity region around the cord from Th7 to Th11.
Two B Cell Malignancies in a Patient

Bone Marrow (81bp)

5′ACACGGCTCTGTTATTACTGTGCAAGAGGGTAACCTATAGGTACGGCTGGTTTGCTTACTGGGGCCAAGGACCCCTGGTCAC3′

Brain (76bp)

5′ACACGGCTCTGTTATTACTGTGGAGAGTGGACATTGGCGCATATTATTGTATTACTGGGGCCAAGCACCCTTGCTCAC3′

Paravertebral Tumor (81bp)

5′ACACGGCTCTGTTATTACTGTGCAAGAGGGTAACCTATAGGTACGGCTGGTTTGCTTACTGGGGCCAAGGACCCCTGGTCAC3′

Paravertebral Tumor (64bp)

5′ACACGGCCCTGTATTACTGTGCAACCTTCGACACGGAGCTGGGGCCAAGGACCCCTGGTCAC3′

Figure 4. Polymerase chain reaction (PCR) and DNA sequence analysis of paraffin-embedded sections. A: Polyacrylamide gel analysis of immunoglobulin heavy chain (IgH) complementarity determining region III (CDR III) PCR products in bone marrow, brain tumor and paravertebral tumor (Lane 1–3: bone marrow; Lane 4–6: brain tumor; Lane 7–9: paravertebral tumor). B: DNA sequence of the IgH CDR III PCR products of bone marrow, paravertebral tumor and brain tumor. PCR products of bone marrow and paravertebral tumor showed identical lengths of around 80 and 60 bp, while PCR product of brain tumor in polyacrylamide gel showed a different length of around 75 bp. By sequencing of each reamplified monoclonal IgH gene rearrangement detected in bone marrow, brain tumor and paravertebral tumor, the presence of different and independent monoclonal rearrangement was confirmed in brain tumor. The underlined sequence represents the complementary strand of the PCR primers.

References


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