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Case Study

Orbital MALT Lymphoma, Abdominal Hodgkin lymphoma, and Systemic Diffuse Large B-cell Lymphoma Develop Sequentially in One Patient

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In February 2002, a 42-year-old woman developed ocular adnexal extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), MALT lymphoma, in the bilateral orbits involving lacrimal glands. She underwent 30 Gy external beam irradiation to the orbital lesions on both sides. She was well until November 2008 when she developed abdominal lymphadenopathy and transabdominal excisional biopsy showed mixed cellularity classical Hodgkin lymphoma at stage II. She underwent standard combination chemotherapy. In July 2010, she developed systemic lymphadenopathy and was diagnosed with diffuse large B-cell lymphoma (DLBCL) by cervical lymph node biopsy. She underwent rituximab monotherapy and finally allogeneic hematopoietic stem cell transplantation in October 2010, but died of renal failure in February 2011. Amplification by polymerase chain reaction of the immunoglobulin heavy chain gene gave rise to dominant discrete fragments of the same size between the orbital lesion with MALT lymphoma in 2002 and the cervical lymph node lesion with DLBCL in 2010. The sequential development of MALT lymphoma, Hodgkin lymphoma, and DLBCL in the long-term course of this patient suggests the common origin of the neoplastic cells, changing their pathological faces in response to irradiation and combination chemotherapy. 〔*J Clin Exp Hematopathol 52(1) : 41-49, 2012*〕

Keywords: ocular adnexal lymphoma, Hodgkin lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), diffuse large B-cell lymphoma, clonal (clonality)

INTRODUCTION

The division into Hodgkin lymphoma and non-Hodgkin lymphoma is reminiscent of the past classification of lymphoma. At present, Hodgkin lymphoma, or more accurately, classical Hodgkin lymphoma, is a still-utilized and well-established clinical and pathological entity that is characterized by the presence of mononucleated and multinucleated neoplastic cells, designated as Hodgkin cells and Reed-Sternberg cells, respectively. These neoplastic cells are admixed with non-neoplastic inflammatory and accessory cells.

On the basis of the past classification into Hodgkin lymphoma and non-Hodgkin lymphoma, simultaneous or sequential development of Hodgkin lymphoma and non-Hodgkin lymphoma, at the same anatomical site or different sites of the same patient, has been reported as case reports or case series to discuss the origin of neoplastic cells.[1-12] The historically used term of composite lymphoma was initially reserved to describe the two types of lymphoma occurring at the same anatomical site. Around the publication of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues in 2001, several reports have described that Hodgkin lymphoma is associated with B-cell lineage lymphomas, including diffuse large B-cell lymphoma (DLBCL),[8,11] follicular lymphoma,[5,6] mantle cell lymphoma,[9] and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT), MALT lymphoma for short.[4,7,8,10,12]

The ocular adnexa is one of the main sites involved with extranodal lymphoma, and the major histopathologic type is MALT lymphoma.[13,14] In this study, we describe a patient who initially developed bilateral ocular adnexal MALT lymphoma and then abdominal Hodgkin lymphoma, which finally culminated in systemic involvement with DLBCL.

CASE REPORT

A 42-year-old woman with one-year history of upper eyelid swelling on the left side was referred to Okayama

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University Hospital in February 2002. Her upper eyelids on both sides were swollen and a mass was palpable at the superotemporal edge of the orbital bone on the left side. The visual acuity was 1.2 in both eyes. The anterior segments and fundi in both eyes were normal. Magnetic resonance imaging showed orbital masses involving lacrimal glands and extending posteriorly on both sides (Fig. 1A). Gallium scan demonstrated an abnormal uptake site only on the left side of the orbit, with no other abnormal uptake noted systemically (Fig. 1B). Excisional biopsy of the orbital mass on the left side revealed MALT lymphoma (Fig. 2). She underwent external beam irradiation at a total dose of 30 Gy to orbital lesions on both sides.

Whole-body 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography fused with computed tomography (PET/CT), carried out as a follow-up check-up in September 2007, showed no abnormal uptake (Fig. 1C & 1D). She was well until November 2008 when she noticed stuffiness in her stomach. Abdominal lymphadenopathy was found and transabdominal excisional biopsy showed mixed cellularity classical Hodgkin lymphoma, stage II (Fig. 3). She underwent 4 courses of ABVd (adriamycin, bleomycin, vinblastine, and dacarbazine) combination chemotherapy regimen, resulting in partial response, and then 4 courses of C-MOPP (cyclophosphamide, vincristine, procarbazine, and prednisolone) regimen,[15] leading to stable disease. In February 2010, she underwent external beam irradiation at a total dose of 40 Gy to residual right renal hilar lesions, which showed high uptake on FDG-PET/CT. However, the patient showed progressive disease. In April 2010, she developed pancytopenia and was diagnosed with secondary myelodysplastic syndrome.

In July 2010, she showed systemic lymphadenopathy (Fig. 1E) and was diagnosed with DLBCL by cervical lymph node biopsy (Fig. 4). She underwent 8 courses of rituximab monotherapy. In August 2010, she had cataract surgeries in both eyes for radiation cataract. She still showed progressive disease and finally underwent allogeneic hematopoietic stem cell transplantation[16] with myeloablative pretreatment by total body irradiation at a total dose of 12 Gy in October 2010. She died of renal failure in February 2011. Autopsy was not carried out.

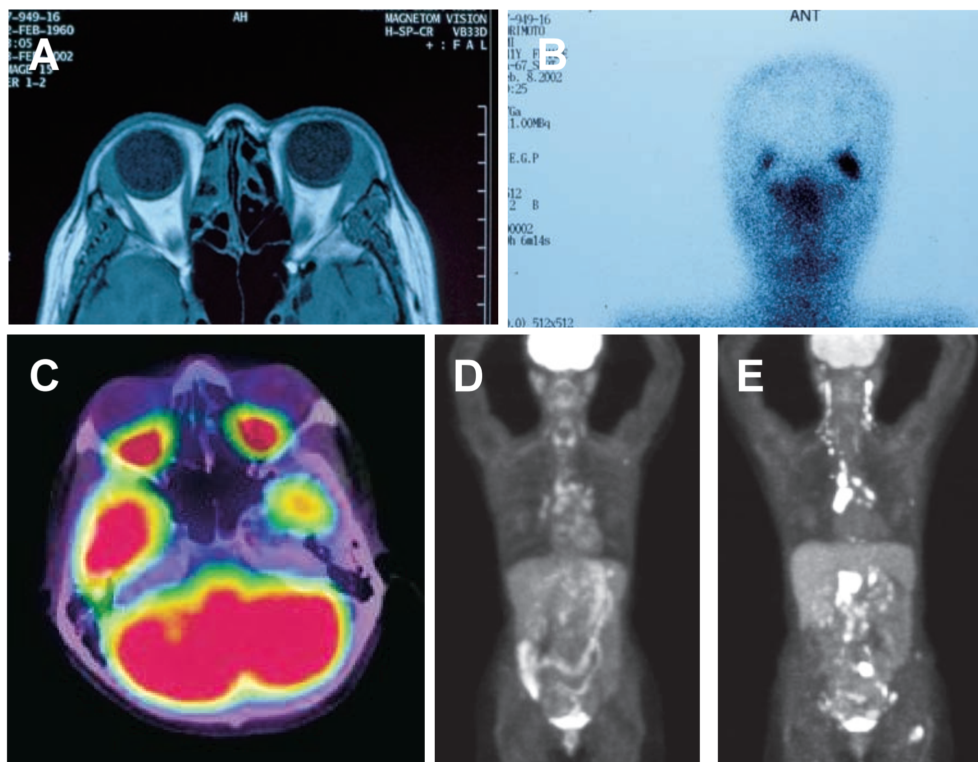

Fig. 1. T1-weighted magnetic resonance imaging (*1A*), showing bilateral orbital lesions involving lacrimal glands in February 2002. Gallium scan (*1B*) showed abnormal uptake only in the orbit on the left side. Whole-body 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG) positron emission tomography fused with computed tomography (PET/CT) in September 2007 showed no abnormal uptake in the bilateral orbits (*1C*) and systemically (*1D*). FDG-PET/CT in July 2010 before allogeneic hematopoietic stem cell transplantation showed numerous abnormal uptake sites in cervical, mediastinal, and abdominal lymph nodes (*1E*).

METHODS

Histopathology, immunohistochemistry, and in situ *hybridization*

Pathological diagnoses were based on hematoxylin-eosin staining and immunohistochemical staining of 4-μm-thick sections from the excised tissues fixed with 10% formalin and embedded in paraffin. Immunohistochemistry was performed and repeated for older tissues, using an automated slide stainer (BenchMark XT, Ventana Medical Systems, Inc., Tucson, Arizona, USA). Tissue sections were processed by standardized heating pretreatment for antigen retrieval prior to entering the usual immunohistochemical procedures.[14,18] *In situ* hybridization was automatically performed by an integrated machine (LEICA BOND-MAX, Leica Microsystems, Newcastle Upon Tyne, UK), using fluorescein-conjugated oligonucleotide probe (Bond ISH Probe, EBER Probe, Epstein-Barr virus-encoded RNA probe, Leica Microsystems).

Immunoglobulin heavy chain gene rearrangement

Immunoglobulin heavy chain gene rearrangement was detected by polymerase chain reaction (PCR).[14,17-20] Briefly, unstained, formaldehyde-fixed, paraffin sections placed on slide glasses were deparaffinized with xylene and graded ethanol series, and samples for genomic DNA isolation were cut out from at least two different areas of the deparaffinized section. Genomic DNA was also isolated from frozen tissues of the left orbital lesion and cervical lymph node lesion, stored at −80℃. The amplification of *immunoglobulin heavy chain* genes was performed by semi-nested PCR, using primers directed to the framework 2 region (FR2A : 5’-TGGRTCCGMCAGSCYYCNGG-3’ for both the first and the second PCR) and to the joining region (LJH : 5’-TGAGGAGACGGTGACC-3’ for the first PCR, and VLJH : GTGACCAGGGTNCCTTGGCCCCAG-3’ for the second PCR). At least two DNA samples from each paraffin section were separately subjected to PCR with TAKARA Ex Taq (Takara Bio Inc., Otsu, Japan). The amplified products from each patient were electrophoresed in parallel in 3% agarose gel. The determination of ‘clonal’ was made only when a single or dominant discrete fragment was consistently generated from different specimens.[17-20]

For the sequencing of the clonal bands, the PCR products were purified with ExoSAP-IT (USB, Cleveland, OH, USA) and used as a template for direct sequencing with the ABI 310 Genetic Analyzer (Perkin-Elmer, Foster, CA, USA) using the BigDye Terminator Cycle Sequencing Kit (Perkin-Elmer) and either of the two primers (FR2A and VLJH) to sequence in both directions.[14]

RESULTS

Histopathology and immunohistochemistry

The left orbital lesion (Fig. 2) in March 2002 showed diffuse infiltration with monotonous cells, admixed with a small number of large cells. Predominant cells were positive for CD79a, admixed with CD3-positive cells. The number of Ki67-positive cells was small. No cell was positive for CD30 (BerH2) or CD15 (Leu M1). Most cells were positive for ϰ light chain while only a small number of cells were positive for λ light chain, indicating ϰ monotype. These features were consistent with the diagnosis of MALT lymphoma.

The abdominal lymph node lesion (Fig. 3) in December 2008 showed Hodgkin and Reed-Sternberg cells with large nuclei and prominent nucleoli, which were positive for CD30 (BerH2), but negative for CD15 (Leu M1), and weakly positive for CD79a. The Hodgkin and Reed-Sternberg cells were negative for Epstein-Barr virus (EBV)-encoded small RNAs by *in situ* hybridization and also negative for pax-5 by immunohistochemistry. These large cells were surrounded by eosinophils and also by CD3-positive small cells and CD20-positive small cells. These features were consistent with the diagnosis of mixed cellularity classical Hodgkin lymphoma.

The cervical lymph node lesion (Fig. 4) in July 2010 showed diffuse infiltration with large cells that were positive for CD20. A small number of CD3- and CD5-positive cells were present. The rate of Ki67-positive cells was high. Small foci of CD30-positive cells were present in the background of a large part of diffuse infiltration with CD30-negative cells. These features were consistent with the diagnosis of DLBCL.

Immunoglobulin heavy chain gene rearrangement

The left orbital lesion with MALT lymphoma in 2002 gave rise to a dominant discrete fragment by PCR amplification of the *immunoglobulin heavy chain* gene from both the paraffin sections and the frozen tissue stored at −80℃ (Fig. 5). The cervical lymph node lesion with DLBCL in 2010 gave rise to a dominant discrete fragment only from the frozen tissue, but not from the paraffin sections. No fragment was generated from the paraffin sections of the abdominal lymph node lesion with Hodgkin lymphoma in 2008.

The fragment generated from the left orbital lesion with MALT lymphoma was the same in terms of size as that from the cervical lymph node lesion with DLBCL. These two fragments from the different lesions shared the same sequences as far as the partial sequences, obtained against a rather high background level, were concerned.

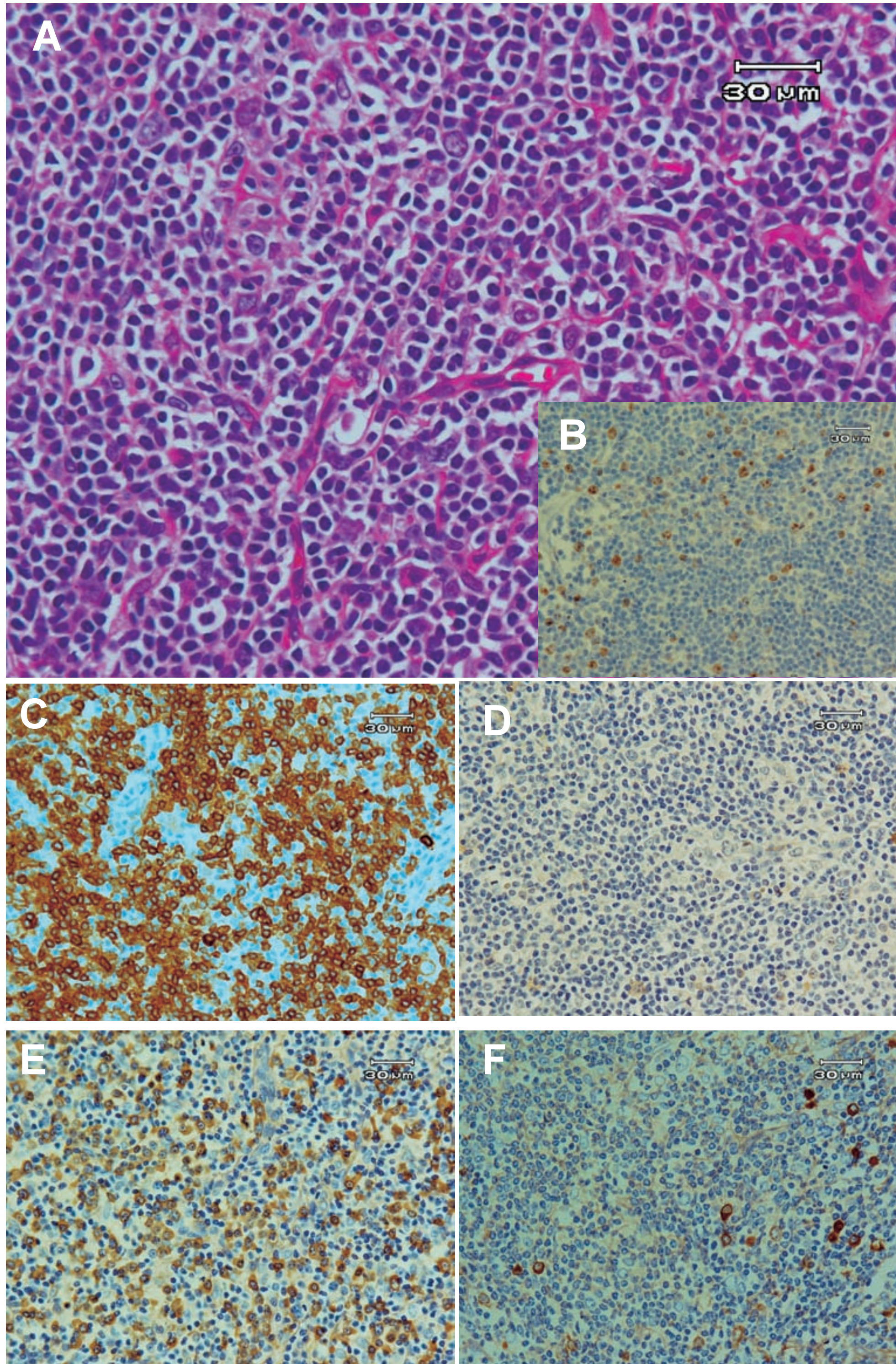

Fig. 2. Histopathology and immunohistochemistry of the orbital lesion on the left side in March 2002, obtained by excisional biopsy. Diffuse infiltration with monotonous cells, admixed with a small number of large cells in hematoxylin-eosin stain (*2A*). The neoplastic cells were occasionally positive for Ki67 (*2B*), predominantly positive for CD79a (*2C*), but negative for CD30 (*2D*). The cells were predominantly positive for Igκ (*2E*), and occasionally positive for Igλ (*2F*), indicating Igκ monotype. *Bar* = 30 μm.

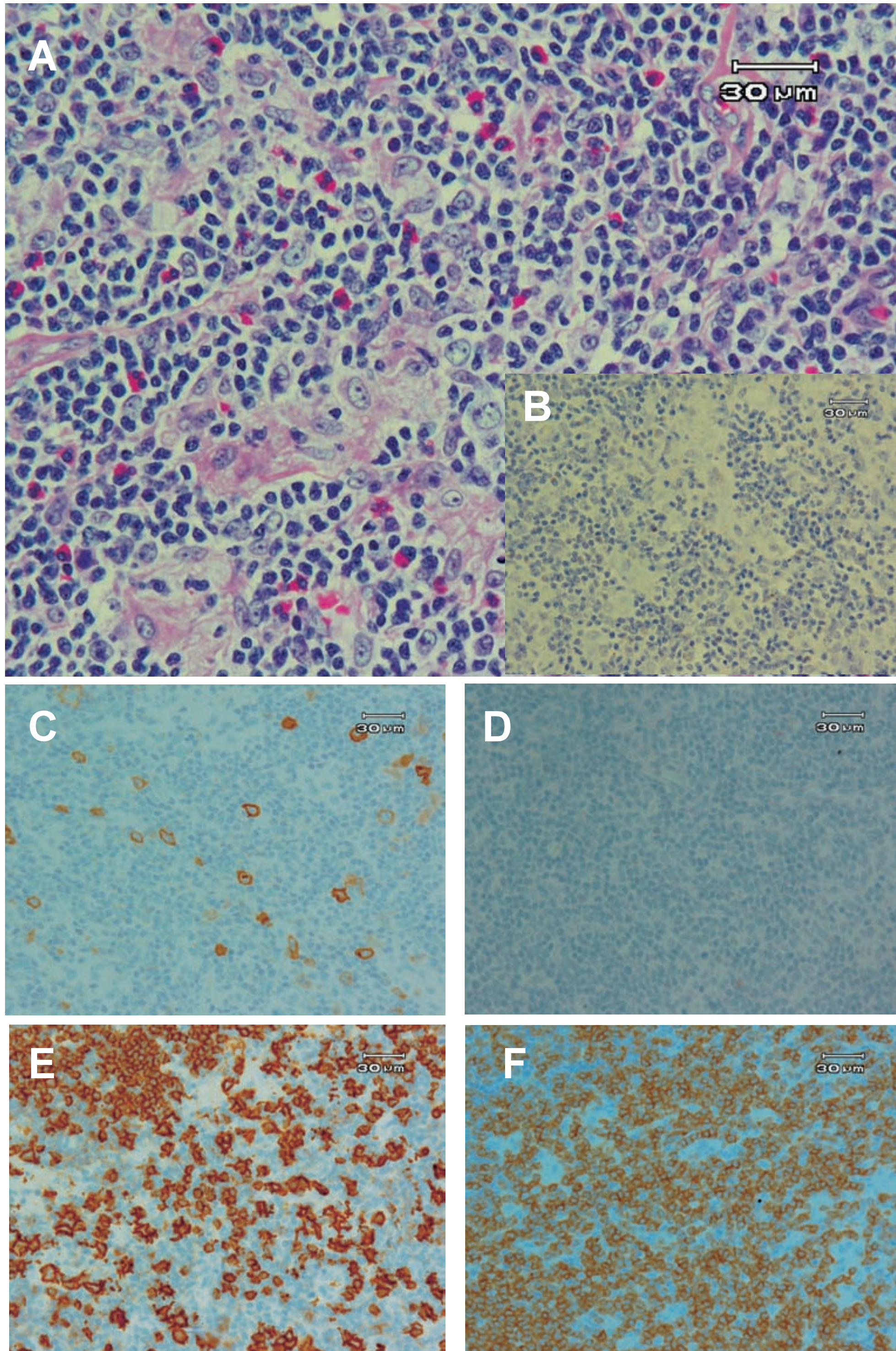

Fig. 3. Histopathology and immunohistochemistry of the abdominal lymph node lesion in December 2008, obtained by transabdominal excisional biopsy. A small number of Hodgkin cells and Reed-Sternberg cells with large nuclei and prominent nucleoli, surrounded by eosinophils and monotonous small cells, in hematoxylin-eosin stain (*3A*). Hodgkin and Reed-Sternberg cells were negative for Epstein-Barr virus (EBV)-encoded small RNAs (EBER) by *in situ* hybridization (*3B*), positive for CD30 (BerH2) (*3C*), and negative for CD15 (Leu M1) (*3D*) by immunohistochemistry. Predominant small cells were positive for either CD20 (*3E*) or CD3 (*3F*). *Bar* = 30 μm.

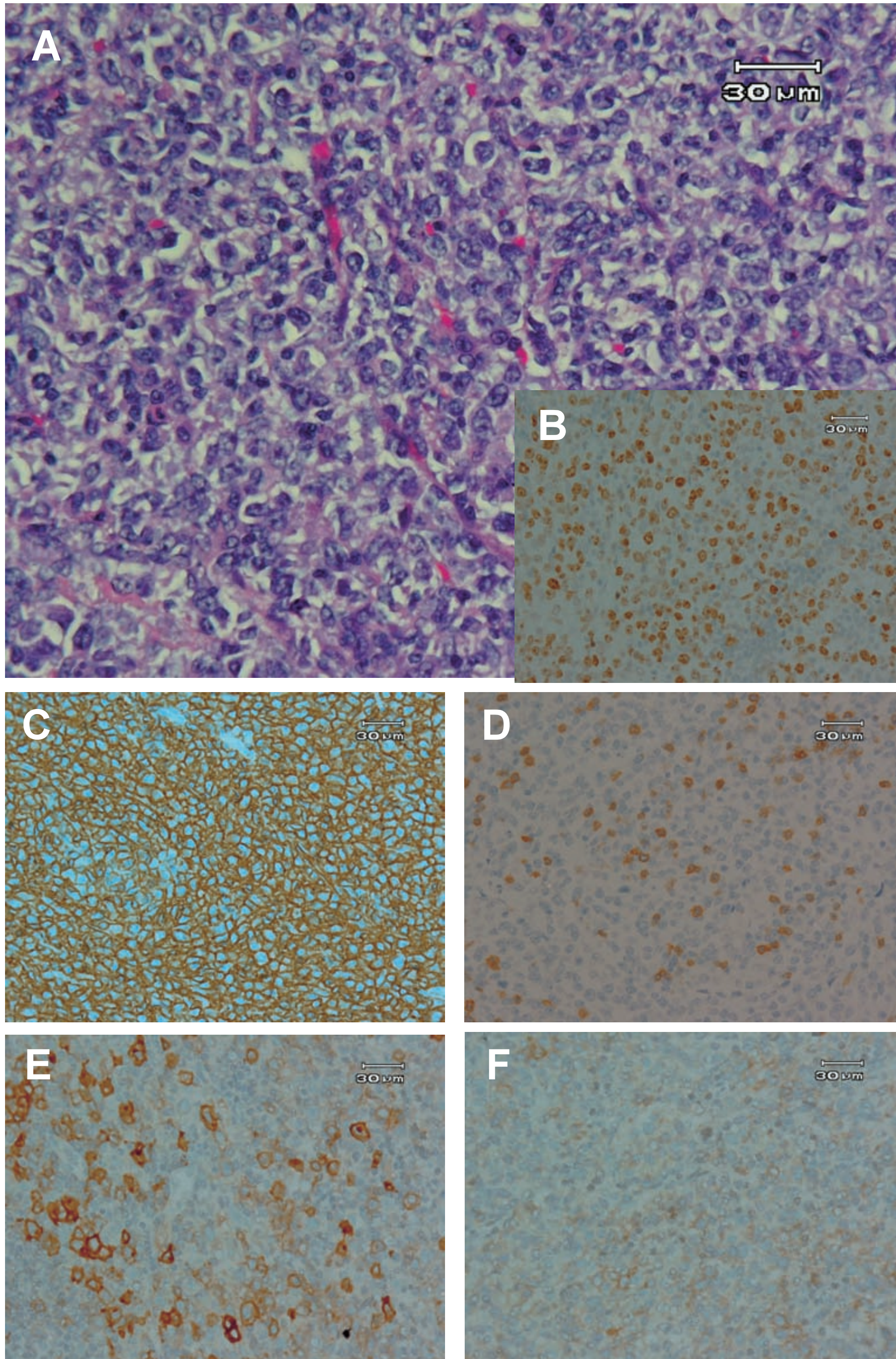

Fig. 4. Histopathology and immunohistochemistry of the cervical lymph node lesion in July 2010, obtained by excisional biopsy. Diffuse infiltration with large cells in hematoxylin-eosin stain (*4A*), which were highly positive for Ki67 (*4B*). Most cells were positive for CD20 (*4C*), admixed with CD3-positive cells (*4D*). CD30-positive cells formed a small focus (*4E*), but were basically rare in a large part of the lesion (*4F*). *Bar* = 30 μm.

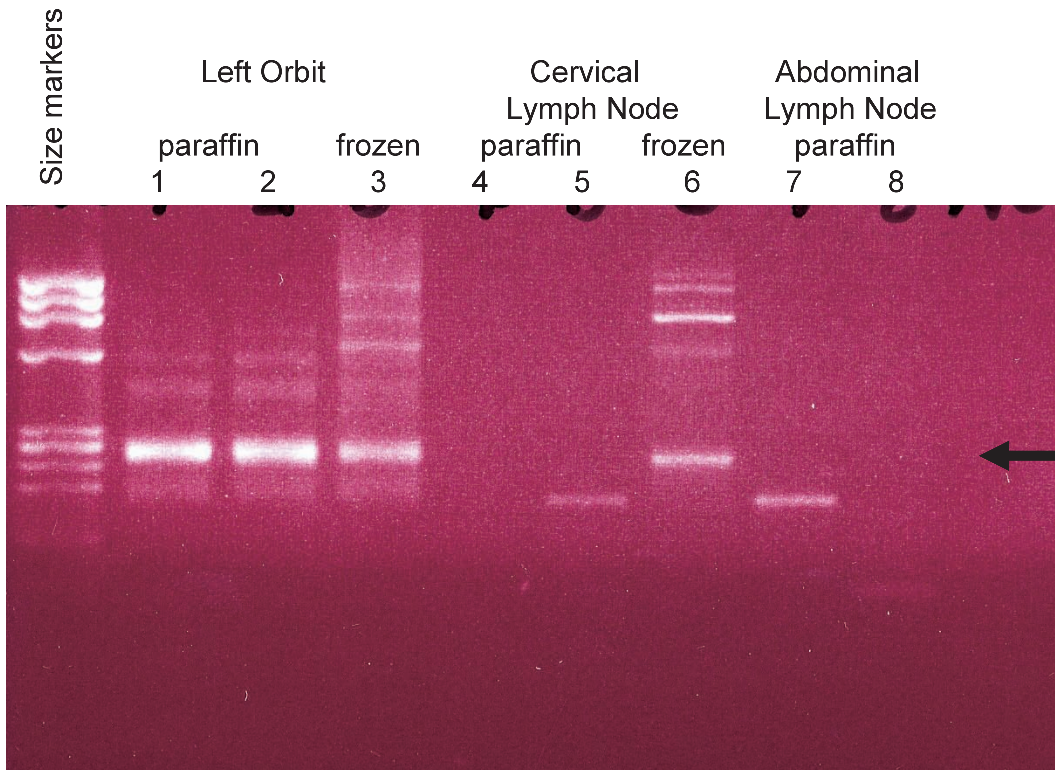

Fig. 5. Polymerase chain reaction amplification of the *immunoglobulin heavy chain* gene from lymphoma lesions. Lanes 1-3, derived from genomic DNA of paraffin sections (designated as paraffin in Lanes 1 and 2) and frozen tissue (designated as frozen in Lane 3) of the left orbital lesion with MALT lymphoma in 2002. Note that dominant discrete DNA fragments of the same size (*arrow*), indicating clonality of cells, were generated from paraffin sections and frozen tissue. Lanes 4-6, derived from genomic DNA of paraffin sections (designated as paraffin in Lanes 4 and 5) and frozen tissue (designated as frozen in Lane 6) of the cervical lymph node lesion with diffuse large B-cell lymphoma in 2010. Note that a dominant discrete fragment was amplified only from the frozen tissue (Lane 6), but not from the paraffin sections (Lanes 4 and 5). Lanes 7 and 8, derived from paraffin sections (designated as paraffin) of the abdominal lymph node lesion with Hodgkin lymphoma in 2008, showing no fragment. Frozen tissue was not available for the abdominal lymph node. Size markers : phage X 174 DNA Hae III digest (1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 base pairs).

DISCUSSION

Sequential development of MALT lymphoma in the ocular adnexa, Hodgkin lymphoma in the abdominal lymph nodes, and finally, DLBCL in systemic lymph nodes in this patient suggests that the same clonal cells of B-cell lineage could change their morphological and immunohistochemical phenotypes in the long-term follow-up with standard modalities of treatment. A similar event, sequential development of MALT lymphoma in the tonsil, Hodgkin lymphoma in mediastinal lymph nodes, and DLBCL in the colon was reported in one patient.[8] The flow from MALT lymphoma, and Hodgkin lymphoma, to DLBCL might be a destined pathway for neoplastic cells derived from the B-cell lineage.

DLBCL is well known to occur as a relapse of MALT lymphoma.[19] In the present patient, we confirmed the absence of EBV-encoded small RNAs by *in situ* hybridization in the abdominal lymph node lesion of Hodgkin lymphoma to exclude the diagnosis of EBV-positive DLBCL of the elderly. Partially and weakly positive CD79a expression in the Hodgkin cells and Reed-Sternberg cells in the lesion suggests that these cells would be derived from the B-cell lineage, although the expression of pax-5, another marker for the B-cell lineage, was absent.

It should be noted that a small number of large cells were admixed with predominant monotonous cells in ocular adnexal MALT lymphoma of the present patient. These large cells were phenotypically the same as predominant large cells in systemic DLBCL, which developed later in the course. The large cells in ocular adnexal MALT lymphoma might survive the irradiation. Hodgkin cells and Reed-Sternberg cells were positive for CD30 (BerH2) but negative for CD15 (Leu M1)

in Hodgkin lymphoma of the present patient. Either CD30 or CD15 was negative in ocular adnexal MALT lymphoma, which preceded Hodgkin lymphoma. In contrast, large cells in small foci of DLBCL, which followed Hodgkin lymphoma in this patient, did express CD30, but not CD15. The results suggest that part of the large cells in DLBCL would retain some features of Hodgkin cells, such as CD30 expression, from the preceding Hodgkin lymphoma. The expression of CD30 in DLBCL, following Hodgkin lymphoma, was also described in a previous patient who took a similar course.[8]

In the present patient, ocular adnexal MALT lymphoma was treated by external beam irradiation at a total dose of 30 Gy while abdominal Hodgkin lymphoma was treated by standard combination chemotherapy (ABVd and C-MOPP).[15] In the previous patient with a similar course, tonsillar MALT lymphoma was treated by combination chemotherapy with cyclophosphamide, vincristine, and prednisolone, followed by external beam irradiation at a total dose of 45 Gy while mediastinal Hodgkin lymphoma was treated with standard combination chemotherapy with ABVD and MOPP, followed by mantle field (40 Gy) and inverted Y area (32 Gy) irradiation.[8] These similar courses of treatment in the present patient and in the previous patient[8] might serve as the background for sequential development of MALT lymphoma, Hodgkin lymphoma, and DLBCL in the same sequence of events.

Clonal analysis by PCR amplification of the *immunoglobulin heavy chain* gene showed fragments of the same size between the ocular adnexal MALT lymphoma and systemic DLBCL, suggesting the same clonal origin of both types of lymphoma in the present patient. No amplification of the fragment of the *immunoglobulin heavy chain* gene in abdominal Hodgkin lymphoma could be attributed to a small quantity or poor quality of genomic DNA derived from the paraffin sections. This interpretation would be further supported by the fact that the amplification of the fragment was obtained only from genomic DNA of the frozen tissue, but not from genomic DNA of the paraffin sections, of the cervical lymph node lesion with DLBCL.

In conclusion, this is the second case report, to the best of our knowledge, to show that MALT lymphoma, Hodgkin lymphoma, and DLBCL developed sequentially in the same patient. Standard treatment such as combination chemotherapy and irradiation might be a driving force for these phenotypic changes of neoplastic cells. Changing patterns of the phenotypes of lymphoma cells in the same patient should be borne into mind in the era of long-term survival of patients with lymphoma.

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