Case Report

Primary Type3 (Non-ABC, Non-GCB) Subtype of Extranodal Diffuse Large B-Cell Lymphoma of the Thyroid Bearing No *MYD88* Mutation by Padlock Probe Hybridization

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Keywords

Primary extranodal malignant lymphoma · Diffuse large B-cell lymphoma · *MYD88* gene mutation

Abstract

Primary extranodal malignant lymphoma of the thyroid is a rare entity composed of mostly neoplastic transformation of germinal center-like B cells (GCB) or memory B cells. Other B-cell-type malignancies arising primarily in the thyroid have rarely been described. Immunohistochemical examination of autopsied primary malignant lymphoma of the thyroid in an 83-year-old Japanese female revealed the presence of a non-GCB subtype of diffuse large B-cell lymphoma (DLBCL) without the typical codon 206 or 265 missense mutation of *MYD88*. The lack of the highly oncogenic *MYD88* gene mutation, frequently observed in DLBCL of the activated B-cell (ABC) subtype, and the detection of an extremely aggressive yet local clinical phenotype demonstrated that the present case was an exceptional entity of the type3 (non-GCB and non-ABC) subtype.

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Introduction

Primary lymphoma of the thyroid is a rare entity, representing approximately 1–2% of all extranodal lymphomas and <2% of all thyroid malignancies [1, 2]. Herein, we describe an autopsy case of thyroid diffuse large B-cell lymphoma (DLBCL) with nongerminal center-like B cells (non-GCB) harboring no *MYD88* mutation.

Case Presentation

An 83-year-old Japanese female had been receiving regular outpatient treatment by a general physician for follow-up of hypothyroidism over the previous 10 years. A rapidly growing thyroidal mass and swelling of the thyroid gland causing severe dyspnea were noted 1 month before she was admitted to our hospital. While the size of the thyroid glands increased continuously, administration of antibiotics brought about temporal improvement of the symptom symptoms. Clinically, the condition was assumed to be thyroidal anaplastic carcinoma. The patient died of cardiopulmonary arrest on day 10 after admission; autopsy was carried out 2 h postmortem. The size of the thyroid tumor was 13 × 13 × 8 cm (Fig. 1a). Tumor cells infiltrated the trachea, esophagus (Fig. 1b), and the middle of the right lobe of the lung (Fig. 1c). The patient was compromised with stomach cancer comorbidity (Fig. 1d). Pathological diagnosis of the resected thyroid specimen revealed diffuse infiltration of round cells with scant cytoplasm (Fig. 1e). On the other hand, histological analysis of the coincidental stomach cancer revealed signet-ring cell carcinoma (Fig. 1f). Immunohistochemical examination of thyroid tissue disclosed cells positive for CD20 (Fig. 2a) and MUM1 (Fig. 2b), and negative for CAM5.2 (Fig. 2c), CD3 (Fig. 2d), Bcl-6 (Fig. 2e), and CD10 (Fig. 2f).

To determine whether the non-GCB type of DLBCL was activated B-cell like (ABC) or of type3 (non-GCB and non-ABC) [3], immunohistochemical analysis was conducted to check for the presence of the *MYD88* mutation [4] in the lymphoma cells. DNA samples from 5-mm-thick sections of formalin-fixed paraffin-embedded specimens were selectively prepared from microdissected lymphoma cells by the agarose-bead-mediated technique [5] and subjected to PCR. Since codons 206 and 265 are the 2 hot spots of somatic *MYD88* mutations recurrently observed among specific subsets of B-cell malignancies, especially in the ABC type of DLBCL [4], 2 sets of primers were used for PCR to cover 2 genomic regions: sense, 5'-CCGTGGCCTTCTAGCCAAC-3' and antisense, 5'-GATGTCCTGCCTGGCACCTG-3' for codon 206; and sense, 5'-TCTCTCCAGGTGCCCATCAGA-3' and antisense, 5'-CAAGTACAAGGCAATGAA-GAA-3' for codon 265. PCR products were ligated and cloned into T vector and sequenced. No typical c.794T>C mutation causing the p.L256P mutation (Fig. 3a) and c.618T>A mutation causing p.S206S silent mutation were found in 12 independent clones (Fig. 3b). To confirm that *MYD88* harbored no missense mutation, padlock probe hybridization was carried out, followed by hyperbranched rolling-circle amplification, as previously described [6]. For target DNA sequencing, 2 genomic regions were used: no mutation, TCTCCAGGTGCCCATCA-GAAGCGACTGATCCCCATCAAGTACAAGGCAAT and c.794T>C mutation causing p.L256P mutation, TCTCCAGGTGCCCATCAGAAGCGACCGATCCCCATCAAGTACAAGGCAAT. For the padlock probe, 2 genomic regions were used: wild type, GTCGCTTCTGATGGGCACCT...CTTGT-ACCTGATGGGGATCA and mutant type GTCGCTTCTGATGGGCACCT...CTTGTACTTGATGG-GGATCG. In the positive control DLBCL case with known p.L256P mutation, signals were detected in both the wild-type padlock probe (Fig. 3a) and the mutant-type padlock probe (Fig. 3b). In the present case, however, signals were detected in the wild-type padlock probe

(Fig. 3c) but not in the mutant-type padlock probe (Fig. 3d). The present case was therefore confirmed to harbor no missense mutation and was finally diagnosed as an exceptional entity of type3 (non-GCB and non-ABC) subtype of DLBCL.

Discussion

Compared with secondary lymphoma with systemic involvement, primary malignant lymphoma of the thyroid is a rare entity and is mostly secondary to chronic thyroiditis [1, 2]. As other extranodal lymphomas, primary malignant lymphoma of the thyroid is classified on the basis of pathological subtypes, each with a different prognosis.

Among the fewer than 150 cases described in the literature to date, the GCB subtype of DLBCL is the most common [7]. While cases with DLBCL, accounting for up to 70% of all primary malignant lymphomas of the thyroid, are aggressive subtypes associated with a poor prognosis, those with mucosa-associated lymphoid tissue usually follow an indolent course [8].

Since the first report of the highly oncogenic *MYD88* p.L265P mutation in the ABC subtype of DLBCL [4], L265P mutation has been recurrently detected in primary central nervous system lymphoma, in primary cutaneous leg-type DLBCL [9], and in Waldenstrom macroglobulinemia [10], indicating that *MYD88* is 1 of the target genes of certain subsets of B-cell malignancies at an early stage of oncogenesis. In the present case, while immunohistochemical examination of the tissue specimens revealed the presence of the non-GCB subtype of DLBCL (CD10 [–], Bcl-6 [–], MUM1 [+], Fig. 2), genetic analysis by both PCR and in situ morphological analyses showed a lack of typical codon 206 or 265 missense mutation (Fig. 3). The present case was finally diagnosed as type3 (non-GCB and non-ABC), a subtype of DLBCL that may account for a characteristic clinical course of a rapidly growing tumor, locally aggressive, yet without systemic involvement of distant organs.

Statement of Ethics

All procedures followed were in accordance with the ethical standards of the Ethical Committee of the Ehime University Hospital on human experimentation and with the Helsinki Declaration of 1964 and later versions. Informed written consent was obtained from the patient's family for this study.

Disclosure Statement

All authors declare that they have no conflicts of interest to disclose.

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Fig. 1. **a** Enlarged thyroïdal mass of 13 × 13 × 8 cm prominent at autopsy. The section of the cervix reveals a whitish, solid lesion with necrotic foci (**b**). Although systemic involvement was not observed, the tumor infiltrated the surrounding esophagus and the trachea (**b**, asterisk). **c** Infiltration of the 3-cm malignant lymphoma is also seen in the middle of the right lobe of the lung (arrowheads). **d** Coincidental finding of stomach cancer on the side of the lesser curvature of the body. **e** Histopathological analysis of the resected thyroïdal tissue specimen, showing diffuse infiltration of the round cells with scant cytoplasm and a few atrophic and destroyed thyroïdal glands (HE, ×200). On the other hand, the stomach cancer is a typical signet-ring cell carcinoma (**f**, HE, ×400).

Fig. 2. **a** Immunohistochemical examination showing infiltrating cells positive for CD20 ($\times 200$) and MUM1 (**b**, $\times 200$) and negative for CAM5.2 (**c**, $\times 200$), CD3 (**d**, $\times 200$), Bcl-6 (**e**, $\times 200$), and CD10 (**f**, $\times 200$), confirming the diagnosis of nongerminal center-like B-cell type of diffuse large B-cell lymphoma.

Fig. 3. PCR conducted with the use of formalin-fixed paraffin-embedded samples to explore a genetic alteration of the *MYD88* gene. The PCR products were ligated and cloned into T vector and sequenced. No typical c.794T>C mutation causing p.L256P mutation (**a**, arrow) or c.618T>A mutation causing p.S206S mutation (**b**, arrow) is seen in 12 independent clones. Hyperbranched rolling-circle amplification was conducted to confirm PCR sequencing. In the positive control specimen from a diffuse large B-cell lymphoma case with a known typical c.974T>C mutation, signals are seen in both the wild-type padlock probe (**a**) and mutant-type padlock probe (**b**). In the present case, however, signals are seen in the wild-type padlock probe (**c**), but no signals are seen in the mutant-type padlock probe (**d**).